International Journal of Pharmaceutical Development & Technology

e ISSN - 2248 - 910X

www.ijpdt.com

Print ISSN - 2248 - 9096

DESIGN AND OPTIMIZATION OF ANTIDIABETIC DRUG CONTAINING SOLID LIPID NANOPARTICLES

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ABSTRACT

The aim of this study was to develop and optimize solid lipid nanoparticles (SLNs) anti diabetic drug glibenclamide. SLNs offer several advantages such as improved drug stability, controlled release, and enhanced bioavailability. In this STUDY, optimize the formulation variables including lipid concentration, surfactant concentration, and homogenization speed for the preparation of SLNs. The optimized SLNs exhibited a mean particle size, a narrow size distribution, and a high drug encapsulation efficiency. The characterization studies confirmed the successful incorporation of glibenclamide into the SLNs, along with sustained drug release. Additionally, the SLNs demonstrated excellent stability over a period of 3 months. In conclusion, the developed SLNs loaded with glibenclamide offer a promising strategy for improving the therapeutic efficacy of the antidiabetic drug. These findings provide a foundation for further investigations and potential translation into clinical applications for the treatment of diabetes mellitus.

Keywords: Design, Optimization, Anti diabetic, Glibenclamide, Solid Lipid Nanoparticles.

INTRODUCTION

SOLID LIPID NANOPARTICLES

A wide variety of pharmaceutical dosage forms are used to deliver drugs for treatment of acute and chronic illnesses, including solid, semi-solid, liquid dosage form, suppositories, creams, ointments, injections, and aerosols [1]. With the development of colloidal drug delivery systems, pharmaceuticals and drugs have been targeted in a whole new way. Emulsions, nanoparticles, micro particles, and micro gels are a few examples of colloidal delivery systems. Solid colloidal particles are termed nanoparticles because the active principle is dissolved, entrapped or attached to them [2]. Nanoparticles are ideal for drug delivery because they are small, have large surfaces, and can change their surface properties. Drugs can be targeted with nanoparticles, sustained over time, marketed intravenously, dissolved for intravascular delivery, and improved against enzyme degradation with nanoparticles [3].

As alternative drug delivery vehicles, solid lipid nanoparticles (SLNs) are used instead of oil-in-water emulsions, liposomes, microparticles, and polymeric nanoparticles. The particles are composed of spherical lipid particles in a range of sizes. Hydrophilic and lipophilic drugs are incorporated into SLNs for controlled and targeted delivery. Lipid solids, emulsifiers, and co emulsifiers constitute solid lipid nanoparticles. It is not uncommon for solid lipids to melt at temperatures higher than body temperature (37°C) when they are used in such delivery systems. Numerous studies have been done on lipids, such as fatty acids, steroids, waxes, triglycerides, and acylglycerols. Lipid dispersion can be stabilized using any class of emulsifier, either alone or in combination [4].

Microparticles advantages in Solid Lipid Nanoparticles

In the body, the smallest blood capillaries are approximately 5-6 millimeters in diameter, so particles in the bloodstream should be smaller than 5 millimeters in order to minimize embolism. In this regard, SLNs have the advantage of being suitable for intravenous delivery.

Liposomes advantages in Solid Lipid Nanoparticles

- Solvents organic when possible should be avoided.
- Producing large quantities at a reasonable cost and with excellent reproducibility.
- An extremely unique feature of SLNs is the ability to control their release and target drugs using ligand coatings and attachments [5].
- A one-year increase in product stability has been achieved.

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Polymeric Nanoparticles advantages in Solid Lipid Nanoparticles

A lipid's biodegradability makes it more biocompatible [6].

Organic solvents should be avoided when possible. Sterilization and large-scale production are feasible.

As a preparation method, high pressure homogenization provides excellent reproducibility at a reasonable cost [7].

Production techniques for Solid Lipid Nanoparticles

There were high concentrations of microparticles in the nanoparticles created by Speiser. A high shear mixing or ultrasonication process was used by Domb to produce lipospheres [8]. Both Speiser's and Domb's nanopellets and lipospheres, however, contained microparticles. Scientists have recognized the potential of SLN technology in the last decade, and their research efforts have improved the synthesis of SLN's.

- High pressure homogenization technique
- Technique of hot homogenization
- Technique of cold homogenization
- Technique based on microemulsions
- Solvent Emulsification Evaporation Technique
- Solvent Displacement Technique
- A Technique based on Emulsification Diffusion

In order to prepare solid lipid nanoparticles, Muller and Lucks first used high pressure homogenization (HPH) [9]. Nanoemulsions for parenteral nutrition like Intralipid and Lipofundin have been produced with homogenizers for several years now [10]. Therefore, scaling up is less problematic and more cost-effective than other approaches. It has been proposed by several researchers that this technique can be used to create better solid lipid nanoparticles.

Diabetes Mellitus

A wide variety of disorders are associated with insulin resistance, ranging from autoimmune destruction of pancreatic beta -cells to abnormalities in the -cells that result in an insulin deficiency. Diabetes is characterized by abnormalities in carbohydrates, fats, and proteins metabolism due to impaired insulin action. At one or more points along the complex pathways of hormone action, inadequate insulin secretion and diminished tissue responses to insulin lead to inadequate insulin action.

Type 1 diabetes

Diabetes mediated by the immune system. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular- mediated autoimmune destruction of the beta-cells of the pancreas.

Type 2 diabetes

It is characterized by insulin resistance and usually has relative (rather than absolute) insulin deficiency and accounts for 90-95% of diabetes cases. This form of diabetes was previously known as non-insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes. These individuals do not require insulin treatment to survive at least initially, and often throughout their lives. Diabetes of this type is probably caused by many different factors. Despite not knowing the specific cause, patients do not have the other causes of diabetes listed above or below, including autoimmune destruction of beta cells.

MATERIALS AND METHOD

MATERIALS

Glibenclamide, Potassium bromide, Hydroxy Propyl Methyl Cellulose, Ammonium oxalate, Span 80, Polaxomer, Polysorbate 80, Manitol, Potassium Di Hydrogen Phosphate, Sodium Hydroxide.

DRUG PROFILE

GLIBENCLAMIDE [11, 12]

Synonyms	: Diabeta, Glynase, Micronase
Drug class	**:** Antidiabetic medication
Formula	**:** $C_{23}H_{28}ClN_3O_5S$
Molar mass	**:** 494.00 g·mol^{-1}

Chemical structure

Glibenclamide, also known as glyburide, is an antidiabetic medication used to treat type 2 diabetes. It is recommended that it be taken together with diet and exercise. It may be used with other antidiabetic medication. It is not recommended for use by itself in type 1 diabetes Common side effects include nausea and heartburn. Serious side effects may include angioedema and low blood sugar. It is generally not recommended during pregnancy but can be used during breastfeeding. It is in the sulfonylureas class of medications and works by increasing the release of insulin from the pancreas.

Medical uses

Glibenclamide is indicated as an adjunct to diet and exercise to improve glycaemic control in adults with type 2 diabetes. It is not as good as either metformin or insulin in those who have gestational diabetes.

PREPARATION OF Glibenclamide SOLID LIPID NANOPARTICLE

Identification of drug by UV-spectrophotometric method:

Preparation of standard calibration curve for Glibenclamide in phosphate buffer 7.4 pH

Calibration curve:

Preparation of buffer solution [13, 14]

Preparation of 0.2 M Sodium hydroxide (NaOH): Dissolve sodium hydroxide in water to produce a 40 to 60% w/v solution. Precaution has to be taken to avoid absorption of carbon dioxide, siphon off the clear supernatant liquid and dilute with carbon dioxide free

water which has a suitable volume of the liquid containing 8.0 g of NaOH in 1000 ml water, and finally it is standardized.

Preparation of 0.2 M Potassium Dihydrogen Phosphate (KH_2PO_4):

Dissolve 27.218 g of Potassium Dihydrogen Phosphate in water and dilute with water of 1000 ml.

Preparation of Phosphate buffer solution pH 7.4:

Place 50 ml of 0.2 M KH_2PO_4 in 200 ml volumetric flask and add 39.1 ml of 0.2 M NaOH and make up the volume with distilled water. For 1000 ml phosphate buffer place 250 ml of 0.2 M KH2PO4 and add 195.5 ml of 0.2 M NaOH and make up the volume with distilled water

Preparation of Stock Solution:

10 mg of Glibenclamide was accurately weighed and transferred to a100 mL volumetric flask for the preparation of 100 µg mL−1solution using 0.1 N NaOH. After volume make-up, the stock solution was sonicated for 5 min to remove air bubbles.

Selection of Wavelength:

Intended for the wavelength selection, the stock solution of Glibenclamide 100 µg mL^{-1} was smartly run on a UV spectrometer in the range of 200–400 nm with fast scanning speed. The maximum absorbance of the solution was achieved at 229 nm, thus the λmax is used for further proceeding.

Linearity and Calibration Curve:

For the linearity and calibration curve, the absorbance of serially diluted aliquots of GLB was measured at 229 nm to achieve the linearity and calibration curve of developing method. Adding up with this, for the confirmation of Beer's Lambert law, statistical measurements of the y-intercept and regression correlation were also calculated by the side of the linearity graph [78].

Drug and excipients compatibility studies

FTIR studies

The chemical interactions between the medications (Glibenclamide) and other constituents in the composition, such as polymer and surfactants, were determined using FTIR analyses. Glibenclamide and a physical combination were studied using the potassium bromide (KBr) pelletization process. The drugs (0.2%) were ground with the KBr, and the combination was then squeezed using a tiny KBr pellet press at a pressure of around 7 tonnes by repeatedly rotating the press handle. In the FTIR instrument (Bruker, Germany) equipped with the OPUS Spectrum software, prepared KBr pellets are scanned throughout a wave number range of 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}. Samples were placed on the sample stage using a force gauge of 100 N, ensuring regular contact between both the specimen and the crystal holder for scanning.

Differential scanning Calorimetry (DSC) studies

The melting point of samples was determined using DSC tests. It aids in the reporting of drug purity, drug-excipient compatibility, and the crystalline quality of Solid lipidnanoparticle formulations. The DSC-70, a Schimadzu model equipment, was used to study drug and drug-loaded Solid Lipid Nanoparticle. The samples were measured at 5 mg and cooked in aluminium pans at a rate of 20 °C/min with dry nitrogen as the effluent gas at a temperature of 20-200 °C. The melting point was measured as an exothermic or endothermic peak [15].

High-speed homogenization followed by ultrasonication method - preparation of Solid Lipid Nanoparticle (SLN)

The required amount of Glibenclamide was homogeneously dispersed in various concentrations of polymeric solution (ranging from 80 to 5%), which was made by dissolving different concentration of surfactant and co-surfactant in deionized water and heating if necessary. The aqueous phase was homogenised for 10 minutes at 15000 RPM in a High-Speed Homogenizer before slowly dispersing the medication into the aqueous phase. As an outcome, Solid Lipid Nanoparticle precipitated in the form of an emulsion. Using a Probe Ultrasonicator, the resulting up with this, for the confirmation of Beer's Lambert law, statistical measurements of the y-intercept and regression correlation were also calculated by the side of the linearity graph [16].

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Evaluation parameters of GLIBENCLAMIDE solid lipid nanoparticle

Particle size and particle size distribution

A Horiba Nanoparticle size analyzer was used to determine the particle size distribution, mean particle size (PS -Z average in nm), and Polydispersity Index (PI) of Solid Lipid Nanoparticle (SZ-100 Nanopartica series).
Solid Lipid Nanoparticle should have a particle size of 10 to 100 nm and a PI of less than 0.5, indicating a unimodel or uniform monodisperse size distribution. All measurements were done in triplicate (n=3) [17].

Zeta potential (ζ)

The Horiba Nanoparticle size analyzer was used to measure the Zeta Potential, or surface charge potential (SZ-100 nanopartica series). An electrophoretic cell with an 80-mV electric field was used to transport the diluted Solid Lipid Nanoparticle into the probe. Using the Smolochowski equation, the Zeta potential was then directly calculated from the eqn.

$$\zeta = \varepsilon\mu/\eta$$

Where, ζ - Zeta Potential, μ - Electrophoretic mobility; Ɛ- Electric permittivity of the liquid; η is the viscosity of the liquid [18].

Surface morphology studies - Scanning electron microscope (SEM) studies

The Scanning Electron Microscope was used to examine the surface morphology of the Solid Lipid Nanoparticle for the selected optimum Glibenclamide Solid Lipid Nanoparticle (Hitachi S-3000 N). Lyophilized Solid Lipid Nanoparticle powder sections were stained with 600 platinum using a sputter coater and analysed using a scanning electron microscope (SEM).

Encapsulation efficiency studies

The centrifugation method was used to determine encapsulation efficiency. In this investigation, 1 ml of Solid Lipid Nanoparticle dispersion with a molecular weight of 12,000–14,000 Daltons and a pore size of 2.4 nm was placed in dialysis bags (Himedia). The concentration of Glibenclamide in the withdrew sample was measured using a UV Spectrophomotometer set to 229 nm. The blank solution was made using the same method and ingredients as the medication solution, but without the drug. The experiment was repeated three times (n=3). The below equation was used to calculate percentage entrapment efficiency.

$$\%EE = \frac{Xs - Xt}{Xs} X\,100$$

Where, Xs - Total amount of drug used for formulation; Xt - Amount of drug in 5 ml saline.

***In-vitro* drug release studies**

The percentage amount of the drug released from Solid Lipid Nanoparticle dispersion performed out using the dialysis membrane technique is referred to as in-vitro drug release. 1 ml of Solid Lipid Nanoparticle dispersion was put into the dialysis membrane with 0.45 m pore size after one end of the dialysis membrane was closed or tied firmly. Both ends of the dialysis membrane were tightly knotted after it was filled.

***In-vitro* release kinetic study**

The drug release survey of SLNs was fixed in various release kinetic parameters such as first order (time vs. log percent drug remaining); zero order (time vs. percent cumulative release); Higuchi's model (square root of time vs. cumulative percent drug release); Peppa's model (Time Vs. log of drug concentration) and their regression (r^2) and k values were determined in order to acquire a linear regression analysis to verify the impact and process of release over time.

Stability studies

This study used an optimized Solid Lipid Nanoparticle dispersion. Each formulation was split into two batches for testing. Three lots of samples were collected in test tubes for each batch. Each test tube was labelled with the months 3rd, 6th and 12th. The findings of each formulation were examined for consistency.

Table 1. Comparison of physiochemical properties of optimized Glibenclamide Solid lipid nanoparticle after stability studies.

Evaluation parameters	Optimized Solid lipid Nanoparticle (GSLN7)	After storage at 25ºC ± 2ºC / 60% RH ± 5% RH for 6 months	After storage at 4ºC±2ºC for 6 months
Particle size in nm	195.1 ± 2.66	199.8 ± 4.12	197.6 ± 3.68
Zeta potential mV	-33.4 ± 2.32	-32.8 ± 3.66	-33.8 ± 2.56
Polydispersity index	0.242 ± 0.20	0.244 ± 0.26	0.244 ± 0.42
Entrapment Efficiency (%)	87.24 ± 3.76	85.48 ± 3.28	86.98 ± 3.46
%CDR at 24 h	90.42 ±3.018	89.36 ± 3.44	90.12 ± 3.42

Figure 1. Solid Lipid Nanoparticles.

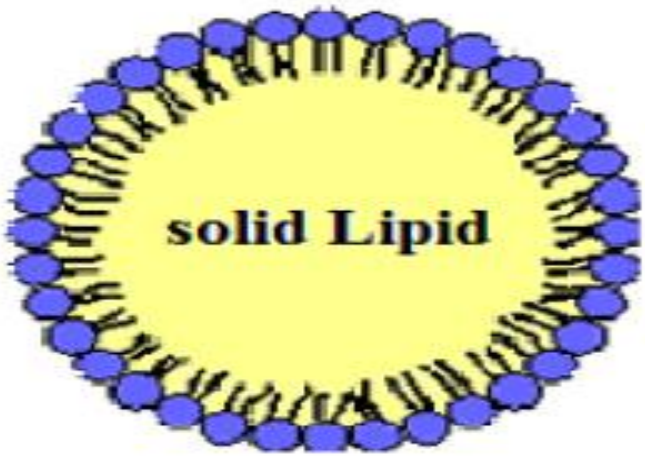


Figure 2. Metabolite therapy for diabetes management includes multiple therapeutic targets.

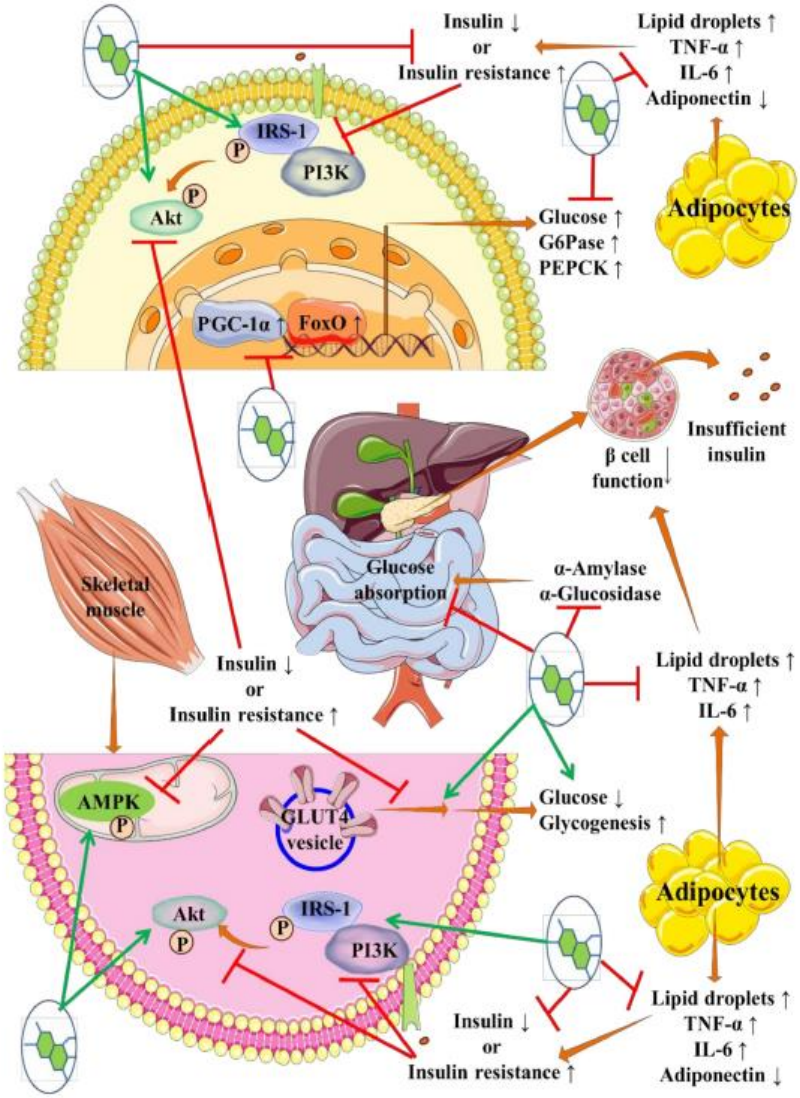


Figure 3. Chemical Structure of Glibenclamide.

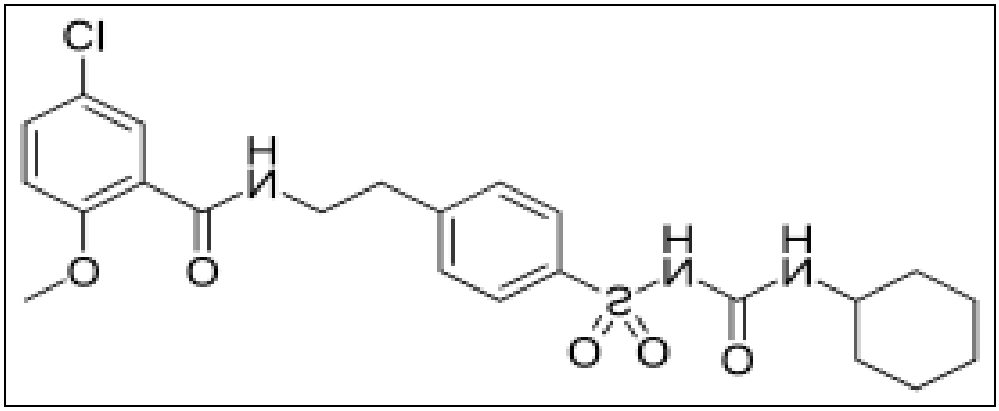


Figure 4. Calibration curve of Glibenclamide in pH 7.4 phosphate buffer at 229 nm.

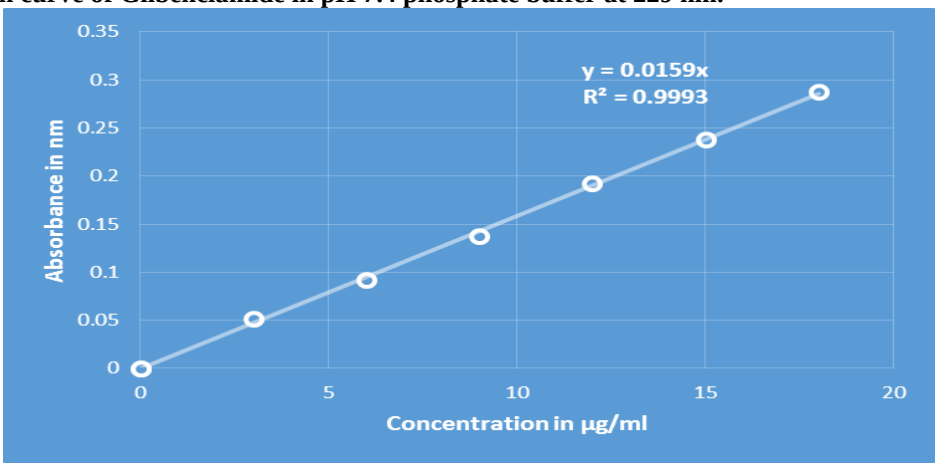


Figure 5. Drug excipients compatibility studies - FTIR studies of (A) Glibenclamide and (B) Optimized Glibenclamide SLN.

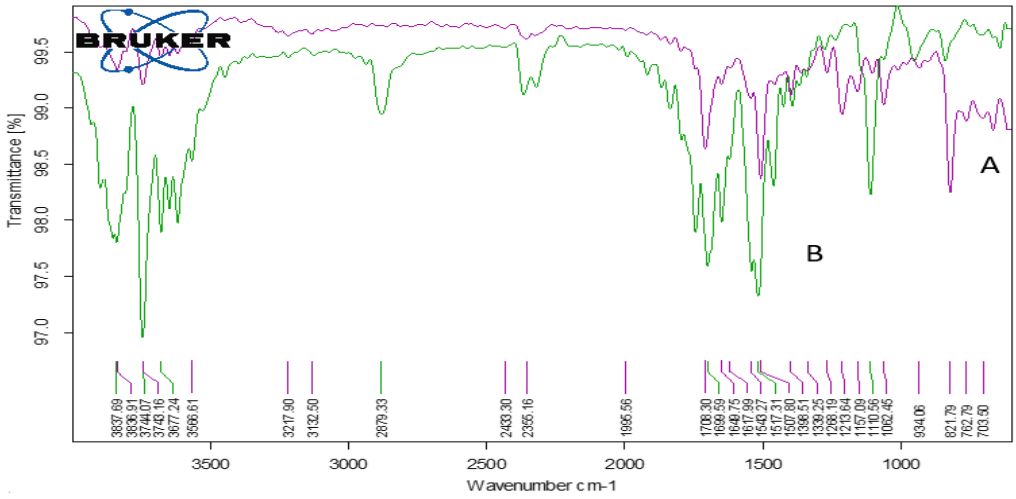


Figure 6. DSC Thermogram for Glibenclamide and Glibenclamide Solid Lipid Nanoparticle

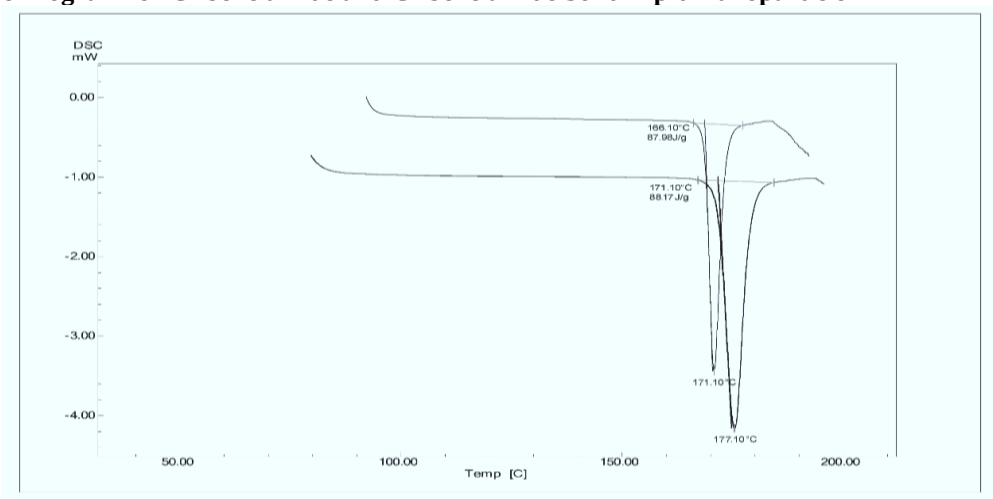
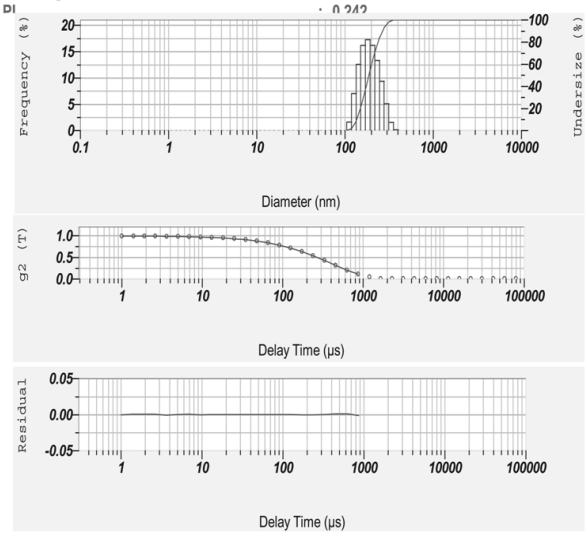


Figure 7. Glibenclamide GSLN 7 (A) Particle Size & Polydispersity index report; (B) Zeta potential report.

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	195.1 nm	49.9 nm	182.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	195.1 nm	49.9 nm	182.0 nm

Cumulant Operations

Z-Average : 180.0 nm



Peak No.	Zeta Potential	Electrophoretic Mobility
1	-33.4 mV	-0.000258 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -33.4 mV

Electrophoretic Mobility Mean : -0.000258 cm^2/Vs

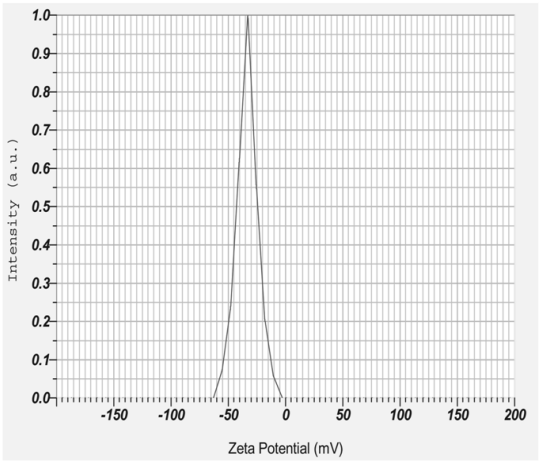


Figure 8. SEM studies of optimized Glibenclamide SLN.

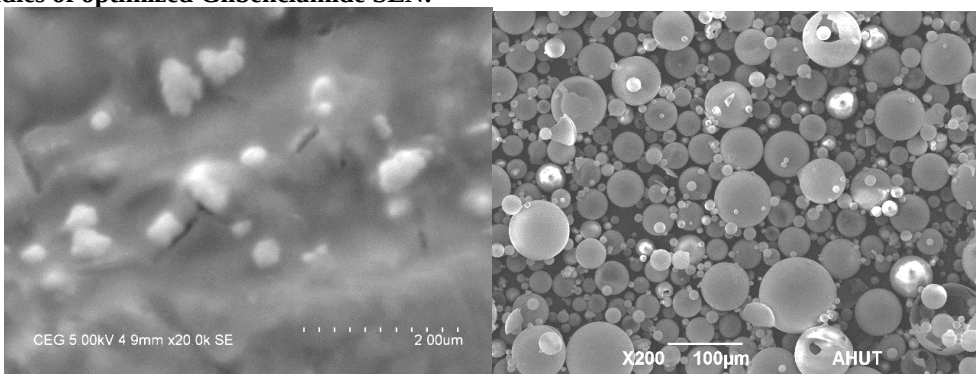
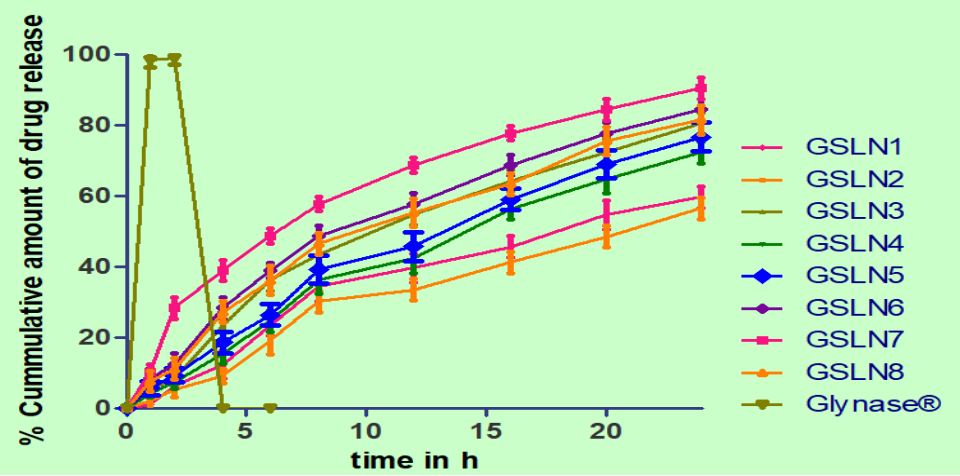


Figure 9. Comparative In-vitro drug release studies between Solid Lipid Nanoparticle vs. marketed Glynase ® tablet (mean ± SD, n=3).



Discussion:

The range of concentrations of 3–18 µg/ml (as per Beer's law) as we generated a calibration curve using pH 7.4 phosphate buffer at 229 nm. The serial dilution are prepared from the stock solution by serial dilutions such as 3, 6, 9, 12, 15 and 18 µg/ml concentration. The correlation and linearity are outstanding, and the r^2 value is 0.9993 in pH 7.4 phosphate buffer with levels ranging from 3 to 18 µg/ml.

Drug excipients compatibility studies - FTIR studies

On comparing pure Glibenclamide to the data collected from FTIR spectra, as shown in Figure 5 it was determined that the appropriate frequencies of fingerprint regions were replicable in a Glibenclamide SLN. It was determined that the drug and excipients included in the formulations were compatible with one another.

Drug excipients compatibility studies - DSC studies

As endothermic peak values in a DSC thermogram, the relevant melting points were observed: Glibenclamide at 166.10 degrees Celsius; Glibenclamide Solid lipid Nanoparticle at 177.10 degrees Celsius.

Particle size

Figure7, show the average particle sizes for all formulations. Based on the impact of the independent variable in the formulation process, particle sizes for all Glibenclamide SLNs formulations were determined to be in the range of 195.1 ± 2.66 to 695.8 ± 3.26nm.

Zeta potential

Figure7, show the zeta potential for all of the formulations. The zeta potential of all Glibenclamide SLNs was determined to be in the range of -16.5 ± 1.72 to -54.0 ± 1.26, owing to the influence of surfactant during the formulation process.

Polydispersity index

The polydispersity index for all formulations. The polydispersity index for Glibenclamide SLNs was reported to be between 0.242 ± 0.20 to 0.788 ± 0.24, owing to the effect of homogenization speed or ultrasonication time in the formulation process. However, for monodisperse nanoparticles, the PI acceptance requirement should be less than 0.7. The formulations GSLN7 and GSLN8, have good polydispersity index 0.242 ± 0.20 and 0.265 ± 0.22 respectively, according to the acceptance requirements. The other formulations were discovered to have a value larger than 0.5.

Optimization of Solid lipid Nanoparticle

The results of independent variables on dependent variables on Glibenclamide SLNs were shown by the 2^3 optimization design based on the foregoing data, it was determined that there was a strong link among particle size and polymer concentration, i.e., increasing the polymer concentration increased the particle size of SLNs. At low -1 level polymer, GSLN7 formulation showed required particle size of around 195.1 ± 2.66nm between all formulations (GSLN1-GSLN8) (5 mg). The reduction in particle size was achieved by combining a low polymer content with a high homogenization rpm and ultra-sonication period. Particle size reduction was also achieved as a result of increased homogenization speed and ultrasonication time, which separated large particles and particle aggregates into small dispersed particles, resulting in particle size reduction.

Percentage entrapment efficiency and percentage yield

For Solid lipid Nanoparticles, the required percentage entrapment efficiency and yield should be greater than 85%. The effectiveness of entrapment was found to be 64.42 ± 3.44 percent to 96.45 ± 2.42 percent, and the percent yield was found to be 56.82 ± 2.84 to 94.28 ± 2.56 percent, according to the results provided in table 5.3. G7 displays the estimated amount of percentage entrapment efficiency and percentage yield by comparing all of the formulations.

Invitro drug release and invitro release kinetics studies

For the GSLN7 optimised formulation, a percentage quantity of drug release experiments was conducted. *In-vitro* drug release studies for the Glibenclamide SLNs (GSLN7) formulation revealed a better-controlled drug release of 90.42 ±3.018 % in 24 hours when compared to the marketed available Glibenclamide tablet dosage form Glynase® tablet 20mg formulation. In 24 hours, the percentage amount of drug released by GSLN7 was discovered to be 90.42±3.018%. Zero order, first order, Higuchi model, Hixson crowell model, and Korsmeyer Peppas model regression values (r^2) were discovered to be 0.982 ± 0.02, 0. 996 ± 0.02, 0.988 ± 0.14, 0.868 ± 0.12 and 0.989 ± 0.08. The zero-order release kinetic model was used in the in-vitro release kinetics experiments of Glibenclamide Solid Lipid Nanoparticle (GSLN7), and the regression values (r^2) were determined to be 0.982 ± 0.02, indicating good linearity. The drug was delivered in a predefined and controlled manner from Glibenclamide loaded SLNs (GSLN7), which matched zero order kinetics. It was validated as the best model for releasing the medicine in order to achieve the desired therapeutic effect without causing any side effects. Higuchi's release kinetic pattern had an r^2 of 0.996, indicating that the medication was released by diffusion. It meant that drug release from SLNs was governed by a non-fickian diffusion process, in which the drug was discharged from the polymer by polymer relaxation and diffusion.

Stability studies

The shows the comparative stability study data for GSLN7 before and after conducting stability experiments. GSLN7's PS nm, ZP mV, and %EE during preparation were 195.1 ± 2.66 nm, -33.4 ± 2.32mV, 87.24 ± 3.76% and GSLN7 after performing stability investigations, i.e. after 6 months of storage at 4˚ ± 2˚C, was PS nm was found to be 197.6 ± 3.68 nm, ZP was found to be -33.8 ± 2.56mV, EE was found to be 86.98 ± 3.46%. The stability data of optimised Solid lipid Nanoparticles (GSLN7) are tested for short-term stability at 4˚C±2˚C for 6 months. At three-month intervals, the parameters were assessed.

CONCLUSION

In conclusion, the design and optimization of glibenclamide-loaded solid lipid nanoparticles (SLNs) present a promising approach for the development of an efficient antidiabetic drug delivery system. The use of SLNs offers several advantages, including enhanced drug stability, prolonged release, improved bioavailability, and targeted delivery. Overall, the successful design and optimization of glibenclamide-loaded SLNs hold great promise for the treatment of diabetes mellitus. Further research and development in this field can pave the way for the commercialization of this innovative drug delivery system, benefiting diabetic patients worldwide.

REFERENCES

1. Basu, B., K. Garala, R. Bhalodia, B. Joshi, K. Mehta, Solid lipid nanoparticles: A promising tool for drug delivery system. Journal of Pharmacy Research, 2010. 3(1): p. 84-92.
2. Muller, R.H., K. Mäder, and S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. European journal of pharmaceutics and biopharmaceutics, 2000. 50(1): p. 161-177.
3. Kreuter, J., Colloidal drug delivery systems. Vol. 66. 1994: CRC.
4. Bhattacharya, R. and P. Mukherjee, Biological properties of. Advanced drug delivery reviews, 2008. 60(11): p. 1289-1306.
5. Lockman, P.R., et al., Brain uptake of thiamine-coated nanoparticles. Journal of controlled release, 2003. 93(3): p. 271-282.
6. Mehnert, W. and K. Mäder, Solid lipid nanoparticles: Production, characterization and applications. Advanced drug delivery reviews, 2001. 47(2-3): p. 165-196.
7. Gohla, S. and A. Dingler, Scaling up feasibility of the production of solid lipid nanoparticles (SLN). Die Pharmazie, 2001. 56(1): p. 61.
8. Domb, A.J. and M. Maniar, LIPOSPHERES FOR CONTROLLED DELIVERY OF SUBSTANCES. 1996, EP Patent 0,502,119.
9. Lucks, S. and R. Müller, Medication vehicles made of solid lipid particles (solid lipid nanospheres-SLN). 1999, EP Patent 0,605,497.
10. Zur Mühlen, A., C. Schwarz, and W. Mehnert, Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism. European journal of pharmaceutics and biopharmaceutics, 1998. 45(2): p. 149-155.
11. Quintanar-Guerrero, D., et al., Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. International journal of pharmaceutics, 1999. 188(2): p. 155-164.
12. Trotta, M., F. Debernardi, and O. Caputo, Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique. International journal of pharmaceutics, 2003. 257(1-2): p. 153-160.
13. Souza LG, Silva EJ, Martins AL, Mota MF, Braga RC, Lima EM, Valadares MC, Taveira SF, Marreto RN. Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. Eur J Pharm Biopharm 2011; 79 (1):189-96.
14. Silva AC, Gonzalez-Mira E, Garcia ML, Egea MA, Fonseca J, Silva R, Santos D, Souto DB, Ferreira D. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. Colloids Surf B Biointerfaces. 2011; 86 (1):158-65.

15. Bunjes H, Unruh T. Characterization of lipid nanoparticles by differential scanning calorimetry, X-ray and neutron scattering. Adv Drug Deliv Rev 2007;59 (6):379-402.
16. Shaikh MV, Kala M, Nivsarkar M. Formulation and optimization of doxorubicin loaded polymeric nanoparticles using Box-Behnken design: ex-vivo stability and in-vitro activity. European Journal of Pharmaceutical Sciences 2017; 100: 262-272.
17. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int J Pharm 2008; 346 (1-2):124-32.
18. Mukherjee S, Ray S, Thakur RS. Design and evaluation of itraconazole loaded solid lipid nanoparticulate system for improving the antifungal therapy. Pak J Pharm Sci 2009; 22(2):131-8.