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Preparation and Characterization of Glipizide Loaded Palmitic Acid Coupled Pluronic F127 Nanoparticles

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The objective of this research work is preparation and evaluation of Glipizide (GPZ) loaded nanoparticles using palmitic acid-coupled pluronic F127 (PF127) copolymer. GPZ is comes under second generation category medicine which is intended for treatment of type II diabetes mellitus. Copolymer PAF127 was prepared and characterized by various analytical techniques. Glipizide nanoparticles (GPZNPs) was prepared using solvent evaporation technique and characterized for particle size, polydispersity index (PDI), zeta potential, drug entrapment, percentage yield, in vitro drug release, and stability studies. The morphology of GPZNPs was studied by scanning electron microscopy (SEM). The compatibility between drug and excipients was examined by Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC). The particle size of prepared GPZNPs was found to be in ranged from 220.4±5.15 to 970.20±5.42 nm, PDI value from 0.162±0.01 to 0.710±0.03, and zeta potential was recorded in between -20.4±0.62 to -6.42±0.24 mV. The drug entrapment efficiency of GPZNPs was in range 12.37±4.52 to 62.24±5.24 %. The GPZNP1 batch containing glipizide and copolymer (1:1 w/w ratio) showed most favorable results. The GPZNP1 was found to be spherical shape with smallest crystal size (220.4 nm) compared to other batches. The compatibility studies results revealed that there were no interactions between the glipizide and excipients. The GPZNP1 showed the stability at 5±3°C up to 3 months. In vitro release of drug from GPZNP1 was 32.4% in the 4 hr and remaining drug was released up to 38 h.

Keywords: Glipizide, Nanoparticles, Copolymer, Scanning Electron Microscopy.

1. Introduction

Diabetes mellitus Type-2 (T2DM) is a chronic, metabolic disorder in which blood glucose

level is elevated. Insulin is essential which helps the body to use glucose. T2DM is mainly caused due to inadequate supply or use of insulin because of imperfect working of pancreatic β-cells of the islets of Langerhans. Due to poor supply of insulin, various functions of body are affected and cause diabetes.

Now a day's T2DM is an increasing global health concern. According to International Diabetes Federation (IDF) about 240 million citizens survive without undiagnosed diabetes and about half of the adult suffering from diabetes being unaware about the diabetes illness [Magliano D et al., 2021]. The DM is mainly predominance in that population whose lifestyle has changed from traditional to more modern, overweight or obese person, family history of DM, alcoholic and smoking person. Hypertension, stroke, cardiac arrest, hyperlipidemia, renal dysfunctions, organ failures, and micorovascular disorders are also associated with T2DM [DeFronzo RA et al., 2015, Pinhas-Hamiel O et al 2007]. According to the Global Disease Burden 2019, ischemic heart disease and stroke were the primary and second-leading causes of the worldwide disease burden in 2019 [Ong KL et al., 2023].

Glipizide (GPZ) is a second-generation sulfonylurea oral hypoglycemic agent which lowers the blood glucose levels in patient suffering from T2DM, through stimulating insulin secretion from the pancreatic β-cells of the islets of Langerhans. Glipizide also showed several other extra pancreatic effects, like enhancing the sensitivity to insulin and diminishing the glucose production from liver [Thombre AG et al., 1999, Rendell M et al., 2004]. Due to fewer side effects and less therapeutics cost GPZ has widely prescribed by physicians. The only drawback of GPZ is its short duration of action (1-3h), in diabetic patient it decreases the blood glucose level within thirty minutes [Verma RK et al., 2004]. To overcome this drawback of GPZ, there is need to develop newer formulation of GPZ which enhanced the duration of action.

Currently nanotechnology is widely used techniques for oral drug delivery [Barwal I et al., 2013, Elbahwy IA et al., 2017]. Nanoparticles based formulations improved the bioavailability, prolog duration of action, enhance efficacy and ultimately enhance therapeutic activity of drug molecules. Various type of polymer, co-polymers have been reported to use as a drug carrier for preparation of nanoparticles [Shariatinia Z et al., 2017, Rani R et al., 2017].

In this research work we prepared GPZNPs by using solvent evaporation techniques. Prepared nanoparticles were characterized by particle size, polydispersity index (PDI), zeta potential (ZP), drug entrapment, percentage yield, in vitro drug release, and stability studies. The morphology of GPZNP was studied by scanning electron microscopy (SEM).

2. Material and methods

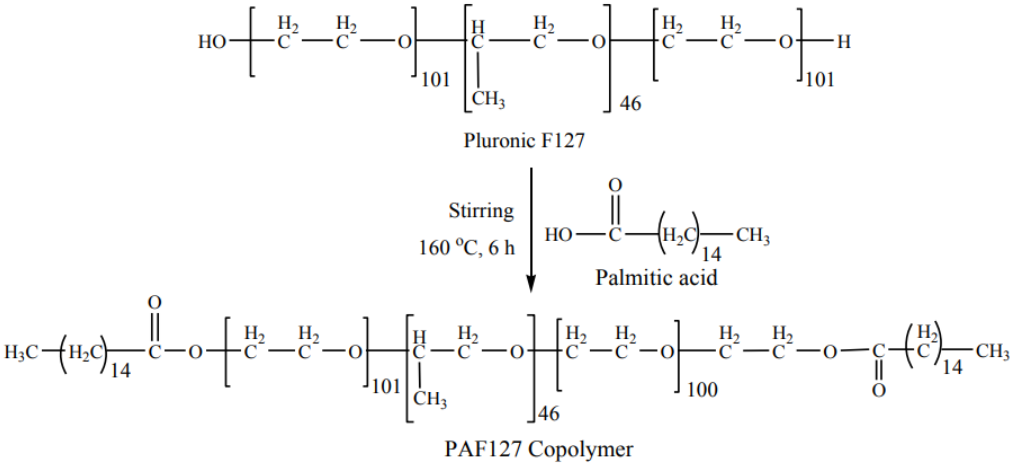
2.1. Materials

Glipizide was a kind gift from USV Ltd (Daman, India), palmitic acid and polyvinyl alcohol was purchased from Sigma-Aldrich, India. All the chemicals and reagents used were of analytical grade.

2.2. Preparation of Pluronic F127 copolymer

Pluronic acid (poly(ethylene oxide)- poly(propylene oxide)-poly-(ethylene oxide)) F127

copolymer was synthesized according to method reported by Kamboj et al., 2019. Synthetic scheme of copolymer are shown in Scheme 1. Take pluronic acid F127 and palmitic acid (P) in equal amount in round bottom flask (RBF). Heat the mixture with continuous stirring on magnetic stirrer to get molten mixture and maintain the temperature 160ºC for 6.5 hr. The unreacted P-F127 copolymer was separated by solvent extraction method using ethyl acetate/ petroleum ether in ratio 1:1 (v/v). Filter the mixture to get PAF127 copolymer, remove the solvent by rota evaporator and dried the synthesized polymer at room temperature under vacuum [Kamboj VK et al., 2019]. The structure of synthesized copolymer was confirmed by Fourier transform infrared (FTIR) and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy.



Scheme 1: Synthesis of PF127 penta block copolymer

2.3. Compatibility studies

2.3.1. FT-IR spectroscopy analysis

The compatibility study between the drug, polymer and other excipients were checked by FT-IR spectral analysis. FT-IR gives specific peak for different chemical functional groups (Mukherjee et al., 2005). FTIR spectra of of pure glipizide, polymer PF127, PVA, physical mixture, and glipizide nanoparticles were recorded in the range of 4000-400 cm^{-1} on Perkin Elmer RX1 Fourier transform spectrophotometer using KBr pellets.

2.3.2. Differential scanning calorimetric (DSC) analysis

DSC thermogram of sample was obtained using Pyris 6 DSC thermal analyzers (Perkin Elmer, USA). Dried the 2 mg of sample in a vacuum desiccator and sealed in aluminium pan which is heated at a temperature increment of 10ºC from 20-300ºC under constant nitrogen purging environment. The empty aluminium pan was used as a reference.

2.3.3. X-ray diffractometry (XRD) analysis

The XRD analysis was carried out to know the crystalline and amorphous characteristic of pure glipizide, polymer, PVA, and glipizide nanoparticles. XRD analysis was performed by X-ray diffractometer (D8 Advanced, Bruker AXS, Germany). The instrument uses CuKα

radiation produced at 40 kV voltages with 15 mA current. The diffraction pattern was recorded over a 2θ with angular range of 10 to 70.

2.4. Preparation of glipizide-Loaded PF127 nanoparticles

Glipizide loaded nanoparticles (GPZNPs) were prepared using solvent evaporation method. Required quantity of glipizide and PF127 copolymer was dissolved in dichloromethane (DCM). Polyvinyl alcohol (PVA) surfactant separately dissolved in aqueous solvent. The drug polymer solution was emulsified by adding an aqueous solution of PVA with constant stirring in magnetic stirrer. This emulsion was placed in probe sonicator for 10 minutes to obtained nanosize emulsion. The organic solvent was removed by using rota evaporator. Then the resulting mixture was centrifuged for 30 min in 1000 rpm followed by lyophilization for 2 days, the resulting nanoparticles were collected [Mukherjee B et al., 2008].

2.5. Characterization of prepared nanoparticles (GPZNPs)

2.5.1. Particle size, Poly dispersity index (PDI), and zeta potential

The sample of nanoparticles were suspended in milipore water and characterized for particle size, PDI, and zeta potential at 25°C by using Malvern Zetasizer. The results were reported as the mean ± standard deviation.

2.5.2. Entrapment efficiency and percentage yield

The GPZNPs were subjected to centrifugation in ultra-micor centrifuge (Thermofisher Scientific USA) at 10000 rpm for 10 minutes Take 100 micro litter of supernatant and mixed with phosphate buffer pH 7.4 with continuous stirring. Measured the absorbance using UV-Vis spectrophotometer at 225 nm and compared with the amount of glipizide used in formulation of batch [Emami J et al., 2014]. Drug loading capacity and entrapment efficiency (%) were calculated using Equation 1 and 2, respectively.

$$\text{Drug loading (\%)} = \frac{\text{Amount of glipizide in nanoparticles}}{\text{Amount of nanoparticles recovered}} \text{X } 100 \ldots\ldots 1$$

$$\text{Entaptment efficiency (\%)} = \frac{\text{Amount of glipizide in nanoparticles}}{\text{Amount of glipizide used in formulation}} \text{X } 100 \ldots\ldots 2$$

2.5.3. Surface morphology study

The surface morphology of the optimized GPZNP1 batch was studied by field emission scanning electron microscopy (Ultra Plus, Zeiss).

2.5.4. In vitro drug release studies

In vitro drug release studies were accomplished for optimized batch GPZNP1 and pure GPZ by dialysis sac method [Rani R et al., 2017]. 5 ml of samples were placed in dialysis bags and tied with dialysis thread. The dialysis bags containing GPZNP1 and pure glipizide were immersed separately in a stirring flask with 50 ml of phosphate buffer solution of pH 7.4. Maintained the flask temperature 37±5°C and continuous stirred the sample at 200 rpm. In

each hour, the aliquot of 1 ml was taken from the flask and replaced with a 1 ml of fresh phosphate buffer and measured the absorbance of sample in UV-Vis spectrophotometer at 270 nm and blank sample (nanoparticles without drug) was uses to minimize the error cause by polymer absorbance. A plot was prepared between percentage drug released vs time in hours.

2.5.5. Stability study

The stability studies of GPZNP1 were performed on two temperatures (5±3 and 25°C). The aliquot of GPZNP samples was withdrawn after completion of 1st and 3rd months. These samples were examined for any possible change in particle size, PDI, zeta potential, entrapment efficiency, and color.

3. Result and discussion

3.1. Characterization of PF127 copolymer

The esterification reaction between the carboxyl group of palmitic acid and hydroxyl group of Pluronic F127 yield PF127 surface active copolymer. The successful coupling of palmitic acid with Pluronic F127 was confirmed by FT-IR spectra of copolymer having a band around 1728.96 cm^{-1} which were assigned to the stretching vibration of C=O ester bond. Major features of ^{1}H NMR spectra of PF127 are enlisted in Table 1.

Table 1: Major features of ^{1}H NMR spectra of PF127 copolymer in $CDCl_3$

Type of proton	Chemical shift δ (ppm)
CH_2-O	3.70–3.67
CH_2CH_2-O	2.38–2.35
$CH_2CH(CH_3)$-O	1.63–1.60
$CH_2CH(CH_3)$-O	1.35–1.30
CH_2 in PA	1.20–1.16

3.2. Preparation of polymeric nanoparticles

The glipizide nanoparticles were prepared by an emulsion solvent evaporation technique. Different batches of GPZNPs were prepared by changing the Drug: PF127 copolymer ratio to find the best formulation. The prepared GPZNPs was screened for particle size, PDI, zeta potential, entrapment efficiency, and percentage yield (Table 2).

Table 2: Evaluation of various parameters of different batches of GPZNPs

Batch	Drug/polymer (w/w)	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment Efficiency (%)	Percentage yield
GPZNP1	1:1	220.4±5.15	0.162±0.01	-20.4±0.62	62.24±5.24	85.42±4.24
GPZNP2	1:2	532.43±5.05	0.316±0.03	-18.22±0.24	52.62±5.41	66.45±4.32
GPZNP3	1:3	748.24±5.28	0.522±0.02	-11.32±0.64	39.42±4.50	49.14±5.25
GPZNP4	1:4	857.32±5.14	0.467±0.02	-10.87±0.63	32.39±5.82	38.37±5.15
GPZNP5	1:5	570.34±5.12	0.428±0.03	-9.21±0.45	26.88±5.56	37.41±4.62
GPZNP6	2:1	688.34±6.44	0.543±0.03	-8.57±0.62	22.79±5.54	28.45±4.40

GPZNP7	3:1	838.36±4.33	0.622±0.03	-7.34±0.58	18.37±4.52	22.44±5.52
GPZNP8	4:1	970.20±5.42	0.710±0.03	-6.42±0.24	12.37±4.52	19.44±5.52

n=3, mean values±SD. SD: Standard deviation

The particle size for GPZNP1 was found to be 220.4±5.15 nm (smallest) and for GPZNP8 batch 970.20±5.42 nm (largest). The GPZNP1 and GPZNP8 were showed PDI value 0.162±0.01 and 0.710±0.03, respectively. The values of zeta potential for GPZNP1 were −20.4±0.62 mV, which support the stability of nanoparticles during storage phase. Drug entrapment efficiency and percentage yield of GPZNP1 and GPZNP8 were observed as 85.42±4.21 and 19.44±5.52 %, respectively.

The smallest size particles having higher value of zeta potential and lowest PDI value reported the higher stability during storage [Jain S et al., 2009]. A fixed amount of PVA surfactant used which stabilizes the zeta potential of GPZNPs. Higher the entrapment efficiency of the drug in nanoparticles, the smallest amount of drug get loss during formulation process and increase the nanoparticles yield [Kusum VD et al., 2009]. The nanoparticles with higher concentration of the drug can showed potent therapeutic effect in a small dose and final size of dosage form get reduced which can be administered with more ease to young and geriatric patients.

3.3. FTIR studies

The compatibility studies of drug to excipients were monitored by FT-IR spectroscopy (Figure 1). The characteristic peaks of glipizide were detected shown in Table 3.

Table 3: FT-IR Vibration frequency of pure glipizide

Functional group	Vibration frequency in cm^{-1}
-NH stretching	3300.20
C-H stretching	2889.37
C=O stretching	1666.50
-CONH- stretching	1595.13
C=C aromatic stretching	1514.12
C-H aromatic bending	1485.19
O=S=O stretching	1309.67, 1273.02

O=S=O stretching also detected at 1337.27 and 1160.14 cm^{-1} in the mixture and GPZNP1. No shifting of peak was detected in mixture and optimized GPZNP1 as compared to spectra of PF127, PVA, and pure glipizide (Figure 2). This indicates that the glipizide drug and excipients (polymer, surfactant) used were compatible and did not interact with each other.

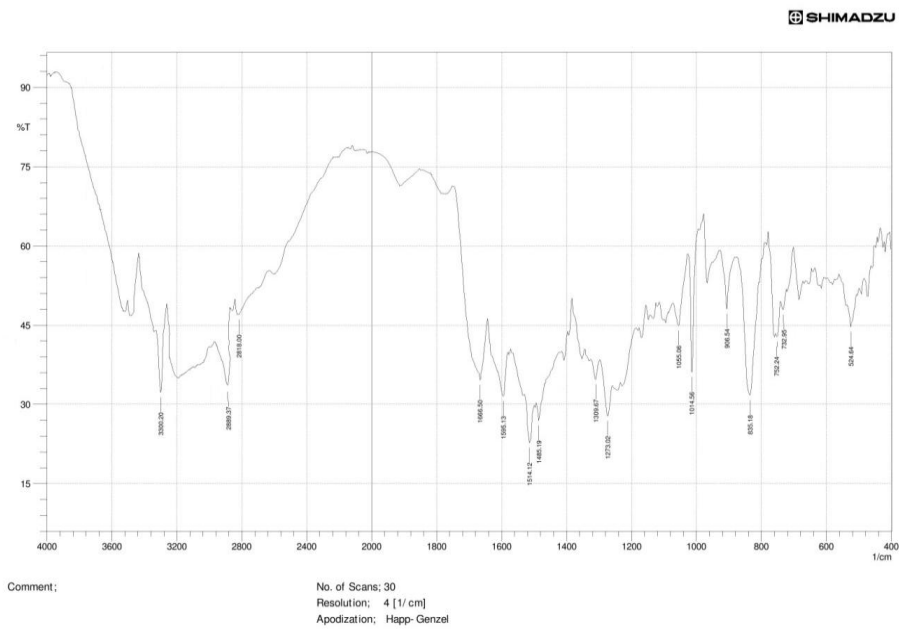


Figure 1: FTIR spectra of pure glipizide

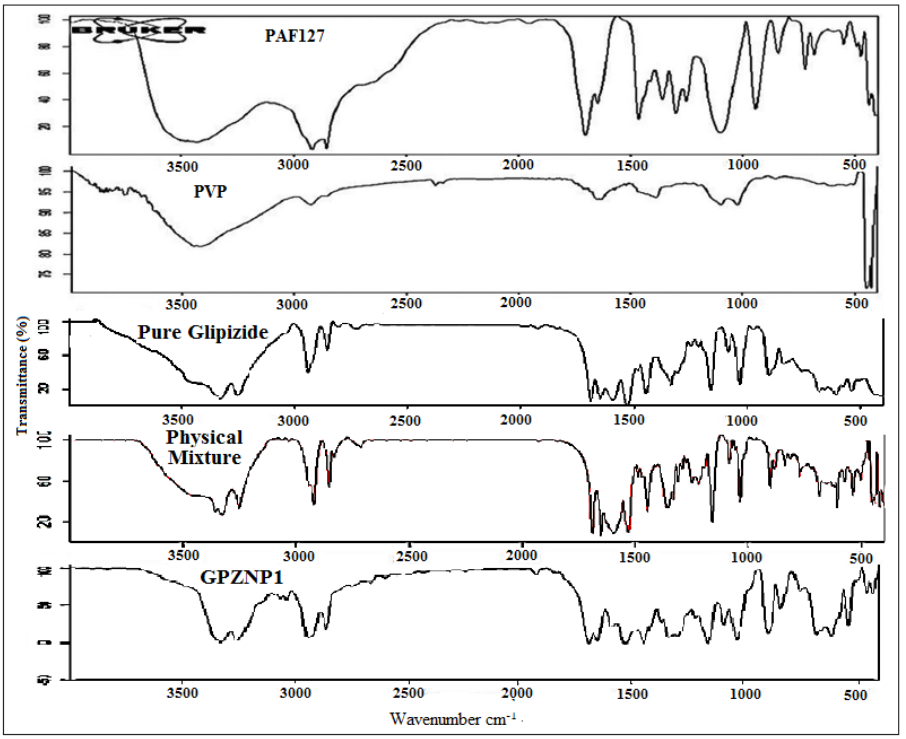


Figure 2: FTIR spectra of PAF127, PVP, Pure Glipizide, Physical mixture and GPZNP1

3.4. DSC analysis

DSC analysis is useful to determine the thermal properties of the nanoparticles. It is also useful for quantitative and qualitative analysis such as for identification of physicochemical state of the drug inside the nanoparticles and drug-excipients interactions [Ramazani et al., 2017].

In pure glipizide a sharp endothermic peak was observed at 211.18 ºC (Figure 3) which was absent in PF127 copolymer (Figure 4). DSC thermogram of PVP surfactant showed an endothermic peak at 98.5 ºC (Figure 4). The peak due to drug and excipients were present in physical mixture. For GPZNP1 small melting peak was observed as compared to pure glipizide. These results revealed that the glipizide was entrapped within nanoparticles and present in the amorphous state [Rawat et al., 2010]. Based on thermal behavior, there was no significant interaction between glipizide and excipients. The excipients were selected based on results of FTIR and DSC analysis and ues for further studies.

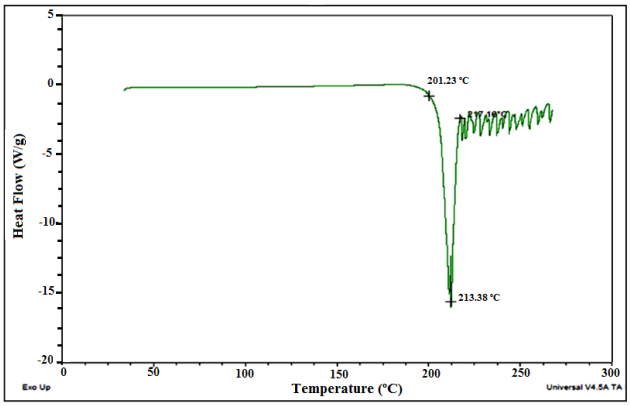


Figure 3: DSC thermogram of pure glipizide

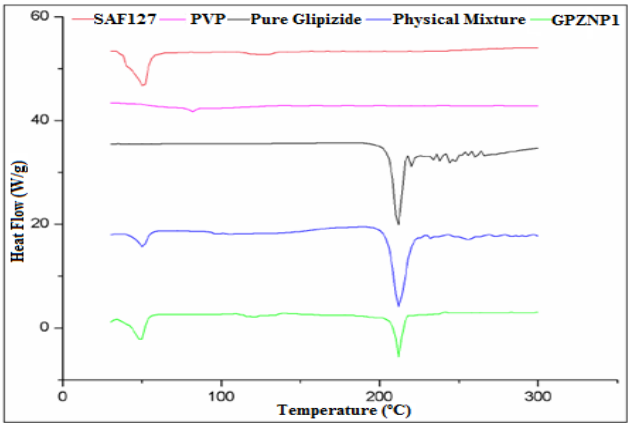


Figure 4: DSC thermogram of PAF127, PVP, Pure glipizide, Physical mixture and GPZNP1

3.5. XRD studies

The XRD patterns of the PF127 copolymer and PVP showed a diffused spectrum having fewer peaks suggested semi-amorphous nature. The XRD spectra of copolymer, surfactant, pure

glipizide, physical mixture and GPZNP1 are shown in Figure 5. In pure glipizide XRD pattern, sharp diffraction peaks was observed which indicate the presence of crystalline state of glipizide [Dash et al., 2015]. The physical mixture showed diffused peaks of PF127 copolymer and PVP. But the XRD of GPZNP1 showed relatively less sharp peak with low intensity and has partially amorphous nature. This reduced peak intensity of GPZNP1 indicates the decrease crystalline properties because of crystal lattices of the drug in polymeric nanoparticles [Mokale et al., 2016].

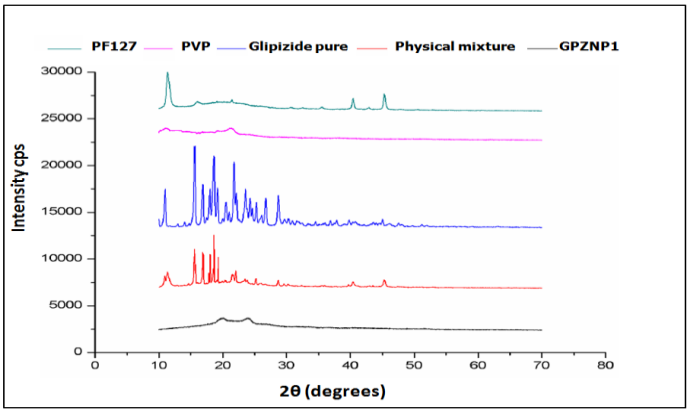


Figure 5: XRD diffraction analysis of PF127, PVA, glipizide pure form, physical mixture and GPZNP1

3.6. In vitro drug release studies

The in-vitro release of the glipizide from GPZNP1 showed first burst release followed by the sustained release compared to pure glipizide. The cumulative drug release from GPZNP1 at 4 hr, 8 hr and 12 hr was observed 32.4 ± 1.6, 49.5 ± 2.4 and 58.3 ± 2.9 %, respectively. The 99.5 % drug from GPZNP1 was released up to 38 hr. The initial burst release of glipizide from GPZNP1 may be due to the loosely associated drug on the interface of the polymeric matrix. The in-vitro drug release graphs between cumulative drug release in percentage verses time is shown in Figure 6.

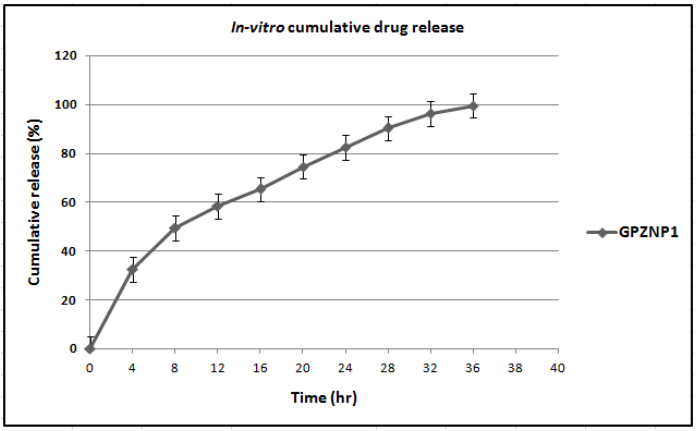


Figure 6: In-vitro cumulative drug release profiles of GPZNP1

3.7. Morphological studies by SEM

The nanoparticles of glipizide (GPZNP1) showed smooth and spherical shaped crystals in SEM, which indicated the proper encapsulation of glipizide in polymer matrix. The physical mixture showed the smooth surface rectangular crystals of glipizide (Figure 7). This smooth surface crystal of nanoparticles confirmed the complete removal of solvents from the glipizide nanoparticles during preparation process and a sign of good quality.

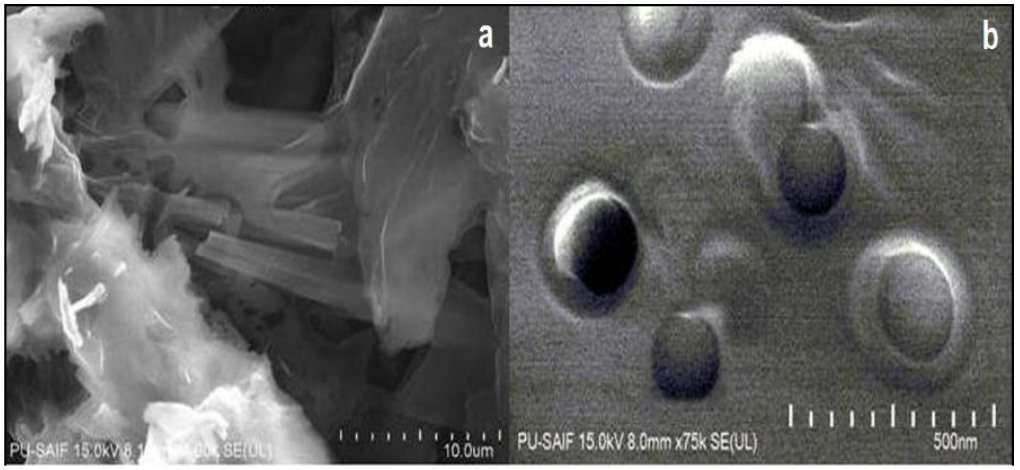


Figure 7: SEM images of (a) Physical mixture; (b) GPZNP1

3.8. Stability studies

The stability studies of GPZNP1 (Table 3) were carried out for three months at two different temperatures 5±3 and 25°C. The particle size, PDI, zeta potential, entrapment efficiency was slightly changed in first and third month at 5±3 ºC temperature whereas at 25°C significant change have been observed. The particles size of GPZNP1 at both temperatures remains nano-sized (< 260 nm). During the three months of storage time, no visual color change was detected. The decreasing entrapment efficiency with increasing particle size might be due to the semi-amorphous characteristics of PF127 copolymer. Due to slight deviation of stability studies parameters at 5±3° this temperature was suggested as optimum storage temperature for storage of GPZNP1.

Table 3: Results of short-term stability studies of GPZNP1

Storage condition	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)	Visual observation
Fresh GPZNP1	220.2±5.12	0.162±0.01	-20.4±0.62	62.24±5.24	Clear suspension
1 month (5 ± 3 ºC)	223.3±4.20	0.164±0.02	-19.9±0.52	61.27±4.20	Clear suspension
3 month (5 ± 3 ºC)	228.2±3.38	0.167±0.02	-19.5±0.44	59.28±5.5	Clear suspension
1 month	233.4±5.22	0.178±0.03	-18.01±0.42	55.43±5.5	Clear

(25 ºC)					suspension
3 month (25 ºC)	242.2±4.78	0.189±0.02	-17.40±0.48	52.20±3.5	Clear suspension

4. CONCLUSION

Glipizide nanoparticles (GPZNPs) were successfully prepared by emulsion solvent evaporation technique using PF127 copolymer and PVA and characterized for particle size, PDI, zeta potential, drug encapsulation efficiency, compatibility study of drug with excipients, stability, in-vitro drug release and percentage yield. The compatibility of drug with excipients was checked by DSC, thermogram of GPZNP1 do not showed any reaction. Particle size, PDI, zeta potential, drug encapsulation efficiency, and percentage yield of GZNPs were found to be affected by copolymer concentration. The PF127 copolymer contains hydrophilic and hydrophobic portions which encapsulated glipizide and formed nano-sized particles (<260 nm). In vitro drug release of glipizide from nanoparticles was significantly prolonged to 36 h compared to pure drug. Further, to explore the application of PF127 copolymer as a nano-carrier for oral glipizide delivery clinical studies is required. This study conclude that the bioavailability of newly prepared GPZNP1 was successfully prolonged and frequent oral administration problems with conventional dosage forms can be defeated for diabetes mellitus.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.K.C.; methodology, R.K.C.; validation, B.P.; formal analysis, B.P.; investigation, B.P.; resources, R.K.C.; data curation, B.P.; writing—original draft preparation, B.P.; writing—review and editing, R.K.C.; visualization, B.P.; supervision, R.K.C.; project administration, R.K.C.; funding acquisition, R.K.C. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data available in article and raw data are available from the corresponding authors upon request.

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