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Research Article

BIOAVAILABILITY AND DISSOLUTION ENHANCEMENT OF GLYBURIDE NANOSUSPENSION

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ABSTRACT

Objective: The main objective was to develop nanosuspension of glyburide (GLY) by quasi emulsification solvent diffusion method and to enhance dissolution and bioavailability characteristics of the drug GLY, an antidiabetic drug which belongs to Biopharmaceutical Classification System-II category.

Methods: In this work, nanoparticles were prepared using polyvinyl alcohol, hydroxypropyl methyl cellulose, and Eudragit RL100. Twelve formulations of GLY (GLY-1–GLY-12) were formulated using the excipients at various compositions. Drug and excipient compatibility studies were conducted using Fourier transform infrared and differential scanning calorimeter. The prepared nanosuspension was analyzed using scanning electron microscopy for surface of the particle analysis, melting point, solubility, particle charge zeta (*mv*), percentage drug entrapment efficiency (%), and *in vitro* drug release. The optimized formulations of nanosuspension were further studied for *in vivo* pharmacokinetic evaluation. Reverse-phase high-performance liquid chromatography method was developed, validated, and used for the study of these formulations in rat plasma.

Results: From these studies, it was confirmed that drugs and excipients chosen were compatible with each other. GLY-8 was the best formulation with a particle size of 85–96 nm with 168.7°C melting point, freely soluble in phosphate buffer pH 7.4, 93.53% drug entrapment, and 90.26±1 mV of zeta potential. This formulation shows percentage drug release of 99.85% in 24 h. *In vivo* pharmacokinetic study for optimized formulation (GLY -8) suggested that there was no reaction with the rat plasma. From the results, it was shown that C_{max} and T_{max} were found to be 0.604±0.03 µg/ml and 2±1.01 h, respectively. The values of $t_{1/2}$ (h), area under the curve (AUC) $_{(0\text{-}t)}$, and AUC $_{(0\text{-}\infty)}$ were found to be 10.04 h, 2.562±0.41 µg.h/ml, and 2.147±0.45 µg.h/ml, respectively.

Conclusion: Based on the results obtained, oral administration of nanosuspension could not only provide the better absorption of poorly water soluble drugs but may also reduce toxicity and provide a new tool in drug delivery system.

Keywords: Glyburide, Scanning electron microscopy studies, *In vitro* drug release, *In vivo* pharmacokinetic evaluation, Dissolution and bioavailability enhancement.

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INTRODUCTION

In the drug discovery and development, poor water solubility is regarded as wide problem. More than 40% of drugs are poorly soluble in water, so they show problems in formulating them in conventional dosage forms. This problem is complex for Class II drugs which are poorly soluble in aqueous and organic media. Nanosuspension preparation is preferred for compounds that are insoluble in water (but are soluble in oil) with high log p value. Various approaches are there to resolve problems of low solubility and low bioavailability such as micronization, cosolvency, oily solution, and salt formation, and some other techniques are liposomes, emulsions, microemulsion, solid dispersion, and β-cyclodextrins inclusion complex. In these cases, nanosuspensions are preferred. It is most suitable for the compounds with high log p value, high melting point, and high dose. Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster (e.g., oral or intravenous administration of the nanosuspension). This is one of the unique advantages that it has over other approaches for enhancing solubility. It is useful for molecules with poor solubility, poor permeability, or both, which poses a significant challenge for the formulators [1]. Glibenclamide which is also known as glyburide (GLY) belongs to second-generation sulfonylurea. It is majorly used to treat non-insulin-dependent diabetes mellitus and administered orally [2]. GLY has been classified as the Biopharmaceutical Classification System (BCS) class-II drug due to its low aqueous solubility, with bioavailability of 100% and huge permeability. The solubility of GLY in gastrointestinal fluids and pH in the gastrointestinal tract impact on its *in vivo* dissolution. A lot of approaches have been made to optimize *in vivo* dissolution and bioavailability. However, to predict the *in vivo* performance of a dosage form, it is necessary to have an *in vitro–in vivo* correlation studies. The main objective of this study was to develop nanosuspension of GLY by quasi emulsification solvent diffusion method and also to enhance dissolution and bioavailability. This manuscript includes 12 formulations of GLY which were prepared with three different polymers such as polyvinyl alcohol (PVA), hydroxypropyl methyl cellulose (HPMC), and Eduragit (EDG) with a stabilizer, i.e. poloxamer 407 with formulation codes from GLY-1 to GLY-12. The best formulation had been identified and further subjected to both *in vitro* and *in vivo* studies [3-5]. The present study brought out the best formulation with an enhanced dissolution and bioavailability.

MATERIALS AND METHODS

Materials

GLY drug sample was procured from Hetero Drugs, Hyderabad. HPMC was procured from Yucca Enterprises, Mumbai. PVA was brought from Yarrow Chem Products., Mumbai. EDG RL 100 was procured from Yucca Enterprises, Mumbai. Poloxamer 407 was procured from SD Fine Chem, Hyderabad. All other chemicals and reagents utilized in the study were with the analytical grade.

Analytical method development

Determination of λ_{max}

Ultraviolet (UV) spectrum of GLY was carried out in phosphate buffer pH 7.4. 10 mg of GLY was weighed accurately and transferred to a 10 ml

volumetric flask. The solution of 10 µg/ml was kept in a fused silica cuvette. UV spectrum was recorded in the range of 200–800 nm by Shimadzu double-beam UV-visible spectrophotometer against blank buffer solution pH 7.4. The wavelength for maximum absorbance was recorded [6].

Preparation of stock solution

Accurately weighed 10 mg of GLY was dissolved in 100 ml of phosphate buffer pH 7.4 to get a concentration of 100 µg/ml.

Preparation of standard solution

From the above stock solution, 1, 2, 3, 4, and 5 ml were transferred to a 10 ml volumetric flask and volume was made up to the mark with phosphate buffer pH 7.4. The final concentrations of 10, 20, 30, 40, and 50 µg/ml were obtained. Absorbance of the above standard solutions was determined in a UV-visible spectrophotometer and calibration curve (CC) was constructed between concentration and absorbance [7].

Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of GLY were studied. GLY and KBR were mixed and compressed into a pellet in the ratio of 1:100 in a motorized pellet press (Ki Maya Engineers, India) at 10:12 tons of pressure. The pressed pellet was scanned in FTIR spectrophotometer. The FTIR spectrum of GLY was compared with the standard pharmacopoeial spectrum of GLY [8].

Preparation of GLY nanosuspension

GLY nanosuspensions were prepared by quasi emulsification solvent diffusion method. GLY (30 mg), PVP, HPMC, and EDG RL100 (0.5, 1.0, 1.5, and 2%) were codissolved in 15 ml of methanol. The solution was slowly injected with a syringe containing thin Teflon tube into 35 ml water containing poloxamer 407. It was maintained at low temperature in ice bath to protect from sun light. During injection, the mixture was stirred well by a high-speed homogenizer at a speed of 5500 rpm. The solution immediately turned into pseudo emulsion in the external aqueous phase. The counter diffusion of methanol and water out of and into the emulsion microdroplets, respectively, results into the formation of nanosuspension. Formulation was prepared with varying polymer ratio. 12 formulations of GLY were prepared with three different polymers such as PVA, HPMC, and EDG with a stabilizer, i.e., poloxamer 407 with formulation codes from GLY-1 to GLY-12. Table 1 gives the formulation of GLY nanosuspensions [9].

Physicochemical evaluation of drugs

Color and appearance

The color and appearance of drug were observed and recorded.

Melting point

The melting point of GLY was determined by open capillary method. The melting point was determined by introducing small amount of substance in the capillary which was then attached to graduated thermometer. Later, constant heat was applied with the assembly suspended in the paraffin bath. The drug sample was tested at the temperature ranging between 200 and 250°C. The temperature required to melt drug was noted.

Solubility studies

Solubility studies were done by adding 100 mg of drug in increments to 10 ml of different buffer solutions. The conical flasks with 250 ml of water were kept on a shaker water bath until saturation occurred. The conical flasks were kept for 5 h at ambient temperature. The samples were centrifuged and filtered and the filtrates were analyzed by UV-visible spectrophotometer [10].

Evaluation of GLY nanosuspension

Size analysis

Scanning electron microscopy (SEM) is a method for high-resolution surface imaging. The SEM uses an electron beam for surface imaging. The advantages of SEM over light microscopy are greater magnification and much larger depth of field. Different elements and surface topographies emit different quantities of electrons, due to which the contrast in a SEM micrograph (picture) is representative of the surface topography and distribution of elemental composition on the surface [11].

Zeta potential of the drug

Zeta potential measurements were run at 25°C with an electric field strength of 23 V/m, using Zetasizer (Nano ZS 90, Malvern Instruments, UK). To determine the zeta potential, samples of drug nanosuspensions were diluted and placed in electrophoretic cell. The zeta potential was calculated as described by Helmholtz–Smoluchowski equation. The formulation of GLY with PVA, HPCM, and EDG RL 100 with a stabilizer poloxamer 407 (Pluronic F127) was done. After formulating the suspension or nanosuspension, the zeta potential was determined to know the stability of the nanosuspension. Zeta potential is very important as it measures stability. Hence, all formulations were determined with zeta potential and recorded [12].

Drug content

$$\%\text{Drug content} = \frac{\text{Obtained amount of drug}}{\text{Theoratical amount of drug}}$$

A weighed amount of each preparation was dissolved in the required amount of methanol and diluted suitably in phosphate buffer of pH 7.4. Spectrophotometrically, drug content was determined at required wavelength. Calculation was done using the following formula [13].

Percentage entrapment efficiency

To determine percentage entrapment, around 2 ml of each formulation was taken in c (10 ml) and was centrifuged in centrifuge machine at 2000–3000 rpm for 4 h. The supernatant layer was filtered through Whatman filter paper no: 41 and diluted with phosphate buffer

Table 1: Formulation of GLY nanosuspensions using emulsification solvent diffusion method

Formula (mg)	GLY	PVA (%)	HPMC (%)	EDG	Poloxamer (mg)	Distilled water (ml)
GLY-1	30	0.5	–	–	200	50
GLY-2	30	1.0			200	50
GLY-3	30	1.5			200	50
GLY-4	30	2.0			200	50
GLY-5	30		0.5		200	50
GLY-6	30		1.0		200	50
GLY-7	30		1.5		200	50
GLY-8	30		2.0		200	50
GLY-9	30			0.5	200	50
GLY-10	30			1.0	200	50
GLY-11	30			1.5	200	50
GLY-12	30			2.0	200	50

GLY: Glyburide, PVA: Polyvinyl alcohol, HPMC: Hydroxypropyl methyl cellulose, EDG: Eudragit

pH 7.4. The resultant solutions were analyzed at a wavelength of nm of respective drug using UV double-beam spectrophotometer. These readings were taken for 3 times and the result was calculated [14]. The percentage entrapment efficiency was calculated according to the following equation or formula:

EE= (Total drug content – free drug content/Total drug content) × 100

Differential scanning calorimetry (DSC)

GLY thermal behavior was assessed by carrying out thermal analysis by DSC (DSC-Hitachi 7020). The samples (8–10 mg) were carefully transferred and heated in a crimped aluminum pan for accurate results. The samples were heated from 100 to 600°C at the rate of 10°C/min. A physical mixture of GLY with polymers (PVA, HPMC, and EDG) in the ratio of 1:1 was assessed by carrying out thermal analysis. Thermogram for drug and polymer mixture was taken by using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland) [15].

In vitro drug release studies

In vitro drug release of the nanosuspension was carried out using USP dissolution apparatus type 2 (paddle type). 5 ml of nanosuspension was taken in a dialysis membrane consisting of spectra or membrane (cutoff: 1200 Da). This dialysis system was tied to paddle and dissolution medium with phosphate buffer pH 7.4. Dissolution was carried in triplicate for 10 h at 37±10°C temperature and 50 rpm. At regular intervals of time, 1 ml of sample from the external medium was taken and replaced with fresh phosphate buffer and all the samples were analyzed at nm of respective drug using U.V spectrophotometer. GLY nanosuspensions were prepared by quasi emulsification method which were analysed by in vitro drug dissolution method [7,11].

Table 2: CC data of GLY in phosphate buffer pH 7.4

Sl. No	Concentration (μg/ml)	Absorbance at 229 nm
1	25	0.298
2	50	0.440
3	75	0.602
4	100	0.732
5	125	0.869
6	150	0.992

GLY: Glyburide, CC: Calibration curve

Table 3: Physical properties of drug

Sl. No	Parameter	Drug (GLY)
1	Color	White
2	Appearance	Crystalline

GLY: Glyburide

Table 4: Melting point determination of drug

Drug name	GLY°C
Reported melting point	165–171
Observed Melting Point	168.7

Mathematical dissolution model for GLY nanosuspension formulations

Various mathematical dissolution models such as Higuchi, Krosmeyer–Peppas, and first order were applied for GLY nanosuspension to evaluate the release kinetics of GLY nanosuspension.

***In vivo* studies**

Bioavailability studies in Wistar rats

Male Wistar rats weighing about 200±20 g were supplied by the Experimental Animal Center of Local Vendor, and animal experiment was evaluated and approved by the Institution of Animal Ethics Committee (1423/PO/a/04/CPCSEA/104/2018). Rats were randomly divided into two groups with six animals each and were caged at least for 3 days before the study. They had free access to both food and water. Food was withdrawn 12 h before start of the study. An optimized formulation of nanosuspension was administrated orally to rats [16].

RESULTS

Analytical method for GLY

Determination of λ_{max}

UV spectra of GLY in phosphate buffer pH 7.4 were determined by double beam UV-Visible spectrophotometer and found to be at 229 nm. Fig. 1 shows the λ_{max} curve of GLY.

CC and physical properties of GLY

CC of GLY was constructed by determining the absorbance of GLY at 229 nm. It was observed that GLY showed good linearity over the concentration range of 25–150 μg/ml. Table 2 and Fig. 2 show calibration and linearity curve data of GLY, respectively. The color of GLY was white and crystalline in appearance Table 3.

Drug-excipient compatibility studies

Drug and excipient compatibility studies were performed using FTIR spectrophotometer and DSC, and no marked incompatibilities were found which was shown in Fig 3 and 4.

Physical properties of GLY nanosuspension

Melting point

Capillary tube method was used to determine melting point and was found to be 168.7°C, GLY. This value is the same as that of the literature citation shown in Table 4.

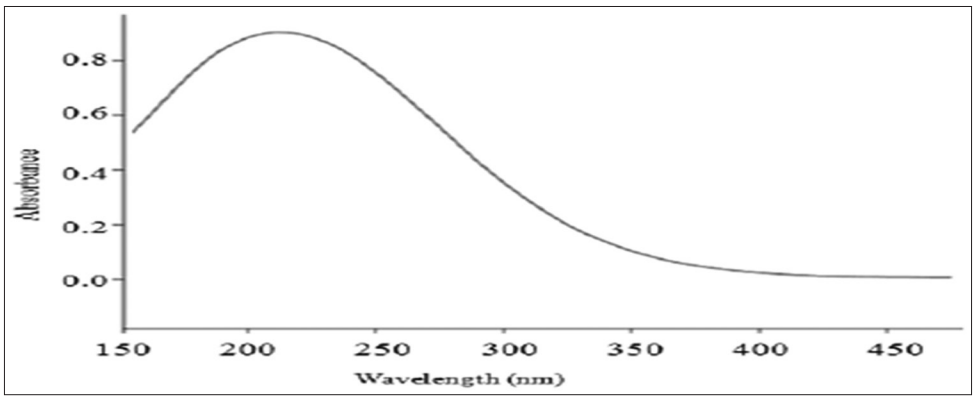

Fig. 1: λ_{max} curve of glyburide in phosphate buffer pH 7.4

Solubility studies

Table 5 shows the results of solubility studies according to pharmacopeia limits

Evaluation of GLY nanosuspension

SEM

SEM of optimized formula (GLY-8) is shown in Fig. 5 and SEM analysis of different formulations is described in Table 6.

DSC

DSC thermograms of pure GLY and GLY plus polymer blend were mentioned in Figs. 6 and 7.

In vitro drug release studies: Table 7 and Fig 8 showed the percentage cumulative drug release of various GLY nanosuspensions.

Mathematical dissolution model for GLY nanosuspension formulations

Various mathematical dissolution models such as Higuchi, Korsmeyer-Peppas, and first order were applied for GLY nanosuspension to evaluate the release kinetics of GLY nanosuspension. Table 8 and Figs. 9-11 give the data and plots of Higuchi, Krosmeyer–Peppas, and first order, respectively.

Table 5: Solubility studies

Medium	mg/100 ml
Water	Insoluble
Phosphate buffer pH (6.8)	Very slightly soluble
Methanol	Soluble
Chloroform ($CHCL_3$)	Soluble

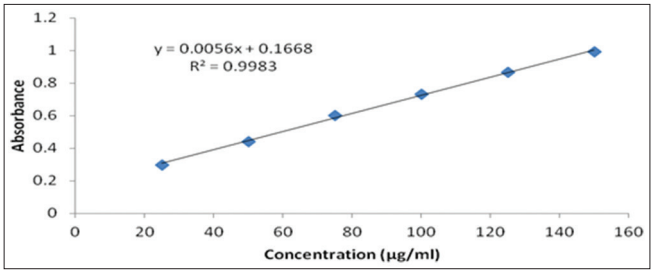

Fig. 2: Calibration curve of glyburide in phosphate buffer pH 7.4

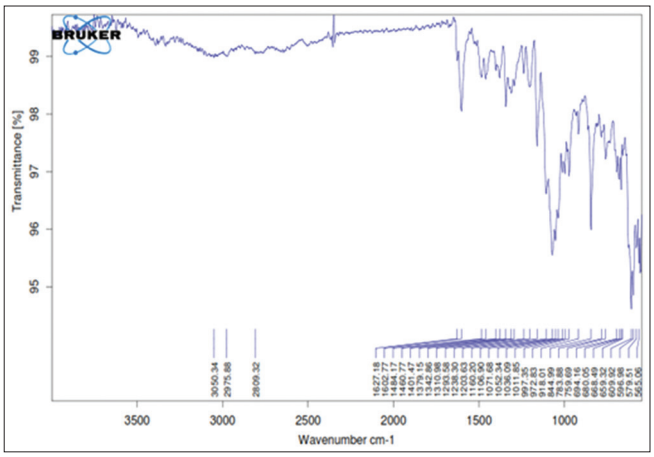

Fig. 3: Fourier transformed infrared spectrum of glyburide

Table 6: Evaluation studies of particle size (nm), drug content, % drug entrapped, and zeta potential (mV) for GLY nanosuspension

S. No	Formulation	Particle size	Drug content	% Drug entrapped	Zeta potential
1	GLY-1	198–225	78.62±0.24	77.11	61.58±1
2	GLY-2	196–210	96.38±0.84	75.90	65.20±1
3	GLY-3	215–230	87.47±1.41	71.89	64.15±2
4	GLY-4	241–236	75.23±0.59	80.75	70.11±2
5	GLY-5	152–163	79.66±0.45	89.48	79.55±1
6	GLY-6	172–189	92.84±0.57	88.80	81.48±2
7	GLY-7	111–125	88.45±1.22	84.76	85.22±1
8	GLY-8	85–96	98.93±0.62	93.53	90.26±1
9	GLY-9	325–315	78.21±0.47	57.52	35.10±2
10	GLY-10	354–385	85.56±0.81	60.76	31.59±1
11	GLY-11	321–361	83.26±0.66	58.75	33.45±1
12	GLY-12	311–320	92.47±1.12	61.16	38.15±2

GLY: Glyburide

Table 7: Percentage cumulative drug release data of glyburide nanosuspension

Time (H)	Percentage cumulative drug release											
	GLY-1	GLY-2	GLY-3	GLY-4	GLY-5	GLY-6	GLY-7	GLY-8	GLY-9	GLY-10	GLY-11	GLY-12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	18.26	15.8	13.44	10.22	17.55	15.22	13.22	8.22	18.22	15.25	13.85	10.43
2	31.52	22.84	23.54	19.52	28.65	24.65	25.36	18.65	34.25	24.65	20.65	24.36
3	49.55	38.41	35.41	28.47	40.01	35.65	34.58	24.15	50.22	34.22	28.69	32.84
4	61.2	55.74	42.68	37.85	59.14	48.52	47.26	30.17	68.24	47.15	35.01	40.21
5	78.55	69.14	54.21	46.24	72.36	60.2	55.3	39.98	79.25	56.84	42.59	48.65
6	88.49	80.14	68.11	57.15	88.24	78.52	65.84	45.21	89.22	61.47	50.22	57.1
7	–	99.16	78.1	65.85	99.28	86.21	74.22	52.02	–	75.26	65.64	65.95
8	–	–	85.68	78.21	–	98.52	84.62	64.22	–	86.22	75.21	72.8
9	–	–	97.02	85.25	–	–	90.11	70.84	–	98.14	88.89	80.41
10	–	–	–	97.11	–	–	99.45	81.58	–	–	99.45	89.79
11	–	–	–	–	–	–	–	90.24	–	–	–	98.02
12	–	–	–	–	–	–	–	99.85	–	–	–	–

GLY: Glyburide

In vivo pharmacokinetic studies

Determination of GLY in rat K_2 ethylenediaminetetraacetic acid plasma

Preparation of CC standards

The stock solutions ranging from 0.1 µg/ml to 1.0 µg/ml were prepared by diluting with mobile phase from main stock solution. Table 9 and Fig. 12 show CC data of GLY in rat plasma.

Evaluation of *In vivo* pharmacokinetic parameters

Based on *in vitro* drug release studies, GLY-8 formulation was selected for further *in vivo* pharmacokinetic characterization in rats.

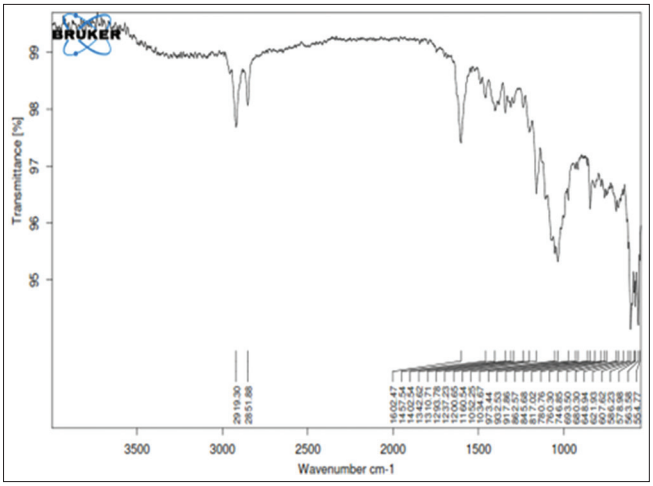

Fig. 4: Fourier transformed infrared spectrum of glyburide plus polymer blend

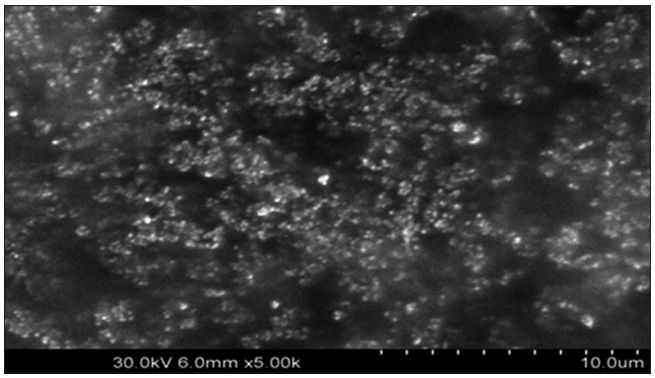

Fig. 5: Scanning electron microscopy photograph of glyburide-8 nanosuspension

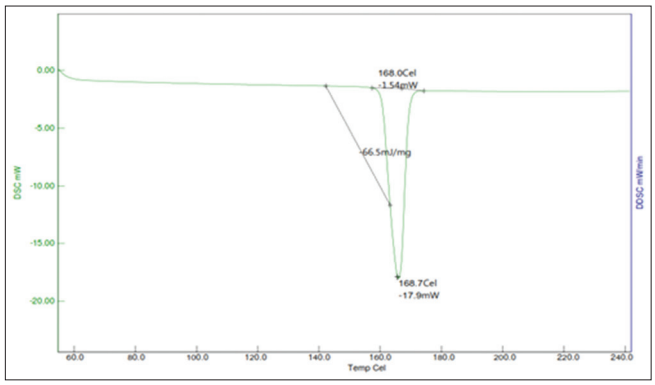

Fig. 6: Differential scanning calorimetry thermogram of pure glyburide

Plasma concentration of GLY in rats (1 mg/kg body weight [b.w.], p.o) is presented in the table. Table 10 summarizes the mean plasma concentration of GLY in rats (1 mg/kg b.w., p.o,) which was considered for computation of pharmacokinetic parameters. Figs. 13 and 14 show the representative chromatogram of GLY from plasma samples.

C_{max}: Peak plasma concentration attained by GLY (GLY) was 0.604±0.03µg/ml following per oral administration.

T_{max}: Time required for attaining peak plasma concentration by GLY (GLY), following per oral administration, was 2±1.01 h.

Area under the curve (AUC): $AUC_{(0-t)}$ was calculated, and it was found to be 2.562±0.41 µg.h/ml for GLY. $AUC_{(0-\infty)}$ was calculated, and it was found to be 2.147±0.45 µg/ml for GLY.

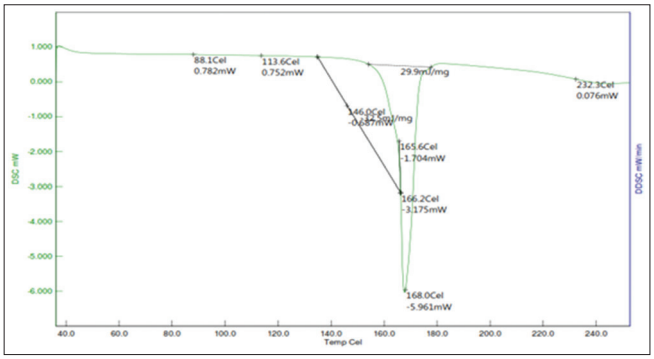

Fig. 7: Differential scanning calorimetry thermogram of glyburide plus polymer blend

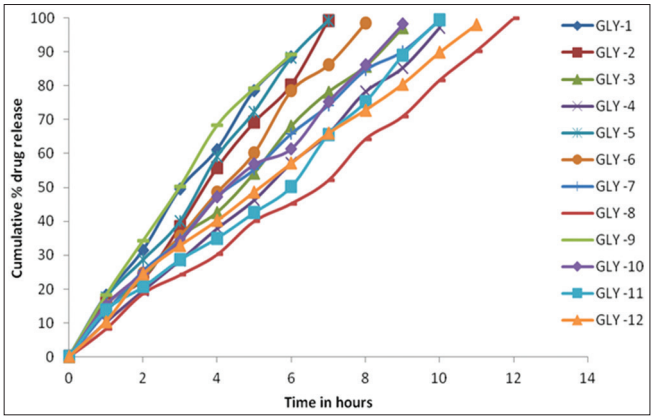

Fig. 8: Percentage cumulative drug release from glyburide nanosuspension

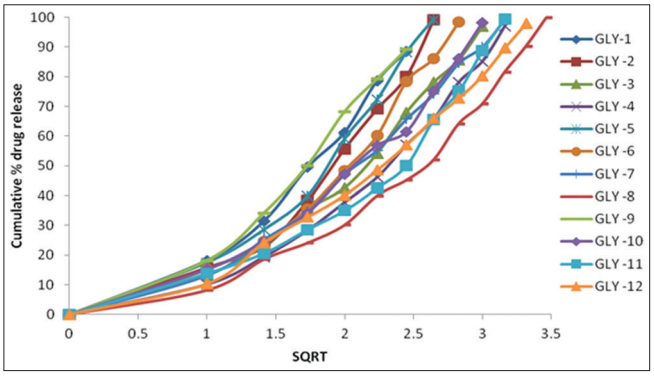

Fig. 9: Higuchi plot

Table 8: Kinetic correlation coefficients and diffusion exponent data of various kinetic models of GLY nanosuspension

Formulation code	Correlation coefficient values (R^2)				Diffusion exponent value (n)
	Zero order	Higuchi's model	Korsmeyer–Peppas	First order	
GLY-1	0.997841	0.965893	0.900845	−0.134	0.900766
GLY-2	0.997247	0.944732	0.980874	−0.13393	0.980738
GLY-3	0.998848	0.962434	0.9099	−0.10033	0.909931
GLY-4	0.9995	0.955341	0.978083	−0.09885	0.978057
GLY-5	0.99842	0.955166	0.921243	−0.12429	0.921226
GLY-6	0.998244	0.95406	0.927203	−0.11286	0.92719
GLY-7	0.996914	0.974591	0.874915	−0.08667	0.874964
GLY-8	0.997	0.950947	0.975553	−0.08486	0.975513
GLY-9	0.995607	0.970394	0.905735	−0.13314	0.905721
GLY-10	0.997745	0.964084	0.85161	−0.09417	0.851576
GLY-11	0.993451	0.94033	0.870733	−0.09121	0.870795
GLY-12	0.997648	0.97353	0.890978	−0.08182	0.890924

GLY: Glyburide

Table 9: CC data of GLY in rat plasma

Concentration (μg/ml)	Peak area 1	Peak area 2	Peak area 3	Mean Peak area	Standard Deviation	% RSD
0.1	659782	652134	659423	657113	4315.68	0.66
0.2	1058864	1037138	1060168	1052057	12936.39	1.23
0.4	1313316	1330325	1326036	1323226	8845.90	0.67
0.6	1706481	1735033	1709901	1717138	15591.29	0.91
0.8	1950506	1941450	1940379	1944112	5563.49	0.29
1.0	2251880	2216008	2213456	2227115	21485.33	0.96

CC: Calibration curve, GLY: Glyburide

Table 10: Mean plasma concentration of GLY following oral administration

Time point (h)	Concentration±standard deviation (μg/ml)
0	0.200±0.05
0.5	0.369±0.06
1	0.440±0.07
2	0.604±0.03
4	0.524±0.04
6	0.316±0.05
8	0.119±0.05
10	0.007±0.01
12	0.200±0.01
C_{max} (μg/ml)	0.604±0.03
T_{max} (h)	2±1.01
$AUC_{(0-t)}$ (μg.h/ml)	2.562±0.41
$AUC_{(0-\infty)}$ (μg.h/ml)	2.147±0.45
$T_{1/2}$ (h)	10.04

GLY: Glyburide, C_{max}: Maximum drug plasma concentration, T_{max}: Time to reach maximum concentration, $T_{1/2}$: Elimination half-life, MRT: Mean residence time, AUC: Area under the concentration-time curve

$T_{1/2}$: Time required for a drug to decrease by half of its content was found to be 10.04 h following oral administration of GLY.

In vivo pharmacokinetic study of GLY-8 formulation in 6 rats following single oral administration of 1 mg/kg b.w. of GLY was performed. The concentration of drug in the plasma samples was obtained from the blood samples which were collected from each rat throughout a period of 12 h and were calculated. Fig. 14 shows the representative chromatogram of GLY from rat plasma. The concentration-time profile of GLY in the rat plasma is shown in Table 10. The mean estimated non-compartmental pharmacokinetic parameters derived from the plasma concentration profiles are summarized in Table 10, and Fig. 15 shows the mean plasma concentrations of GLY across the time points following per oral administration. GLY plasma concentrations calculated at different time intervals for 6 rats showed that the drug was readily absorbed after oral administration and the peak plasma concentration of GLY was reached by 2±1.01 h. The C_{max} of GLY was

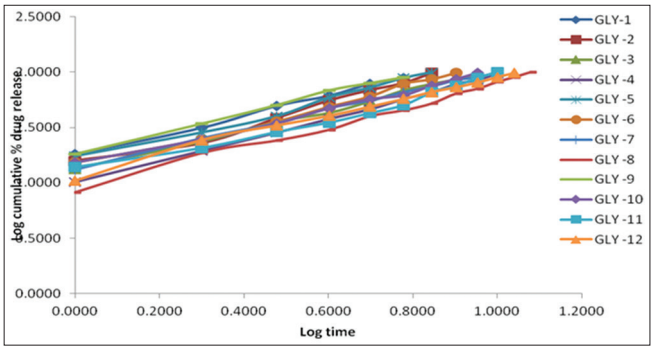

Fig. 10: Korsmeyer–Peppas plot

0.604±0.03. The values of $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were found to be 2.562±0.41 and 2.147±0.45 μg.h/ml, respectively. Till date, an extensive study of GLY pharmacokinetic parameters such as its distribution pattern, mechanism of metabolism, and excretion was not reported and still under investigation.

DISCUSSION

In the current work, an attempt has been made to introduce a new drug delivery system "nano particulates incorporated in suspension." The mechanism of drug release from the nanosuspension release was explained in four steps. They are (1) Formulation of colloidal system, (2) size reduction to nanoparticles through homogenization, (3) counter diffusion mechanism of nano-sized particles into a suspension, and (4) sustained release of the drug and particle detachment.

Drug and excipient compatibility studies were performed using FTIR spectrophotometer and DSC, and no marked incompatibilities were found. GLY has been scanned in the UV spectrophotometer and found λ_{max} at 229 nm. A CC was constructed in the concentration range of 25–150 μg/ml. The curve was the best fit with R^2 value of 0.9983 and slope of 0.0056. The FTIR spectra for pure drug of GLY and with blend are shown in Figs. 1 and 2. Furthermore, the GLY compatibility with polymer blend was proved by DSC. The DSC thermogram of GLY (Fig. 6)

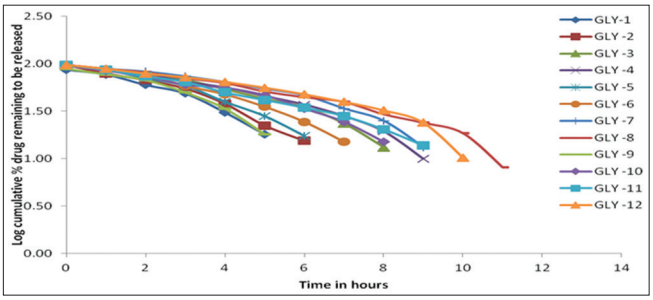

Fig. 11: First-order plot

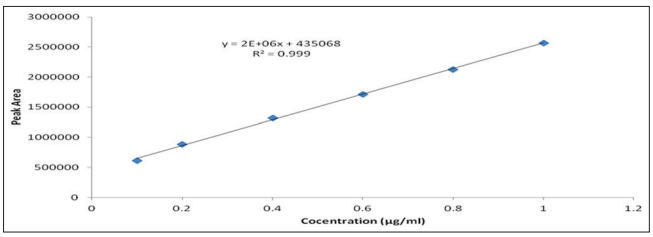

Fig. 12: Calibration curve of glyburide in rat plasma

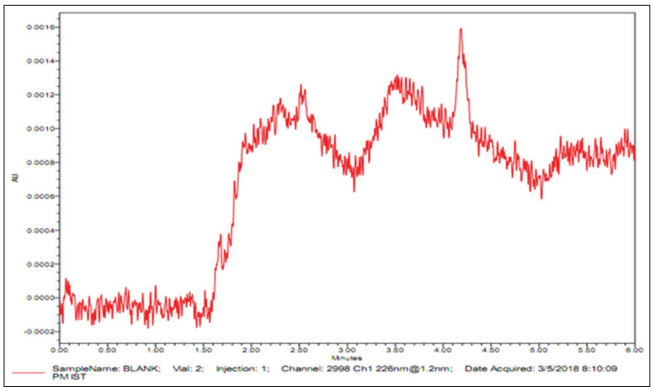

Fig. 13: Blank chromatogram from rat plasma sample

reveals the melting point at 168.7°C, whereas GLY with blend (Figs. 6 and 7) also shows the melting point at 168.0°C which proved that drug had good thermal stability. Nanosuspension formulation of GLY was prepared based on the principle of quasi emulsification solvent diffusion method. With increase in concentration of polymers, the prepared nanosuspension formulations had shown good correlation with size and particle charge (PVP, HPMC, and EDG RL 100). As the concentration of polymer increased, an increase in the entrapment efficiency was observed and average particle size also increased. GLY-8 was the best formulation with a particle size of 85–96 nm, 93.53% drug entrapment, and 90.26±1 mV of zeta potential. This formulation showed percentage drug release of 99.85 % in 24 h. The release exponents showed that value of "n" was 0.975513 (<1) indicating non-Fickian transport mechanism on drug release behavior. The drug relapse followed zero order with regression value near to 0.99 and diffusion type of release from the Higuchi equation regression value of 0.97.

In vivo pharmacokinetic study for optimized formulation (GLY-8) was carried out using the developed and validated reverse-phase high-performance liquid chromatography method on Wistar albino rats. For GLY-8, C_{max} and T_{max} were found to be 0.604±0.03 µg/ml and 2±1.01 h, respectively. The values of $t_{1/2}$ (h), $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ were found to be 10.04 h, 2.562±0.41 µg.h/ml, and 2.147±0.45 µg.h/ml respectively. Based on the obtained results, oral administration of nanosuspension could not only provide the better absorption of poorly water-soluble drugs but may also reduce toxicity and provide a new tool in drug delivery system.

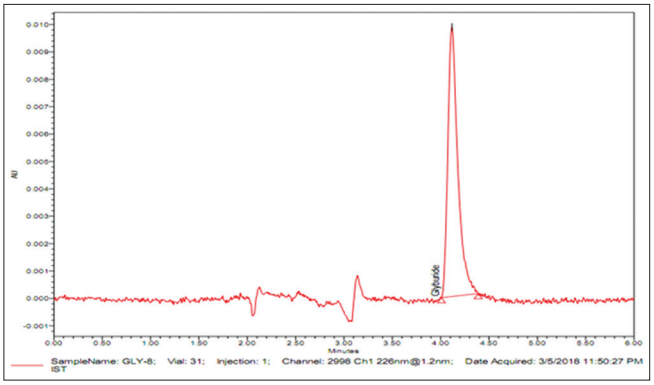

Fig. 14: Blank and C_{max} chromatogram of glyburide from rat plasma sample

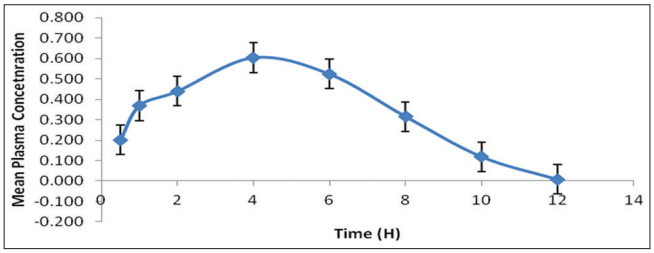

Fig. 15: Mean plasma concentration of glyburide in rat plasma

CONCLUSION

GLY is poorly water-soluble (BCS class II) drug. The poor aqueous solubility of this drug gives rise to many obstacles in the design of pharmaceutical formulations and leads to variable oral bioavailability. Hence, this drug was selected for the present study for enhancing their rate of dissolution and oral bioavailability through nanosuspension technologies. Nanosuspensions were prepared and evaluated by *in vitro* and *in vivo* methods. From the obtained results, it was concluded that all the nanosuspensions prepared were found to be fine and nano range. GLY was taken in phosphate buffer with a pH 7.4 at 229 nm, respectively, and nanosuspensions were prepared. The obtained nanosuspensions were checked for the characterization of size analysis, drug content, entrapment efficiency, drug release studies in *in vitro*, FTIR spectroscopy studies, DSC studies, and SEM studies. FTIR spectra showed no interactions between excipients and drug of the nanosuspension. Among all GLY nanosuspension formulations, GLY-8 formulation was considered as an optimized formulation which showed maximum drug release, i.e., 99.85% in 24 h.

CONFLICTS OF INTEREST

There were no conflicts of interest.

AUTHORS' CONTRIBUTION

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Mr. Pradeep Kumar M collected the data, analyzed the data, performed all the laboratory works, and wrote the introduction, discussion, and the material and method part.

Dr. K. B. Chandra Sekhar, supervisor of this study, helped in selection of the topic, procuring chemicals, and drugs from different laboratories and also helped me a lot in drafting the manuscript and proofreading.

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