DOI: 10.4274/tjps.20082

Palmitic Acid-Pluronic F127-Palmitic Acid Penta-Block Copolymer as a Novel Nanocarrier for Oral Delivery of Glipizide

Vipan Kumar KAMBOJ, Prabhakar Kumar VERMA*

Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak-124001, India

*Correspondence: E-mail: vermapk422@rediffmail.com,

Phone: +91 9992581437,

ORCID-ID: <https://orcid.org/0000-0002-1769-6484>

ABSTRACT

Objective: The aim of the present study is to develop nanotechnology-based oral formulations of Glipizide to enhance bioavailability and to eliminate the frequent oral administration of the conventional dosage form. Glipizide is an antidiabetic drug with short biological half-life with limited oral bioavailability. Noval Palmitic acid-Pluronic F127-Palmitic acid (PAF127) pentablock copolymer based prolonged release glipizide nanoparticles (GN) were prepared and screened for *in vitro* and *in vivo* studies.

Methods: GN was prepared using novel PA-F127 pentablock copolymer by solvent evaporation technique. The prepared nanoparticles were evaluated for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, percentage yield, drug excipient compatibility using FTIR and DSC analysis, XRD, SEM, *In vitro* drug release studies, stability studies and *in vivo* pharmacokinetic studies.

Results: The results of FTIR and DSC analysis revealed the absence of drug-excipient interactions. The optimized GN1 has particle size 242.60 ± 4.20 nm, PDI 0.171 ± 0.014 and zeta potential -21.41 ± 0.462 mV. Prepared nanoparticles were spherical in shape and showed semi-amorphous characteristics. *In vitro* release studies showed 34.43 ± 4.8 % drug was released in first 8 h, 56.11 ± 4.12 % glipizide were released further for 24 h. The GN1 was found to be stable at 5 ± 3 °C up to three months. Pharmacokinetic studies showed that the orally administered GN1 were superior with $C_{\max}$ 2.35 fold, $t_{\max}$ 1.6 fold, area under the curve ($AUC_{0 \rightarrow \infty}$) 3.3 fold and mean residence time (MRT) 1.2 fold as compared to pure glipizide ($p < 0.05$).

Conclusion: The study concluded that the bioavailability of newly developed GN1 was successfully prolonged and frequent oral administration problem with conventional dosage form can be defeated for diabetes treatment.

Keywords: Glipizide, Nanoparticles, Palmitic acid, Pluronic, Bioavailability

INTRODUCTION

Glipizide is a potential second-generation sulfonylurea derivative belongs to Biopharmaceutical Classification System (BCS) Class-II drugs. It is commonly utilized as an oral hypoglycemic agent for the treatment of type II diabetes malitus^{1,2}. Glipizide is most effective insulin secretagogues and presents fewer side effects compared to the first-generation drugs³. It is a weak acid with pka value 5.9 and better absorbed from the acidic medium. Due to very low pH level of glipizide, its aqueous solubility is negligible which causes discrepancies in bioavailability⁴. After absorption from the gastrointestinal tract, glipizide reduces the blood glucose levels in 30 minutes and peak concentration of the drug reaches within 1-3 h³. It is rapidly eliminated from the body due to its small biological half-life (3.4 ± 0.7 h) and hence drug needs frequent oral administration in 2 or 3 doses of 2.5 to 10 mg per day⁵. Due to poor solubility of glipizide, researchers have investigated several drug delivery systems including solid self-nanoemulsifying drug delivery system², microspheres⁵, poly(lactic-co-glycolic acid), eudragit nanoparticles⁶, cyclodextrin complex^{7,8}, chitosan and xanthan beads⁹, nano-suspension¹⁰ to increase solubility and bioavailability of glipizide. Nanotechnology-based drug delivery systems with the use of biodegradable polymers seem to be most convenient for the delivery of any drug due to negligible chances of toxicity and overall improved therapeutic properties¹¹.

Pluronic are A-B-A type triblock non-ionic, biodegradability copolymers enlisted in the British and US Pharmacopoeia as excipients and extensively used in drug delivery systems^{10,12}. Due to amphiphilic nature of Pluronic, they get self-assembled into micelles above the critical micelle concentration in the aqueous solvent¹³. The critical micelle concentration (CMC) value of Pluronic F127 was observed in the range of 0.26-0.8 wt%. The high CMC value indicates the dissociation of nanoparticles occurs before reaching the target site¹⁶. This problem can be overcome by using mixed polymers. The modified block copolymers like stearic acid-coupled F127 nanoparticles of doxorubicin¹² and Pluronic/poly(lactic acid) vesicles for oral insulin delivery¹⁴ have been investigated. These studies inspire us to go further to explore the application of Pluronic in the nanotechnology-based oral drug delivery system for glipizide.

In the present study, we have aimed to develop glipizide nanoparticles with better bioavailability which eliminate frequent dose administration problem. We have prepared orally active GN by using PA-F127 copolymer and optimized for physicochemical properties and evaluated pharmacokinetic parameters in rats. We also analyzed the stability of GN at 5 ± 3 °C and 25 °C for 3 months.

Materials and method

Materials

The pharmaceutical grade glipizide was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, India. Palmitic acid (PA), Pluronic F127 and polyvinyl alcohol (PVA) have been procured from Sigma-Aldrich, India. Other chemical and solvents used were of analytical grade and have been purchased from Molychem, Mumbai.

Synthesis of PA-F127 pentablock copolymer

15 g PA and 15 g Pluronic F127 was added to a 100 ml round bottom flask and the mixture was heated with constant stirring to yield a well-mixed molten phase and reacted at 160 °C for 6 h. The PA-F127 copolymer was recovered by mixing the resulting solution into the ethyl acetate/petroleum ether 1:1 (v/v) solution to eliminate the unreacted PA by filtration. The PA-F127 copolymer was obtained by evaporating the organic solvent at room temperature and dried at 25 °C under vacuum for 24 h. The synthesized copolymer structure was confirmed by the spectrum of FTIR (Bruker 1-206-0280, KBr pellets) and ¹H NMR (Bruker Model Advance II 400; 400 MHz) spectroscopy.

Preparation of glipizide loaded PA-F127 nanoparticles

Glipizide nanoparticles (GN) were fabricated by solvent evaporation technique using PA-F127 and PVA polymeric systems. A mixture of chloroform and methylene chloride (1:1 v/v) was prepared and glipizide was dissolved in it. The PA-F127 copolymer was dissolved in chloroform. The copolymer solution was added to glipizide solution drop by drop with continues stirring. The aqueous phase of PVA 1.0 ml (2%) has been added drop-wise into the organic mixture of drug and copolymer with continuous homogenization (12000 rpm; IKA T25 ultra homogenizer) followed by stirring (700 rpm) for 3 h and obtained nano-suspension was stored in vacuum desiccators overnight at room temperature in order to remove remaining organic solvents. The un-incorporated

glipizide aggregates were removed through filtration process using Whatman 1. The filtrate was centrifuged (14000 rpm; Remi, India) and sediment containing nanoparticles were separated and dried by lyophilization process^{12,15}.

Characterization of prepared GN

Particle size, PDI and zeta potential

The average particle size, PDI and zeta potential of GN were evaluated by using Zetasizer Nano-ZS (Malvern Instruments, UK). The 0.5mg/ml of suspension were prepared in Milli-Q water and analyzed to determine these parameters. The results were described as mean $\pm$ standard deviation (SD) for three replicates¹⁶.

Entrapment efficiency and percentage yield

Accurately weighed GN was dissolved in methylene chloride (20 ml). This solution was added to 100 ml freshly prepared phosphate buffer (pH 7.4) and continuously stirred to extract the glipizide in it. The methylene chloride evaporates during the stirring process¹⁷. The undissolved content was removed by centrifugation at 10,000 rpm (Remi, India) and the supernatant was filtered and amount of glipizide was assessed using UV-Vis spectrophotometer (Lab India 3000*) at 225 nm. Drug entrapment efficiency (%) and percentage yield were calculated using equation 1 and 2, respectively.

$$\text{Entrapment efficiency (\%)} = \frac{\text{Glipizide in supernatant}}{\text{Glipizide in supernatant} + \text{Glipizide in residue}} \times 100 \quad \text{Eq. 1}$$

$$\text{Percentage yield (\%)} = \frac{\text{Glipizide in supernatant}}{\text{Glipizide in supernatant} + \text{Glipizide in residue}} \times 100 \quad \text{Eq. 2}$$

FTIR studies

The interactions between glipizide and excipients were analyzed using FTIR. FTIR spectra of the PA-F127 copolymer, PVA, pure glipizide, physical mixture, and GN1 were taken in KBr Pellet using Bruker (1-206-0280) with software: OPUS-7.2.139.1294 spectrometer and the value of λ max were reported in cm^{-1} (range: 400-4000).

Differential scanning calorimetric analysis

The samples used for FTIR studies were selected for the analysis of thermal properties by using DSC Q10 V9.9, US. The instrument was calibrated using Indium as standard. The samples were sealed in aluminum pans with lids and heated at a rate of 10 $^{\circ}\text{C}/\text{min}$

under the nitrogen environment (60 ml/min). The empty aluminum pan was used as a reference. The heat flow was recorded from 35 to 280 °C.

XRD analysis

X-ray diffraction analysis of selected samples was carried out using Rigaku Miniflex-600 diffractometer. A Cu K α source operation (40 kV, 15 mA) was used. The diffraction pattern of samples was recorded over a 2 θ angular range of 10-70.

Surface morphological studies

The surface morphology of the physical mixture and best-optimized batch was examined by field emission scanning electron microscopy (FE-SEM; JEOL-JSM-7600F, Japan). The samples were dispersed on metallic stubs and then gold coating was done by using the ion-sputtering machine. These samples were vacuum dried before the examination.

In vitro dissolution studies

In vitro dissolution studies were performed for optimized GN1 batch and pure glipizide by modified dialysis sac method¹⁸. Accurately weighed GN1 suspension (equivalent to 5 mg of glipizide) and pure glipizide suspension (5 mg) was placed in dialysis membrane bags (12-14 kDa cut-off, HiMedia, India) and tied with dialysis clips. The dialysis bags were immersed in separate conical flasks containing 150 ml of 0.1 M phosphate buffer solution (pH 7.4). The conical flasks were stirred at 100 rpm with temperature 37.0 $\pm$ 0.5 °C. At fixed time intervals, the aliquot of 1 ml was withdrawn from the conical flask and replenished with 1 ml of fresh phosphate buffer and the assay was performed using UV-Vis spectrophotometer (Lab India 3000⁺) at 225 nm.

Stability studies

It is significant to have an insight into prepared nanoparticles stability. The GN1 suspension was kept in color glass bottle at 5 $\pm$ 3 °C and 25 °C for short-term stability studies. The aliquot of GN1 samples was taken after 1 and 3 months. These samples were analyzed for any possible change in particle size, PDI, zeta potential, entrapment efficiency and color of suspension.

Animals

In vivo studies were accomplished in female Wistar albino rats weighing between 250 to 300 g. The rats were procured from the Lala Lajpat Rai University of Veterinary and

Animal Sciences, Hisar, India. The rats were kept in polypropylene cages and housed in the central animal house of Maharshi Dayanand University, Rohtak under standard environmental conditions (23.0 ± 1 °C, $55 \pm 5\%$ humidity and 12 h/12 h light/dark cycle). The animals were freely accessed to standard animal diet and water *ad libitum*. The protocols of the animal studies were permitted by Institutional Animal Ethical Committee (IAEC 151/57 dated 30/03/2015) and experiments were performed according to CPCSEA guidelines.

Pharmacokinetic evaluation in Wistar albino rats

The overnight fasted rats were (n=6) treated with the single oral dose of freshly prepared GN1 carrying 1.5 mg drug (Group I) and pure glipizide suspension treat Group II (1.5 mg/kg b.w.). The blood samples were withdrawn at different time intervals (0, 0.5, 1, 2, 3, 4, 6, 9, 12 and 24 h) through the tail vein using heparinized tubes. Plasma was separated by centrifugation (Plasto Crafts, India) and frigid at -20 °C until further examination. Rat plasma sample 0.1 ml and 0.1 ml of 0.1 N HCl was vortexed for 3 min and then 3 ml benzene was added for the precipitation of plasma proteins. The mixture was smoothly shaken using cyclo-mixer for 5 min followed by centrifugation for 10 min at 6000 rpm and precipitates were removed by syringe filter (0.22 µm). The organic phase was evaporated under the nitrogen environment and residue was thawed in 0.1 ml of mobile phase by vortex mixing. An aliquot of 20 µl was injected into the column of RP-HPLC by auto-sampler.

Glipizide in rat blood plasma was estimated by HPLC using earlier reported bioanalytical method¹⁹. The pharmacokinetic studies were performed on Dionex UHPLC ultimate 3000 RS containing pump, auto-sampler, column compartment (column: Agilent; 250 mm × 4.6 mm; particle size 5 µm) and diode array detector. The data acquisition was achieved through Chromoleon 6.8 software. The monobasic potassium dihydrogen orthophosphate buffer (20mM; pH 3.5) and acetonitrile were used as mobile phase (65:35 v/v). The mobile phase was filtered through a membrane filter (0.22 µm) and sonicated. The flow rate was kept at 1 ml/min and total run time of the method was set at 15 min. The effluent was monitored at 225 nm.

Statistical Analysis

The pharmacokinetic data were compared by student paired t-test using GraphPad Prism 7 software. The value of $p < 0.05$ was considered as significant.

RESULT AND DISCUSSION

Characterization of PA-F127 pentablock copolymer

The carboxylic group of PA esterified with the hydroxyl groups of Pluronic F127 (Scheme 1). The structure of PA-F127 copolymer was determined by ^1H NMR spectroscopy in CDCl_3 and δ (ppm) of different groups are shown in Table 1. The FTIR spectra of synthesized copolymer having ester band ($\text{C}=\text{O}$ stretching vibration) at 1700.77 cm^{-1} were observed, which confirmed the reaction between PA and F127.

Preparation of glipizide loaded polymeric nanoparticles

The GN was fabricated by the solvent evaporation method with different glipizide to copolymer ratios (Glipizide: PA-F127; 1:1, 1:2, 1:3 and 2:1 w/w) and the fixed concentration of PVA. By this technique, nanoparticles are easily prepared compared to the other methods. A mixture of PA-F127 copolymer and glipizide in organic solvent forms the organic phase. Aqueous phase comprising PVA was added drop by drop into the organic phase. The organic solvents used in these nanoparticles quickly partitioned into the exterior aqueous phase and PVA precipitated around copolymer encapsulated glipizide particles. The evaporation of the entrapped organic solvents leads to the formation of glipizide loaded polymeric nanoparticles¹⁵.

Optimization parameters of prepared GN

The GN was optimized on the basis of morphological properties (in terms of particle size and surface characteristics), entrapment efficiency and percentage yield. Particle size analysis used to characterize nanoparticles and it helps to understand the dispersion and aggregation²⁰. With the reduction of particle size, enhancement of surface area and attractive forces between the particles generate the possibility of aggregation. To defeat such aggregation problems, use of a surfactant in the nanoparticle preparation becomes essential. PVA can encapsulate the nanoparticles and also work as surfactant by reducing the aggregation of nanoparticles which keep them suspended in solution after formation and also re-suspension of lyophilized nanoparticles become easy^{15,21}. The zeta potential of the particles is a significant characteristic, which can demonstrate the

particle stability. Higher the magnitude of zeta potential, irrespective of the charge type (positive or negative), higher stability is anticipated^{20,22}.

The entrapment efficiency and percentage yield is the need of modern nanotechnology-based drug development. Generally, those excipients are selected, which can entrap maximum amount of drug and gives the best yield along with other significant parameters. Higher drug entrapment leads to a reduction in drug loss during the manufacturing process^{22,23}.

The glipizide to PA-F127 copolymer ratios critically affects the particle size as well as other studied parameters. The optimization data of GN (Table 2) exhibited that the nanoparticles produced were of submicron size ranging between 242.6 to 891.2 nm. The zeta potential and PDI values varies between 0.171 to 0.556 and -8.03 to -21.41 mV, respectively. The range of entrapment efficiency and percentage yield was of 35.42 to 81.13 % and 23.2 to 76.4 %, respectively.

Based on the morphological properties, entrapment efficiency and percentage yield, among the five batches, 1:1 ratio (GN1) was chosen as the optimized ratio. The above parameters in other four batches (GN2, 3, 4 and 5) were comparatively less valuable, hence not selected for further studies. In batch GN5, the slight improvement in particle size, entrapment efficiency, and percentage yield was observed over batch GN2, 3 and 4. This happened due to change in the ratios of drug to the copolymer. These preparation trials have been repeated thrice, for reproducibility and uniformity of the results. The particle size and zeta potential analysis of optimized batch GN1 are shown in Figure 1.

FTIR analysis

FTIR spectra provide a distinct idea about interaction(s) between diverse functional groups existing in drug and excipients^{24,25}. The possible interactions between PA-F127, PVA, glipizide, physical mixture and optimized GN1 were investigated by comparing the FTIR peaks (Figure 2).

The IR spectra of pure glipizide exhibited peaks at 3250.44 cm^{-1} (-NH stretching), 2941.02 cm^{-1} (C-H stretching), 1690.44 cm^{-1} (C=O stretching), 1649.88 cm^{-1} (-CONH- stretching), 1591.28 cm^{-1} (C=C aromatic stretching), 1461 cm^{-1} (C-H aromatic bending), 1337.27, 1160.14 cm^{-1} (O=S=O), which are also detected in the physical mixture and

GN1. There was no significant shift in peaks were detected in the physical mixture and optimized GN1 as compared to spectra of PA-F127, PVA, and pure glipizide. This indicates that the glipizide and excipients used were compatible and suitable for current investigation.

DSC analysis

It was found to be useful in the examination of thermal properties of the nanoparticles, providing quantitative and qualitative manifests about the physicochemical state of the drug inside the nanoparticles as well as drug-polymer interactions²⁶.

The characteristic sharp endothermic peak at 212.18 °C was observed for pure glipizide (Figure 3c) which was absent in PA-F127 (Figure 3a) copolymer. PVA (Figure 3b) showed an endothermic peak at 215.31 °C which overlapped with glipizide peak in the physical mixture (Figure 3d) and GN1 (Figure 3e). A close look at overlay in Figure 3 suggested that there was no significant shift in endothermic peaks was detected. Hence, there was no interaction between glipizide and polymeric excipients. The selection of excipients was done on the basis of results of FTIR and DSC analysis and further studies were extended.

XRD studies

The XRD patterns of the PA-F127 copolymer (Figure 4a) and PVA (Figure 4b) showed a diffused spectrum having fewer peaks and suggested semi-amorphous nature. The XRD patterns of glipizide showed several sharp peaks (Figure 4c) which were found to be in line with the previous report²⁷. The characteristic sharp diffraction peaks due to pure glipizide and diffused peaks of PA-F127 copolymer and PVA can be seen in the physical mixture (Figure 5d). After being formulated into nanoparticles, the XRD pattern of GN1 showed comparatively less sharp peaks (Figure 5e) with reduced intensity and has partially amorphous nature. This decreased intensity shows the reduced crystalline properties of the drug²⁸.

Surface morphology by SEM

Smooth surfaced rectangular crystals of glipizide in the physical mixture (Figure 5a) can be seen clearly, which were not visible in optimized GN1 (Figure 5b). The GN1 showed smooth and spherical shaped nanoparticles indicated that the glipizide gets encapsulated in the polymeric matrix. This smooth surface property of nanoparticles

demonstrated the complete removal of solvents from the glipizide nanoparticles and a sign of good quality²⁹.

In Vitro studies

The *in vitro* release of the glipizide from GN1 showed first burst release followed by the sustained release (Figure 6). The release of glipizide from GN1 at 8 and 24 h was $34.43 \pm 4.8 \%$ and $56.11 \pm 4.64 \%$, respectively, whereas on same time interval 53.1 ± 4.6 and $92.1 \pm 4.12 \%$ drug was released from pure glipizide. The initial burst release of glipizide from GN1 may be due to the loosely associated drug on the interface of the polymeric matrix. The drug incorporated into the inner core compartment stayed firmly inside the nanoparticles shows a sustained drug release pattern¹².

Stability studies

Three months stability studies were performed for GN1 at two temperature conditions (4 and 25 °C) and results are enlisted in Table 3. The nano-suspension stored at both temperature remains carry nano-sized particles (< 250 nm) whereas the slight increase in PDI and reduction in zeta potential and entrapment efficiency have been observed. During the storage time, no visual color change was noticed. The reduction of entrapment efficiency and increase in particle size which might be attributed to the semi-amorphous character of the amphiphilic PA-F127 copolymer in GN1. When lipophilic part of copolymer exposed to kinetic energy (temperature or light), semi-amorphous state changes into the more stable amorphous state which leads to increase in particle size and expulsion of drug from the polymeric matrix with the reduction of entrapment efficiency³⁰. The results of stability studies were statistically non-significant. The nanoparticles stored at 5 ± 3 °C showed non-significant variation in studied parameters, which recommend that the above temperature was optimum storage temperature.

Pharmacokinetic studies

The mean plasma concentration of glipizide vs. time profile adopting a single oral dose of GN1 (1.5 mg/kg) and glipizide suspension (1.5 mg/kg) in six rats are presented in Table 4 and Figure 7. The value of peak plasma concentration (C_{max}) of GN1 was found to be 2.35 fold higher than the glipizide suspension ($p < 0.05$). The time required to reach maximum plasma concentration (t_{max}) after oral administration of GN1 and

glipizide suspension was 6.0 and 4.0, respectively. The elimination half-life ($t_{1/2}$) of GN1 was 1.5 fold ameliorated than the glipizide suspension ($p < 0.05$). The area under the curve ($AUC_{0 \rightarrow \infty}$) of GN1 was 3.3 fold higher compared to glipizide suspension ($p < 0.05$). Finally, the improvement (1.2 fold) in mean residence time (MRT) of GN1 over pure glipizide suspension ($p < 0.05$) was recorded. Overall, the oral bioavailability and circulation time of GN1 was improved significantly.

CONCLUSION

In the present investigation, glipizide nanoparticles were prepared using newly synthesized PA-F127 copolymer. The drug and excipients were compatible with each other. The optimized nanoparticles batch (GN1) can be best stored at 5 ± 3 °C without losing its properties. The ameliorated pharmacokinetic parameters of GN1 confirmed the improved bioavailability and circulation time. Therapeutic plasma concentration of drug with single oral dose of GN1 was maintained up to 24 h and frequent oral dose administration (2 or 3 times a day) problem with conventional dosage form can be overcome by the use of GN1. Reported PA-F127 pentablock copolymer could be a suitable carrier for nanotechnology-based oral glipizide.

Acknowledgment

The authors are grateful to University Grant Commission (UGC), New Delhi for providing fellowship under Major Research Project (MRP) Reference No: FN/42/703/2013/SR. Authors are grateful to Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, Ghaziabad for providing chromatographic facilities at ARD Lab.

Conflict of Interest: No conflict of interest was declared by the authors

REFERENCES

1. Lahoti SR, Puranik PK, Heda AA, Navale RB. Development and validation of RP-HPLC method for analysis of glipizide in guinea pig plasma and its application to pharmacokinetic study. *Int J PharmTech Res.* 2010;2:1649-1654.
2. Dash RN, Mohammed H, Humaira T, Ramesh D. Design, optimization and evaluation of glipizide solid self-nanoemulsifying drug delivery for enhanced solubility and dissolution. *Saudi Pharm J.* 2015;23:528-540.
3. Emami J, Boushehri MSS, Varshosaz J. Preparation, characterization and optimization of glipizide controlled-release nanoparticles. *Res Pharm Sci.* 2014;9:301-314.
4. Ammar HO, Salama HA, Ghorab M, El-Nahhas SA, Elmotasem H. A transdermal delivery system for glipizide. *Curr Drug Deliv.* 2006;3:333-341.
5. Patel JK, Patel RP, Amin AF, Patel MM. Formulation and evaluation of mucoadhesive glipizide microspheres. *AAPS PharmSciTech.* 2005;6:E49-55.
6. Naha P, Byrne HJ, Panda AK. Role of polymeric excipients on controlled release profile of glipizide from PLGA and Eudragit RS 100 nanoparticles. *J Nanopharm Drug Deliv.* 2013;1:74-81.
7. Huang H, Wu Z, Qi X, Zhang H, Chen Q, Xing J, Chen H, Rui Y. Compression-coated tablets of glipizide using hydroxypropylcellulose for zero-order release: *In vitro* and *in vivo* evaluation. *Int J Pharm.* 2013;446:211-218.
8. Nie S, Zhang S, Pan W, Liu Y. *In vitro* and *in vivo* studies on the complexes of glipizide with water-soluble beta-cyclodextrin-epichlorohydrin polymers. *Drug Dev Ind Pharm.* 2011;37:606-612.
9. Kulkarni N, Wakte P, Naik J. Development of floating chitosan-xanthan beads for oral controlled release of glipizide. *Int J Pharm Investig.* 2015;5:73-80.
10. Mahesh KV, Singh SK, Gulati M. A comparative study of top-down and bottom-up approaches for the preparation of nanosuspensions of glipizide. *Powder Technol.* 2014;256:436-449.
11. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J Control Release.* 2001;70:1-20.
12. Gao Q, Liang Q, Yu F, Xu J, Zhao Q, Sun B. Synthesis and characterization of novel amphiphilic copolymer stearic acid-coupled F127 nanoparticles for nanotechnology based drug delivery system. *Colloids Surf B Biointerfaces.* 2011;88:741-748.
13. Kamboj VK, Verma PK. Poloxamers based nanocarriers for drug delivery system. *Der Pharm Lett.* 2015;7:264-269.
14. Xiong XY, Li YP, Li ZL, Zhou CL, Tam KC, Liu ZY, Xie GX. Vesicles from Pluronic/poly(lactic acid) block copolymers as new carriers for oral insulin delivery. *J Control Release.* 2007;120:11-17.

15. Dhanalekshmi UM, Poovi G, Kishore N, Reddy PN. *In vitro* characterization and *in vivo* toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. *Int J Pharm*. 2010;396:194-203.
16. Patil P, Bhoskar M. Optimization and evaluation of spray dried chitosan nanoparticles containing doxorubicin. *Int J Curr Pharma Res*. 2014;6:7-15.
17. Lokhande A, Mishra S, Kulkarni R, Naik J. Formulation and evaluation of glipizide loaded nanoparticles. *Int J Pharm Pharm Sci*. 2013;5:147-151.
18. Rani R, Dahiya S, Dhingra D, Dilbaghi N, Kim KH, Kumar S. Evaluation of antidiabetic activity of glycyrrhizin-loaded nanoparticles in nicotinamide-streptozotocin induced diabetic rats. *Eur J Pharm Sci*. 2017;106:220-230.
19. Mutalik S, Udupa N, Kumar S, Agarwal S, Subramanian G, Ranjith AK. Glipizide matrix transdermal systems for diabetes mellitus: Preparation, *in vitro* and preclinical studies. *Life Sci*. 2006;79:1568-1577.
20. Duarah S, Pujari K, Ghosh J, Unnikrishnan D. Formulation and evaluation of metformin engineered polymeric nanoparticles for biomedical purpose. *Res J Pharm Biol Chem Sci*. 2015;6:1005-1019.
21. Birnbaum DT, Kosmala JD, Brannon-Peppas L. Optimization of preparation techniques for poly(lactic acid-co-glycolic acid) nanoparticles. *J Nanopart Res*. 2002;1:173-181.
22. Jain S, Saraf S. Influence of processing variables and *in vitro* characterization of glipizide loaded biodegradable nanoparticles. *Diabetes Metab Syndr Clin Res Rev*. 2009;3:113-117.
23. Kusum VD, Bhosale UV. Formulation and optimization of polymeric nano drug delivery system of acyclovir using 3² full factorial design. *Int J PharmTech Res*. 2009;1:644-653.
24. Mukherjee B, Mahapatra S, Gupta R, Patra B, Tiwari A, Arora P. A comparison between povidone-ethylcellulose and povidone-eudragit transdermal dexamethasone matrix patches based on *in vitro* skin permeation. *Eur J Pharm Biopharm*. 2005;59:475-483.
25. Mukherjee B, Santra K, Pattnaik G, Ghosh S. Preparation, characterization and *in-vitro* evaluation of sustained release protein-loaded nanoparticles based on biodegradable polymers. *Int J Nanomed*. 2008;3:487-496.
26. Ramazani A, Keramati M, Malvandi H, Danafar H, Manjili HK. Preparation and *in vivo* evaluation of anti-plasmodial properties of artemisinin-loaded PCL-PEG-PCL nanoparticles. *Pharm Dev Technol*. 2017;DOI: 10.1080/10837450.2017.1372781.
27. Dash RN, Mohammed H, Humaira T, Reddy AV. Solid supersaturatable self-nanoemulsifying drug delivery systems for improved dissolution, absorption and pharmacodynamic effects of glipizide. *J Drug Deliv Sci Technol*. 2015;28:28-36.
28. Mokale V, Rajput R, Patil J, Yadava S, Naik J. Formulation of metformin

hydrochloride nanoparticles by using spray drying technique and *in vitro* evaluation of sustained release with 3²-level factorial design approach. Dry Technol. 2016;34:1455-1461.

29. Lekshmi UMD, Kishore N, Reddy PN. Subacute toxicity assessment of glipizide engineered polymeric nanoparticles. J Biomed Nanotechnol. 2011;7:578-589.
30. Elbahwy IA, Ibrahim HM, Ismael HR, Kasem AA. Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles. J Drug Deliv Sci Technol. 2017;38:78-89.

Table 1. Major features of ^1H NMR spectra of the PA-F127 copolymer in CDCl_3 .

δ (ppm)	Assign
$\text{CH}_2\text{-O}$ in PEO	3.68-3.66
$\text{CH}_2\text{CH}_2\text{-O}$ in PEO	2.37-2.34
$\text{CH}_2\text{CH}(\text{CH}_3)\text{-O}$ in PEO	1.66-1.62
$\text{CH}_2\text{CH}(\text{CH}_3)\text{-O}$ in PEO	1.31-1.27
CH_2 in PA	1.17-1.14

Table 2. Evaluation parameters of prepared GN

Batch	Glipizide: PA-F127 (w/w)	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment Efficiency (%)	Yield (%)
GN1	1:1	242.60 $\pm$ 4.20	0.171 $\pm$ 0.0143	-21.41 $\pm$ 0.462	81.13 $\pm$ 3.12	76.40 $\pm$ 2.23
GN2	1:2	630.46 $\pm$ 4.05	0.556 $\pm$ 0.0362	-16.33 $\pm$ 0.153	60.41 $\pm$ 4.41	59.31 $\pm$ 4.22
GN3	1:3	721.30 $\pm$ 6.77	0.328 $\pm$ 0.0238	-10.23 $\pm$ 0.513	50.30 $\pm$ 3.34	49.84 $\pm$ 3.41
GN4	1:4	891.20 $\pm$ 7.80	0.471 $\pm$ 0.0264	-8.03 $\pm$ 0.737	35.42 $\pm$ 1.94	23.20 $\pm$ 3.45
GN5	2:1	540.24 $\pm$ 3.51	0.391 $\pm$ 0.0211	-11.5 $\pm$ 0.561	70.60 $\pm$ 2.51	66.34 $\pm$ 4.14

n = 3, mean values $\pm$ SD**Table 3.** Stability Studies of GN1

Storage condition	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment Efficiency (%)	Visual observation
Fresh GN1	242.6 $\pm$ 4.20	0.171 $\pm$ 0.01	-21.41 $\pm$ 0.462	81.13 $\pm$ 3.1	Clear suspension
1 month (5 $\pm$ 3 $^\circ\text{C}$)	244.2 $\pm$ 4.04	0.171 $\pm$ 0.02	-21.20 $\pm$ 0.472	80.43 $\pm$ 2.5	Clear suspension
3 month (5 $\pm$ 3 $^\circ\text{C}$)	246.0 $\pm$ 3.42	0.184 $\pm$ 0.03	-21.01 $\pm$ 0.522	79.25 $\pm$ 4.5	Clear suspension
1 month (25 $^\circ\text{C}$)	246.3 $\pm$ 4.70	0.182 $\pm$ 0.02	-20.84 $\pm$ 0.341	78.64 $\pm$ 4.2	Clear suspension
3 month (25 $^\circ\text{C}$)	249.5 $\pm$ 5.43	0.196 $\pm$ 0.03	-20.35 $\pm$ 0.52	77.25 $\pm$ 3.5	Clear suspension

n = 3, mean values $\pm$ SD**Table 4.** Pharmacokinetic parameters of GN1 and pure glipizide suspension

Sample	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$ (h)	$\text{AUC}_{0 \rightarrow \infty}$ (ng.h/ml)	MRT (h)
GN1	1321 $\pm$ 110	6.0	10.51 $\pm$ 0.2	18574 $\pm$ 96	11.06 $\pm$ 0.4
Glipizide suspension	561 $\pm$ 86	4.0	7.04 $\pm$ 0.1	5688 $\pm$ 102	9.25 $\pm$ 0.3

Mean $\pm$ SD, n=6

Scheme 1. Preparation of PA-F127 copolymer

Figure 1. (a) Zeta potential and (b) particle size analysis of GN1

Figure 2. FTIR spectra of (a) PA-F127, (b) PVA, (c) Pure glipizide, (d) Physical mixture and (e) GN1

Figure 3. DSC thermograms of (a) PA-F127, (b) PVA, (c) Pure glipizide, (d) Physical mixture and (e) GN1

Figure 4. XRD patterns of (a) PA-F127, (b) PVA, (c) Pure glipizide, (d) Physical mixture and (e) GN1

Figure 5. SEM images of (a) Physical mixture (drug + excipients), (b) GN1

Figure 6. *In vitro* release profiles of GN1 and pure glipizide

Figure 7. Mean plasma concentrations of glipizide vs. time graph after single oral administration of GN1