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Original Article

PREPARATION AND CHARACTERIZATION OF NANOMATERIAL BASED GLIPZIDE ORAL FORMULATIONS

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ABSTRACT

Background: *Nanomaterial-based glipizide formulations improve drug delivery, enhance bioavailability, maintain sustained release, and minimize side effects compared to conventional oral forms, offering a promising alternative for type-two diabetes treatment with improved patient outcomes.*

Aim: *To develop controlled-release nanoparticles containing glipizide, a second-generation sulfonylurea used to lower blood glucose in type-two diabetics, by utilizing alginate and chitosan through ionotropic controlled gelation. These nanoparticles aim to enhance drug delivery efficiency and provide sustained release characteristics.*

Objective: *The study 1) Formulate GlACNP for enhanced drug delivery and sustained release. 2) Characterize GlACNP regarding various parameters. 3) Optimize GlACNP properties using statistical methods. 4) Develop a superior glipizide delivery system for type-two diabetes treatment.*

Results: *Preformulation studies confirmed glipizide and excipients' suitability. Partition coefficient and compatibility studies validated glipizide's nature and excipient compatibility. Glipizide-loaded nanoparticles (GN) were optimized, with GN1 (1:1 glipizide to PAF127 ratio) exhibiting favorable attributes, including sustained release. Stability studies over three months at 5 ± 3 ºC showed minimal changes, emphasizing storage significance.*

Conclusion: *This study developed a nanotechnology-based glipizide formulation (GN1) to improve solubility, bioavailability, and dosing frequency in diabetes management. Glipizide-loaded nanoparticles, utilizing PAF127 copolymer, exhibited favorable properties, such as sustained release following the Higuchi model. Compatibility with glipizide suggests potential as carriers in oral formulations. Clinical investigations are required to assess efficacy in managing elevated blood glucose levels in diabetic patients.*

KEY WORDS: *Glipizide, Sulfonylurea, PAF127 copolymer.*

INTRODUCTION

Nanotechnology revolutionizes drug delivery with nano-scale materials and devices. Biodegradable polymer-based nanoparticles stand out for controlled drug release and tissue targeting, particularly in treating diseases like type II diabetes These nanoparticles, derived from natural or synthetic polymers, are prepared using techniques such as solvent and nano-coprecipitation. They promise targeted delivery with minimal side effects across various medical fields. Diabetes mellitus, categorized into type I, type II, and gestational, manifests with symptoms like frequent urination, increased hunger, and thirst.

Anti diabetic drugs are essential for managing elevated blood glucose levels in diabetic patients. They can be categorized into

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various classes: biguanides, thiazolidinediones, sulfonylureas, meglitinides, and α-glucosidase inhibitors. Insulin, crucial for managing hyperglycemia, facilitates glucose passage into cells and affects glucose phosphorylation, oxidative phosphorylation, and lipid and protein catabolism. Major antidiabetic drug classes have distinct mechanisms of action and side effects.

Insulin: Essential for managing hyperglycemia, particularly in type I diabetes patients.

Biguanides: Inhibit glucose production and release from the liver, reducing weight gain and low-density lipoproteins, with side effects like metallic taste and nausea.

Thiazolidinediones: Increase cell sensitivity to insulin, reducing peripheral insulin resistance, but may cause nausea, tiredness, and weight gain.

Sulfonylureas: Stimulate pancreas to produce more insulin, lowering blood sugar levels, with potential side effects including hypoglycemia and weight gain.

Meglitinides: Stimulate rapid insulin production post-meal, with side effects such as weight gain and low blood sugar levels.

α-Glucosidase inhibitors: Block enzymes responsible for starch breakdown, preventing blood sugar spikes, but may cause side effects like loose stools and abdominal pain.

Alternative therapies are urgently needed to address the limitations of current antidiabetic drugs in controlling all pathological aspects of diabetes mellitus.

MATERIALS AND METHODS

Materials

Glipizide: Pharmaceutical grade from HONOUR Labs, Hyderabad.Palmitic Acid, Stearic Acid, Pluronic F127, Polyvinyl Alcohol: Analytical reagent (A.R.) grade from Sigma-Aldrich, India.Polyvinylpyrrolidone K 30: Analytical reagent (A.R.) grade from S.D. Fine Chem Ltd., Mumbai.Streptozotocin Research grade from Sigma, St. Louis, MO, USA.ChloroformMethylene Chloride, Ethyl Acetate, Petroleum Ether: Laboratory reagent (L.R.) grade from Molychem, Mumbai.KBr (Potassium Bromide), Potassium Dihydrogen Phosphate, Potassium Dihydrogen Orthophosphate: Analytical reagent (A.R.) grade from S.D. Fine Chem Ltd., Mumbai.Tween 80: Laboratory reagent (L.R.) grade from Hi-Media Laboratories, Mumbai.

Instruments used:

UV-Vis Spectrophotometer: Lab India-3000+, India FTIR Spectrophotometer: Bruker Alpha, Germany Probe Sonication: Sonic VibraCellTM VCX 750w, USA Homogenization: IKA T25 Ultra Homogenizer, Germany Rotary Evaporator: IKA® RV 10, BS96, Germany Centrifugation: Remi, India Zetasizer: Nano-ZS90, Malvern Instruments, UK DSC (Differential Scanning Calorimeter): Q10 V9.9, Waters, India XRD (X-ray Diffraction): Rigaku Miniflex-600 diffractometer, Japan FE-SEM (Field Emission Scanning Electron Microscope): JEOL, JSM-7600F, Japan Magnetic Stirrer: Remi, India Refrigerator: Samsung RR1914BCASE/TL/2014, India Bath Solicitor: Trans-O-Sonic, Mumbai pH Meter: Contech pH-103, India Centrifugation (Controlled Temperature): Plasto Crafts, India Melting Point Apparatus: Labtech, India Digital Balance: Sartorius, Germany Syringe Filter: Millipore, Billerica.

METHOD

Pre formulation studies

Pre-formulation studies provide crucial data on drug and excipient properties, ensuring product efficacy, stability, and marketability while reducing development costs and time. Parameters include solubility, partition coefficient, ionization constant, polymorphism, powder properties, thermal behavior, spectroscopic profile, drug-excipient compatibility, and stability. Reevaluating physicochemical properties like melting point and purity of glipizide is important before formulation development. Establishing compatibility with excipients is critical, especially for nanoparticle-based formulations.

Organoleptic evaluation

Organoleptic evaluation of pure drug samples included color and odor assessment.

Determination of melting point

The drug samples' melting points were determined using the capillary method with a melting point apparatus. A small amount of drug was placed in a closed-end capillary tube and heated until melting, noting the temperature at which melting began.

Determination of drug solubility

The solubility of glipizide was assessed in water, chloroform, methylene chloride, dimethylformamide, and 0.1 N NaOH. Excess drug was added to each solvent, vortexed, and equilibrated at 37 ± 0.5 °C for over 24 hours. The resulting solutions were filtered and analyzed with a UV-visible spectrophotometer.

Loss on drying

Drug samples weighing 1.00 g were dried in an oven at 105°C, and the change in weight was recorded.

Determination of λmax of glipizide

The wavelength of maximum absorbance (λmax) of glipizide was determined by preparing a solution of 10 µg/ml concentration in phosphate buffer pH 7.4. The solution was scanned at 200-400 nm using a UV-Visible spectrophotometer, and the observed λmax was 274 nm. This wavelength was used for spectrophotometric determination of glipizide during in-vitro release studies.

Preparation of standard curve of glipizide

10 mg of glipizide was dissolved in 20 ml of phosphate buffer (pH 7.4) in a 100 ml volumetric flask, resulting in a stock solution of 100 µg/ml. From this stock solution, dilutions of 2.5, 5.0, 7.5, 10, 12.5, and 15 µg/ml were prepared. The absorbance of each solution was measured using a UV-Visible spectrophotometer at 274 nm.

Determination of partition coefficient

The partition coefficient of drugs was determined using a method by Ghosh and Reddy (2001). 1-Octanol and phosphate buffer (pH 7.4) were saturated by shaking for 24 hours, then separated. Drug was added to each, shaken for 24 hours at 37°C, and allowed to separate for 2 hours. Further separation was achieved by centrifugation. Drug assays were performed at respective λmax using a UV-Visible spectrophotometer. The same procedure was repeated for 1-octanol and water, with readings taken in triplicate (n=3).

Drug-excipient interaction studies

FTIRanalysis

FTIR analysis assessed drug purity and compatibility by analyzing spectra of pure drugs, excipients, and drug mixtures. Prepared nanoparticles were also examined, comparing peaks with starting materials and mixtures. Samples were mixed with dried KBr, pressed into disks at 20000 psi, and examined using an FTIR instrument.

Differential scanning calorimetric analysis

Samples, excipients, and final formulations were analyzed for thermotropic properties using a DSC Q10 V9.9 Build 303 instrument (Waters, India), calibrated with Indium. Two milligrams of each sample were sealed in standard aluminum pans and scanned from 30 to 300 °C at a rate of 10 °C/min under a nitrogen environment (60 ml/min). An empty aluminum pan served as the reference.

XRD analysis

X-ray diffraction analysis was conducted using a Rigaku Miniflex-600 diffractometer with a Cu Kα source operating at 40 kV and 15 mA. The diffraction pattern was recorded over a 2θ angular range of 10-70°.

PreparationofglipizideloadedPAF127nanoparticles

Glipizide nanoparticles were prepared using the solvent evaporation technique with PAF127 and PVP K30 polymeric systems. Glipizide was dissolved in a mixture of chloroform and methylene chloride, while PAF127 copolymer was dissolved separately in chloroform. The glipizide solution was added dropwise to the copolymer solution with continuous stirring. Then, the aqueous phase of PVP K30 was added dropwise into the organic mixture with continuous homogenization. After solvent evaporation and vacuum desiccation, the nano-suspension was filter sterilized to remove unincorporated glipizide aggregates. The filtrate was centrifuged, and the sediment containing nanoparticles was lyophilized.

Characterization of prepared nanoparticles

Particle size, PDI and zeta potential

The particle size, PDI, and zeta potential of nanoparticles were assessed using Zetasizer Nano-ZS. A suspension of 0.5 mg/ml was prepared in Milli-Q water and analyzed. Results were reported as mean ± standard deviation for three replicates.

Drug loading and entrapment efficiency of glipizide nanoparticles

GN was dissolved in methylene chloride (20 ml) and added to freshly prepared phosphate buffer (pH 7.4). Continuous stirring facilitated glipizide extraction, with methylene chloride evaporation. Centrifugation at 10,000 rpm removed undissolved content, and the supernatant was filtered. Glipizide content was assessed using a UV-Vis spectrophotometer (Lab India 3000+) at 225 nm. Drug loading (%) and entrapment efficiency (%) were calculated using equations 1 and 2, respectively.

Surface morphological studies

The surface morphology of the physical mixture and the optimized batch was analyzed using field emission scanning electron microscopy (FE-SEM, JEOL- JSM-7600F, Japan). Samples were dispersed on metallic stubs, gold-coated using an ion-sputtering machine, and vacuum-dried before examination.

In-vitro release studies of glipizide nanoparticles

In vitro drug release studies for the optimized GN1 batch were conducted using a modified dialysis sac method. GN1 suspension equivalent to 5 mg of glipizide was placed in dialysis membrane bags (12-14 kDa cut-off, HiMedia, India) and immersed in 150 ml of 0.1 M phosphate buffer solution (pH 7.4) in conical flasks. The flasks were stirred at 100 rpm and maintained at 37.0 ± 0.5 ºC. At specified time intervals, 1 ml aliquots were withdrawn, replaced with fresh phosphate buffer, and analyzed using a UV-Vis spectrophotometer at 274 nm.

In-vitro release kinetic evaluation

The drug release was analyzed using various kinetic equations: zero-order, first-order, Higuchi, and Korsmeyer-Peppas. Regression coefficients (R^2) were determined from plots of drug release data. For the Korsmeyer-Peppas model, the release exponent "n" was calculated to indicate the drug release mechanism. An "n" value of 0.45 suggests Fickian diffusion, 0.45 < n < 0.89 implies anomalous diffusion, n = 0.89 indicates case II transport, and n > 0.89 suggests super case II transport.

Stability studies

Short-term stability studies were conducted by storing the best batch nanoparticles (glipizide) suspensions in color glass bottles at 5 ± 3 ºC and 25 ºC. After 1 and 3 months, 5 ml aliquots from each sample were analyzed for changes in particle size, PDI, zeta potential, entrapment efficiency, and suspension color.

RESULTS AND DISCUSSION

Glipizide nanoparticles were prepared using PAF127 and SAF127 copolymers and characterized for in-vitro and in-vivo activities.

Glipizide nanoparticles

Preformulation studies of glipizide

The glipizide drug and excipients were standardized according to British Pharmacopoeia specifications, where applicable. Excipients not listed in pharmacopoeias were standardized based on manufacturer specifications.

The information presented in a concise tabular format:

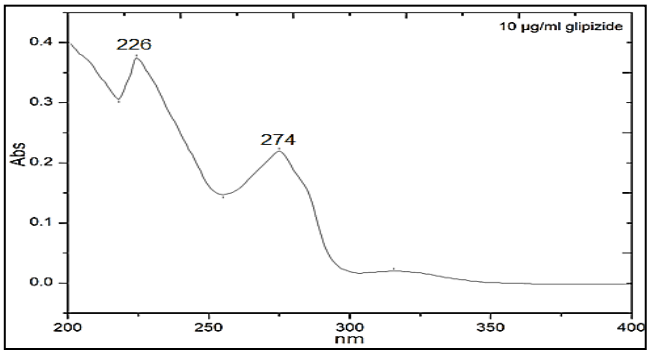
Parameter	Result
Physical appearance	White, odorless crystalline powder
Melting point	208 ± 2°C
Solubility	Practically insoluble in water; slightly soluble in methylene chloride and acetone; soluble in chloroform; dissolves in 1 M sodium hydroxide.
Loss on drying	Passed as per British Pharmacopoeia, 2015

Confirmation of λmax

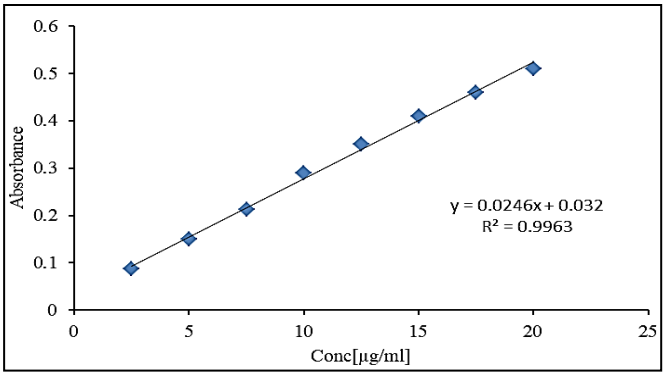
Glipizide in methanol solution was scanned by UV-Vis spectrophotometer from 200 to 400 nm, revealing λmax peaks at 226 and 274 nm (Figure 4.1). These peaks were consistent with the reported UV spectrum of glipizide (British Pharmacopoeia, 2015a).

Preparation of standard curve of glipizide

The standard graph (Figure4.2) of glipizide in phosphate buffer at pH 7.4 was prepared (2.5-20µg/ml) to calculate the amount of drug released from glipizide nano particle formulations in in-vitro drug release study. The λmax 274 was used for analysis. Regression coefficient (r2=0.9963) indicates the accuracy of the estima



UV-Visible Spectra of pure glipizide(10 µg/ml)



Standard graph of glipizide in phosphate buffer solution pH 7.4 (2.5-20µg/ml)

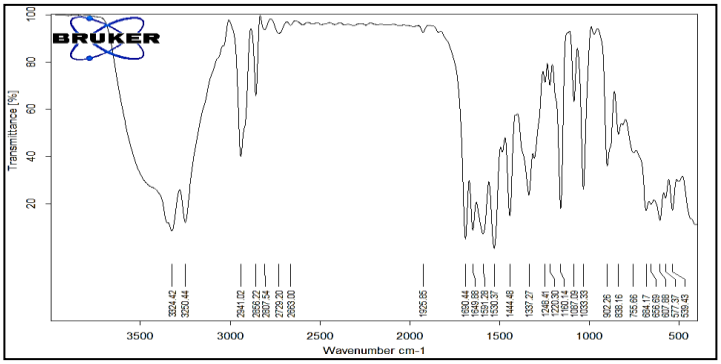
Partition coefficient

The partition coefficient of a drug, like glipizide, affects its permeability through biological membranes. Glipizide's partition coefficients in 1-octanol:water and 1-octanol:phosphate buffer (pH 7.4) were 1.634 ± 0.141 and 1.576 ± 0.172, respectively, indicating its lipophilic nature.

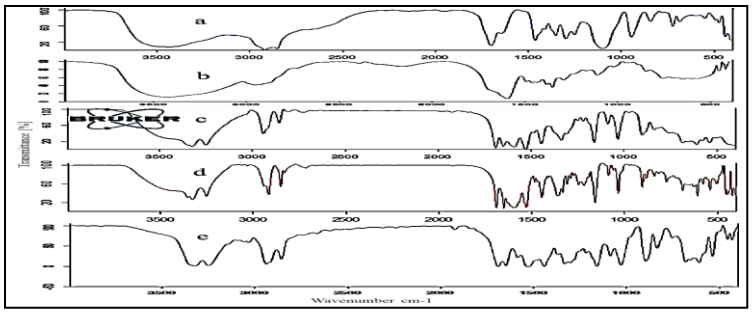
Compatibility studies

FTIR analysis

FTIR spectra analysis revealed peaks corresponding to functional groups in glipizide and excipients. Peaks consistent with glipizide were observed in physical mixture and optimized formulation GN1, indicating compatibility with excipients. No significant peak shifts were noted, affirming compatibility of glipizide and excipients for the study.



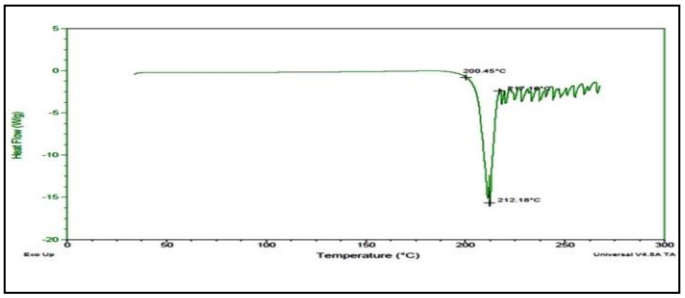
FTIR spectra of pure glipizide



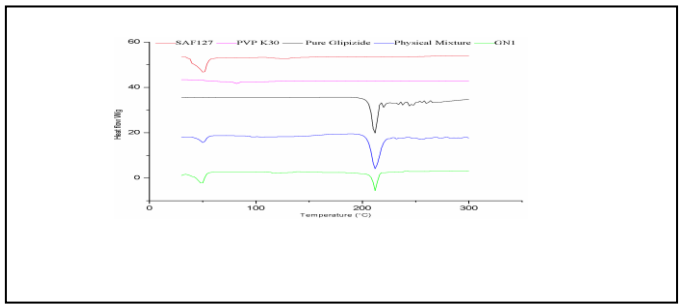
FTIR spectra of (a) PAF127, (b) PVP K30, (c) pure glipizide, (d) physical mixture and (e) GN1

DSC analysis

Thermal analysis, including Differential Scanning Calorimetry (DSC), provided insights into the physicochemical state of glipizide within nanoparticles and its interactions with excipients. Pure glipizide exhibited a sharp endothermic peak at 212.18 ºC, absent in PAF127 copolymer but present at 98.3 ºC in PVPK30. GN1 showed a small melting peak, suggesting glipizide entrapment within nanoparticles in an amorphous or molecular state without significant interaction with polymeric excipients. Excipient selection was based on FTIR and DSC results, guiding further studies.



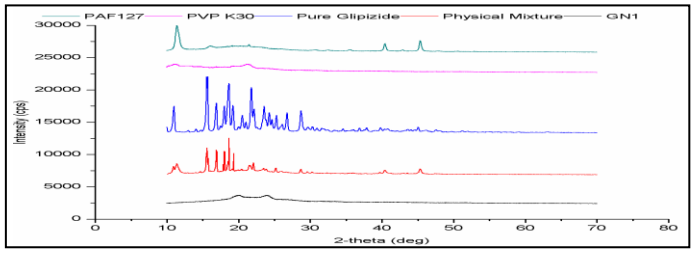
DSC thermogram of pure glipizide



DSC thermograms of PAF127, PVP K30, pure glipizide, physical mixture and GN1

XRD ANALYSIS

XRD analysis revealed semi-amorphous characteristics in PAF127 copolymer and PVP K30, while glipizide appeared crystalline. The physical mixture exhibited both patterns. Formulating into nanoparticles (GN1) reduced peak intensity, suggesting partial amorphous transformation due to crystal lattice distortion. This change could impact drug properties such as solubility and bioavailability.



XRD patterns of PAF127, PVA, pure glipizide, physical mixture and GN1

Preparation of glipizide loaded polymeric nanoparticles

Glipizide-loaded nanoparticles (GN) were fabricated using solvent evaporation, varying glipizide to PAF127 copolymer ratios while PVP K30 concentration remained constant. The method involved an organic phase with copolymer and glipizide, combined with an aqueous phase containing PVA, leading to GN formation upon solvent evaporation.

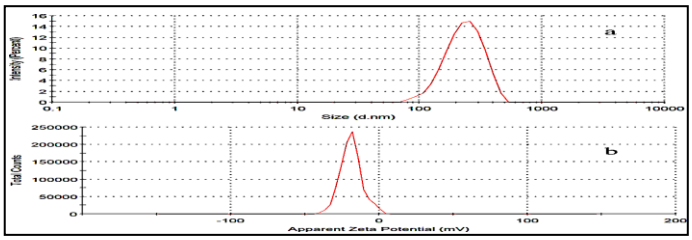
Optimization focused on morphological properties, drug loading, and entrapment efficiency, with particle size aiding dispersion analysis. PVP K30 prevented aggregation, and zeta potential ensured particle stability. Higher drug loading and entrapment efficiency were sought for improved drug delivery.

Glipizide to PAF127 ratios significantly impacted particle size, with GN1 (1:1 w/w) chosen as optimal. Preparation trials were repeated thrice for consistency. Optimized GN1 was analyzed for particle size and zeta potential.

Results of evaluated parameters of prepared glipizide nanoparticles

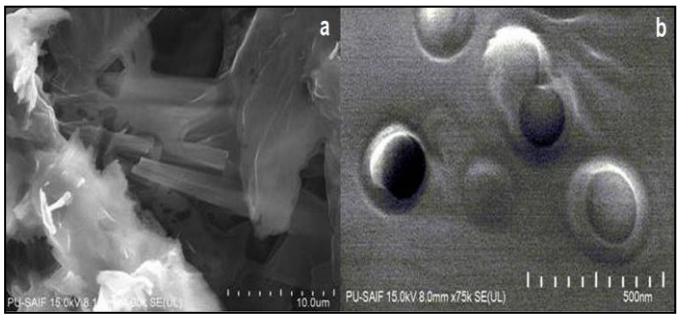
Batch	Glipizide : PAF127 (w/w)	Particle size (nm)	PDI	Zeta potential (mV)	Drug loading (%)	Entrapment efficiency (%)
GN1	1:1	240.4 ± 5.30	0.172 ± 0.01	-19.6 ± 0.62	59.34 ± 5.24	81.42 ± 4.25
GN2	1:2	634.63 ± 5.35	0.542 ± 0.02	-15.83 ± 0.24	47.62 ± 5.41	55.45 ± 5.32
GN3	1:3	729.44 ± 5.28	0.346 ± 0.03	-10.39 ± 0.64	31.42 ± 4.50	42.14 ± 5.25
GN4	1:4	899.36 ± 5.24	0.495 ± 0.02	-8.81 ± 0.45	20.39 ± 5.82	32.37 ± 5.15
GN5	2:1	549.38 ± 5.64	0.416 ± 0.02	-10.21 ± 0.63	23.48 ± 5.56	35.41 ± 5.62
GN6	3:1	691.34 ± 6.42	0.543 ± 0.04	-9.53 ± 0.62	19.29 ± 5.54	24.45 ± 4.42
GN7	4:1	980.2 ± 6.54	0.682 ± 0.03	-6.44 ± 0.58	12.37 ± 4.52	18.44 ± 5.52

n = 3, mean values ± SD



(a) Zetapotential and (b) particle size analysis of GN1

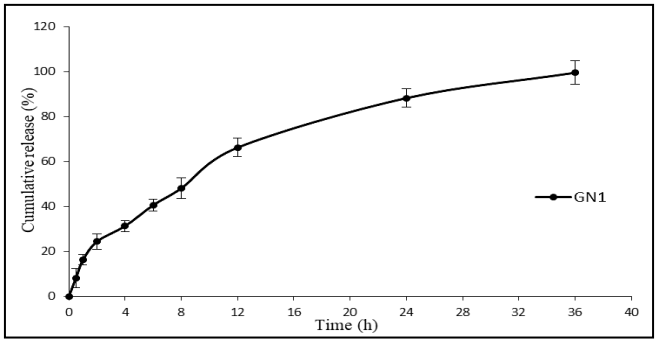
Surface morphology by SEM



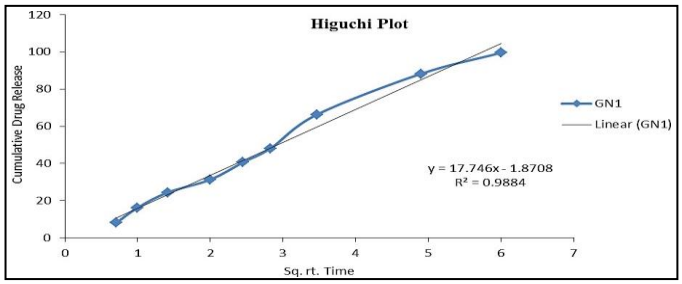
SEM images of (a) Physical mixture, (b) GN1

In-vitro drug studies

The in-vitro release of glipizide from GN1 exhibited an initial burst release followed by sustained release. Cumulative drug release at 2, 8, and 24 hours was 24.4 ± 3.4%, 48.1 ± 4.6%, and 88.2 ± 4.12%, respectively, with 99.5% released by 36 hours. The initial burst release may be attributed to loosely associated drug on the polymeric matrix interface, while drug incorporated into the inner core compartment showed sustained release.



In-vitro cumulative drug release profiles of GN1



In-vitro drug release kinetic of GN1

The drug release kinetics was studied by using various kinetic models such as zero order, first order, higuchi model and koresmeyer-peppas release kinetics. As per data of regression coefficient, it was inferred that release kinetics of the drug from formulation GN1 was according to higuchi kinetics (r2 = 0.9884). In comparison, zero-order kinetics (r2 = 0.8902), first-order kinetics (r2 = 0.6664) and koresmeyer- peppas kinetics (r2 = 0.9836) showed relatively lower r2 values.

Stability studies

Stability studies for GN1 over three months at 5 ± 3 ºC and 25 ºC revealed minimal changes in particle size, PDI, zeta potential, and entrapment efficiency. At 5 ± 3 ºC, nano-sized particles (<260 nm) remained stable, while at 25 ºC, there was an increase in PDI and reduction in zeta potential and entrapment efficiency. No color change was observed. The decrease in entrapment efficiency and increase in particle size at higher temperature may be due to the semi-amorphous nature of PAF127 copolymer. Storage at 5 ± 3 ºC showed insignificant variations, indicating it as the optimal storage temperature.

Storage Condition	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Visual Observation
Fresh GN1	240.4 ± 5.30	0.172 ± 0.01	-19.6 ± 0.62	82.22 ± 4.35	Clear suspension
1 month (5 ± 3 ºC)	243.1 ± 4.23	0.173 ± 0.02	-19.2 ± 0.53	81.17 ± 4.52	Clear suspension
3 month (5 ± 3 ºC)	245.0 ± 3.42	0.181 ± 0.02	-18.9 ± 0.43	78.31 ± 5.5	Clear suspension
1 month (25 ºC)	249.2 ± 5.25	0.191 ± 0.03	-17.54 ± 0.51	75.54 ± 5.3	Clear suspension
3 month (25 ºC)	258.2 ± 4.84	0.198 ± 0.02	-16.35 ± 0.52	72.25 ± 3.5	Clear suspension

n = 3, mean values ± SD

SUMMARY

Diabetes mellitus, a prevalent metabolic disorder globally, is rising notably in urban areas of India due to factors like sedentary lifestyle, aging, poor nutrition, stress, and obesity. It involves defects in insulin usage, leading to elevated blood glucose levels. Glipizide, a second-generation sulfonylurea, is commonly used to manage hyperglycemia in type II diabetes mellitus. It belongs to BCS class II drugs, with limited aqueous solubility due to its low pH.Glipizide effectively lowers blood glucose levels within 1-3 hours but requires frequent oral administration due to its short biological half-life. Conventional oral administration lacks sustained release, leading to fluctuating drug levels and decreased patient compliance.

CONCLUSION

Nanotechnology-based oral delivery offers potential for improved antidiabetic medication. This study developed a nanotechnology-based glipizide formulation to enhance solubility, bioavailability, and reduce dosing frequency. Glipizide-loaded nanoparticles, utilizing PAF127 copolymer, demonstrated favorable properties (GN1) including particle size, PDI, zeta potential, drug loading, and entrapment efficiency. In vitro studies confirmed sustained release following the Higuchi model, with GN1 exhibiting smooth, spherical nanoparticles and semi-amorphous nature in XRD patterns. Compatibility of synthesized pentablock copolymers with glipizide suggests their utility as carriers in nanotechnology-based oral formulations. Clinical investigations are necessary to assess efficacy in managing elevated blood glucose levels in diabetic patients.

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