RESEARCH ARTICLE

Design and Optimization of Solid Lipid Nanoparticles for Enhanced Therapeutic Activity of Linagliptin

Swathi Yambadi*, Vasudha Bakshi

School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal-Malkajgiri, Hyderabad, Telangana, India.

Received: 15th March, 2024; Revised: 23rd May, 2024; Accepted: 13th August, 2024; Available Online: 25th September, 2024

ABSTRACT

To augment oral bioavailability, linagliptin (LGP) loaded solid lipid nanoparticles (SLNs) have been formed using the solvent evaporation technique of emulsification. Utilizing the Box Behken design (BBD), examining the relation between independent and dependent variables was possible. Particle size (PS), entrapment efficiency (EE), and *in-vitro* drug release (DR) studies were considered response factors, whereas the various concentrations of lipid carrier, surfactant, and co-surfactant were considered process variables for optimization. The design expert program was used to obtain the optimized batch. At 24 hours, it was discovered that the optimized batch expected responses for PS, %EE, and %DR were 105.83 nm, 81.19, and 96.69%, respectively. The observed PS, %EE, and %DR responses of the optimized batch at 24 hours were determined to be 101.36 nm, 80.56, and 96.31%, respectively. There is no interaction seen in the basic physical mixing of drug and polymer, according to the FTIR spectra of pure drugs, polymers, and drug and polymer mixtures. The optimized formulation's average size was found to be 138 nm by the particle size analysis. Similarly, a value of 0.255 was found by the polydispersity index (PDI). This suggests that the SLN in the formulation has a homogeneous size dispersion. With a zeta value of -32.8 mV, stability is indicated. Linagliptin was effectively developed as a drug with a 24-hour sustained release. Wistar rats were used in the *in-vivo* evaluation of linagliptin SLN formulations. The drug bioavailability increased five times (a highly significant fivefold increase) in LGP-loaded SLNs ($AUC_{0-t} \approx 134.5$ µg*h/mL) compared to raw LGP ($AUC_{0-t} = 27.23$ µg*h/mL).

Keywords: Linagliptin, Solid lipid nanoparticles, Box Behken design, Optimization.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.59

How to cite this article: Yambadi S, Bakshi V. Design and Optimization of Solid Lipid Nanoparticles for Enhanced Therapeutic Activity of Linagliptin. International Journal of Drug Delivery Technology. 2024;14(3):1668-1675.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

A chronic metabolic condition with high blood sugar levels is called diabetes mellitus [1-2]. Diabetes affects 425 million people globally at the moment, and by 2040, it is predicted that the number of cases and prevalence of this lifestyle disease will reach 642 million. Many kinds of oral anti-diabetic drugs have been used as treatment alternatives.[3] Many of the current treatments have drawbacks, including the potential for GI side effects, the risk of hypoglycemia and weight gain, or the fact that in individuals with modest to severe renal impairment, they should not be administered. Scientists studying pharmaceuticals are currently working very hard to discover more innovative approaches to managing diabetes. Dipeptidyl-peptidase (DPP)-4 inhibitors are recommended as one of the top treatment choices for diabetes management by the American Diabetes Association. As a result, they are increasingly being used in clinical practice.

The US Food and Drug Administration (FDA) authorized LGP, one of these strong DPP-4 inhibitors, in 2011 for the management of type II diabetes mellitus.[4-6] The drug has a low oral bioavailability of 29.5% because of P-gp efflux, inadequate penetration, and first-pass metabolism. A few researchers have tried to address some of the issues related to LGP's bioavailability. Due to their associated benefits, SLNs have drawn more attention as novel nanoparticulate drug carriers.[7,9] When taken orally, SLNs demonstrate lymphatic drug transport as a primary drug absorption mechanism. The drug's increased bioavailability may allow for a decrease in negative effects as well as a reduction in dosage and administration frequency.[10-12] This work aimed to create LGP-SLNs using the emulsification solvent evaporation (ESE) Process. Particle size, zeta potential, PDI, entrapment efficiency, and *in-vitro* drug release experiments were assessed for the optimized SLNs. The

*Author for Correspondence: deanpharmacy@anurag.edu.in

in-vivo evaluation of linagliptin SLN optimized formulation was carried out using Wistar rats.

MATERIALS AND METHODS

Materials

Linagliptin was purchased from Honour Lab Ltd, Sangareddy, India. Glyceryl monostearate (GMS), stearic acid (STA), palmitic acid (PMA), and polysorbate 20,40,60,80 were acquired from S.D. Fine Chem Ltd., Mumbai. Compritol 888 ATO and Precirol ATO 5 from Gattefosse India Pvt. Ltd, Mumbai. Poloxamer 188 from Sigma Aldrich.

Methods

Selection of lipid

Lipids are selected and screened via solubility experiments. Using lipids such as GMS, glyceryl behenate, STA, PMA, and precious ATO 5, the solubility of the drugs was evaluated. A screw-capped vial containing 20 mg of linagliptin was given out.[13] Every lipid was heated above its melting point separately. Using a vertex mixer, the melted lipids were gradually added to vials containing the drug and vigorously shaken while maintaining a consistent temperature. The endpoint of solubility is shown by a clear, yellowish fluid of melting fat. It was proposed that the lipid with the highest drug adsorbing potential was the one in which the drug was most soluble and was used for additional research.

Selection of surfactant

After SLNs are prepared using various surfactants and specific lipids, PS and %EE are computed. The PS was analyzed using Zetasizer Nano-ZS.[14] The surfactant with the maximum percentage of EE and the least amount of PS is chosen.

Preparation of LGP-SLN by emulsification solvent evaporation method

Linagliptin and lipids were dissolved in a solution of ethanol and chloroform. Dropwise, additions of the aforementioned lipid phase, were made to a solution containing dissolved surfactant and co-surfactant. Using a sonicator, the resultant pre-emulsion was then ultrasonicated to reduce the globular diameters to the desired nanometric scale.[15,16] The generated mixture was mechanically stirred at room temperature at a speed of around 400 revolutions per minute to encourage the evaporation of the lipid medium and the subsequent creation of SLNs.

Design & optimization of process and formulation variables with box-behnken design

A set of statistical and mathematical tools called a design of experiment (DoE) approach is used to identify the optimal formulation. Various approaches to experimental design are used, leading to a statistical recommendation for the best course of action for each variable under investigation. SLNs containing model pharmaceuticals were optimized by the use of BBD (Design-Expert 12 program, Minneapolis, USA; Stat-Ease Inc.). Three dependent responses, PS (Y1) and %EE (Y2), and drug release at 24 hours (Y3), are chosen, together with three independent parameters, solid lipid (X1), surfactant (X2), and co-surfactant (X3). Using the above data in BBD software, 15 experimental runs with three center points low, medium, and high, were shown in Table 1. The polynomial equation fit was utilized to evaluate how the independent variables affected the examined responses.

The best-fitting model is identified by comparing the adjusted R^2 value with the expected R^2. Less than 0.05 was the *p-value* criterion for statistical significance. A mathematical optimization method based on a desired model was utilized to discover the optimal SLNs with the lowest PS, highest %EE, and maximal drug release at 24 hours.

In-vitro drug release study

The dialysis membrane bag was then filled with SLN dispersion, which is equal to 5 mg of linagliptin drug, tied at both ends and stored in a beaker with 100 ml of phosphate buffer solution pH 7.4 with 0.5% v/v Tween 80. Using a magnetic stirrer, the temperature and speed were maintained at 37 ± 0.5°C and 100 rpm, individually. The concentration of linagliptin determined with a UV spectrophotometer was used to calculate the amount of linagliptin released.

Characterization

Fourier transform infrared (FTIR) spectroscopic analysis

FTIR (Perkin Elmer, India) was used to analyze the interactions between the formulation ingredients to detect compatibility. Pellets were formed using the hydraulic press method after a sample was introduced to potassium bromide (KBr). A functional grouping of the sample was determined and plotted on a graph. Samples of LGP, GMS, Tween 80, and LGP-loaded SLNs were examined individually and analyzed by FTIR spectra in the 5000 to 500 cm^{-1} range.

Determination of particle size, polydispersity index (PDI), and zeta potential (ZP)

Malvern Zetasizer, ATA Scientific, USA, was used to measure ZP, PDI, and average particle size.

Scanning electron microscope (SEM) study

SEM was used to examine the topography of optimized formulations. To prepare the drug samples for the SEM study, a double-sided carbon tape is secured with an aluminum (Al)

Table 1: Box-Behnken design for linagliptin loaded SLN

Independent variables	*Coded points*		
	Low	*Medium*	*High*
X1= Lipid (mg)	10 (-1)	20 (0)	30 (+1)
X2=Surfactant (mL)	5 (-1)	10 (0)	15 (+1)
X3=Co-surfactant (mL)	3 (-1)	5 (0)	7 (+1)
Dependent variables			
Y1=PS	Minimize		
Y2=EE%	Maximize		
Y3=Drug release at 24 hours	Maximize		

stub that has been gold-coated (200–500Å thick) using a gold sputtering unit in a rotavap while being exposed to an argon environment. The SLN dispersion is then gently sprinkled onto the tape. Following coating, the material was examined using a Quanta 200 E SEM.

In-vivo testing for the optimum SLN formulation

By assessing the pharmacokinetic parameters following a single oral dose, the *in-vivo* test is used to further assess the ideal SLN formulation.

Animals

Male adult Wistar rats were given by the Jeeva Life Sciences animal facility and housed in the animal house. The regular chow diet, tap water, and ambient temperature were provided for the rats. The Institutional Animal Care and Use Committee's criteria (Project no. CPCSEA/IAEC/JLS/21/04/24/025) were followed when conducting the experiments.

Pharmacokinetics Study Protocol

Experimental design

Male healthy Wistar rats of 160 to 190 g are available at all times during their eighteen-hour fast. In a parallel design, ten rats were split into two groups of five rats each. A lyophilized formula (OPT-LGP-SLN) was given to group 1 and raw linagliptin powder was given to group 2, both at a dose equivalent to 10 mg/kg of linagliptin .

To avoid the serious hypoglycemia that could arise from taking linagliptin, before being ingested, the dosages were split up into 2 mL of distilled water and vortexed for 10 seconds, but no more than 60 seconds. 1-mL of a glucose solution at a dose of 1-µg/kg was given. Oral gavage was utilized to provide the doses. The animals were given free access to water during the experiment. Before allowing the animals to eat, food was withheld for the first 6 hours of the experiment (until the 6 hours sample was withheld). At 2, 4, 6, and 24 hours after the pre-dose, each rat had a 0.5 mL blood sample drawn into an EDTA tube via retroorbital vein puncture.

Pharmacokinetic parameters

Using the Microsoft Excel add-in PKSolver, which evaluates pharmacokinetic and pharmacodynamic data. The linear trapezoidal approach was used to calculate the area under the plasma concentration-time profile ($AUC_{0\text{-}inf}$ and $AUC_{0\text{-}24}$) .

Stability study

The optimal SLN formulation underwent stability tests for six months, during which it was kept at two different temperatures: 5 ± 2°C for refrigeration and 25 ± 2°C for ambient temperature (60 ± 5% RH). Changes in the percentage of EE and *in-vitro* drug release were monitored at regular intervals .

RESULTS AND DISCUSSION

Selection of Lipid

LGP had the maximum solubility of 115 mg/g in GMS out of all the lipids used. Therefore, GMS was chosen as the lipid phase.

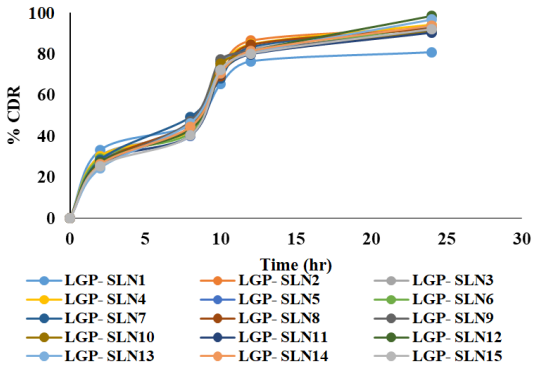

Figure 1: *In-vitro* cumulative drug release (CDR) of all Formulations (F1-F15)

Selection of Surfactant

Tween 80 was discovered to provide the smallest particle size (252 nm) and the highest entrapment effectiveness (67.32%).

***In-vitro* Drug Release**

When drug molecules are released from SLNs, there is often a rapid discharge of the encapsulated LGP after a phase of prolonged drug release. Phase separation caused by lipid crystallization may have contributed to the initial burst of LGP release by accumulating the drug in the outer shell, causing fast drug release from the particle surface. On the other hand, the drug's diffusion, phase partitioning, or matrix degradation causes the LGP molecules trapped in the center of the NPs to gradually release, which is when the controlled release starts, as shown in Figure 1.

Optimization of Process and Formulation Variables

The design displayed 15 experimental runs with three center points. as depicted in Table 2. To assess the impact of the independent variables on the responses that were examined, a polynomial equation that is appropriate for every response was developed using the best-fitting model. Less than 0.05 was the P-value criterion for statistical significance.

For ascertaining the relevance and extent of the influences of variables and associated connections,an ANOVA was used.

Independent Variables' Effect on Particle Size

Figure 2 illustrates the effect of independent variables on particle size. As the temperature at which lipids melt is elevated, particle size tends to increase. The particles' increased viscosity brings on this phenomenon. By dissolving melting lipid droplets, Surfactants prevent the formation of aggregates and produce smaller particles and a more stable dispersion.

Higher concentrations of surfactant and co-surfactant resulted in larger particle sizes. One argument is that supersaturation at the interface may result from an excess of surfactant, which is caused by the diffusion process.

Influence of Independent Variables on %EE

The encapsulation efficiency of the various batches varied from 53.56 to 84.30%. EE rises in response to an increase

Table 2: Experimented responses for 15 Runs of LGP- SLNs using BBD

Formulation code	*Drug (mg)*	*Lipid (X1)*	*Surfactant (X2)*	*Co-surfactant (X3)*	*Particle size (nm)*	*EE%*	*DR% at 24 hr*
LGP-SLN1	5	10	5	5	107.48	71.83	80.81
LGP -SLN2	5	10	10	3	125.60	76.21	92.53
LGP -SLN3	5	10	10	7	135.83	78.59	93.16
LGP -SLN4	5	10	15	5	98.64	53.56	94.21
LGP -SLN5	5	20	5	3	144.85	79.65	90.53
LGP -SLN6	5	20	5	7	239.48	80.41	93.48
LGP -SLN7	5	20	10	7	220.16	72.46	92.68
LGP -SLN8	5	20	10	5	212.73	65.49	92.31
LGP -SLN9	5	20	10	3	184.67	63.65	91.47
LGP -SLN10	5	20	15	3	175.21	61.48	90.73
LGP -SLN11	5	20	15	7	163.38	58.89	90.32
LGP -SLN12	5	30	5	5	254.98	84.40	98.45
LGP -SLN13	5	30	10	3	241.79	83.65	96.68
LGP- SLN14	5	30	10	7	132.76	82.21	93.73
LGP -SLN15	5	30	15	5	116.92	80.65	92.06

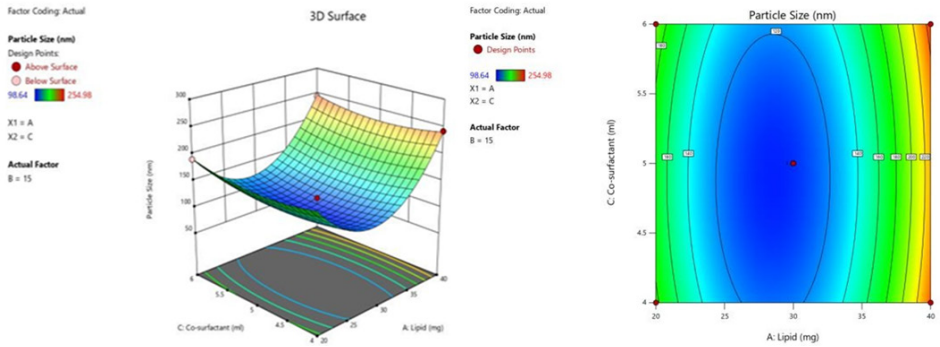

Figure 2: Response surface plots & countor plot displaying the impact of the independent variable on particle size

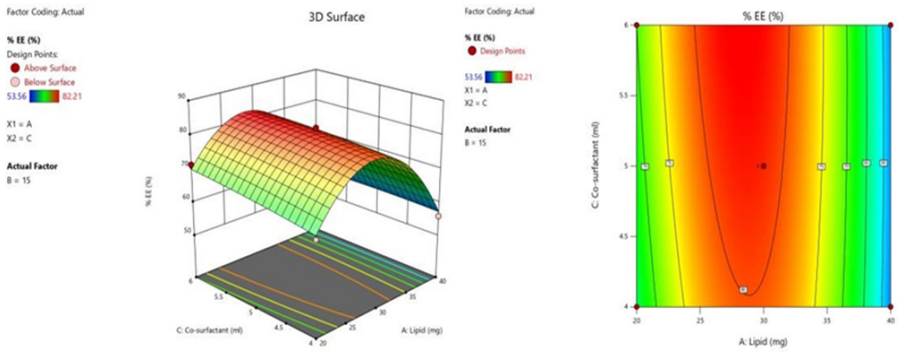

Figure 3: Response Surface Plots & Countor plot displaying the impact of the independent variable on EE%

in lipid content. Because there was more room for drugs in the lipid matrix, the %EE went up. Surfactant displayed a biphasic nature on %EE. The drug raised %EE via increasing solubility and decreasing drug partition in the aqueous phase. As the concentration was raised further, micelles formed, the drug's solubility in the aqueous phase system increased, and the percentage of EE decreased as shown in Figure 3.

Influence of independent variables on Drug Release

It was demonstrated that the percentage of surfactant concentration increases with a rise in phospholipid concentration. The variation in % CDR and the change in % EE had similar patterns. The relationship between surfactant concentration and % CDR was inverse at 24 hours. The drug release may have decreased as a result of a rise in lipid

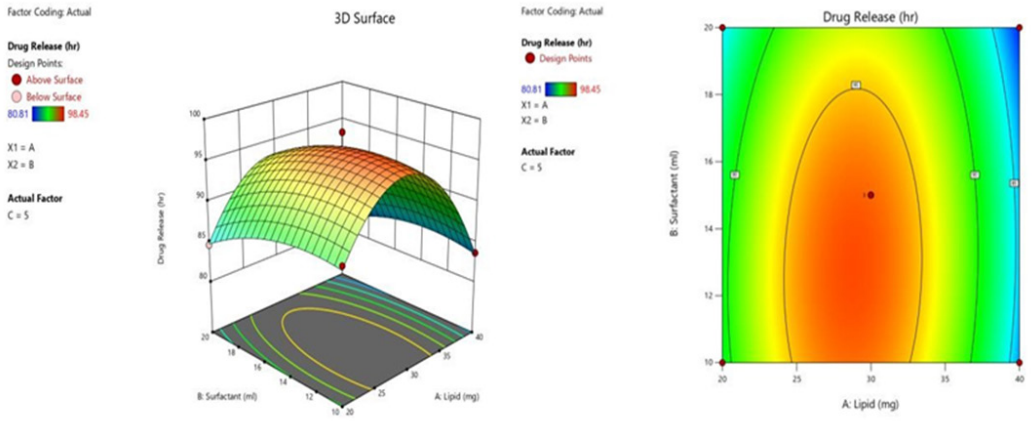

Figure 4: Response Surface Plots & Countor plot displaying the impact of the independent variable on DR% at 24 hr

concentration, which produced larger nanoparticles with a reduced surface area for interaction with the release medium as shown in Figure 4.

Optimization of the Formulation

The forecasted overlay plot, depicted in Figure 5, displays the optimized batch with optimal values that were obtained using design expert software. To validate the optimized procedure, the optimized batch was prepared using the values obtained for the concentration of lipid, surfactant, and co-surfactant (30 mg, 15 ml, and 5 ml). For research purposes, the optimized batch was further characterized as shown in Table 3.

The FTIR spectra of LGP show the following absorption bands: 3421 cm^{-1} (NH2 stretching), 2935 and 2854 cm^{-1} (aliphatic C-H stretching), 1687 cm^{-1} (C=O stretching), 1579 cm^{-1} (C=N stretching), 1296 cm^{-1} (C-N stretching), and 769 cm^{-1} (C≡C bending) .

The spectrum indicates no interaction between the drug and the improved formulation depicted in Figures 6 and 7, respectively.

Particle Size of Optimized Formulation

The optimized formulation's average size was 138 nm by the particle size analysis. Similarly, a value of 0.255 was found by the PDI. According to published research, homogeneous dispersion is indicated by a PDI value of less than 0.5. This suggests that the SLN in the formulation has a homogeneous size dispersion, as shown in Figure 8.

As previously mentioned in the literature review, zeta values greater than ± 30 mv are stable and can be distributed without causing stability problems. The zeta value in our investigation was -32.8 mV, is depicted in Figure 9, which denotes stability.

Surface Morphology of Optimized Formulation

The optimized linagliptin-loaded SLN's morphology was examined using a scanning electron microscope. A glass slide was placed over the sample once it had been spread out over the slab surface, and photomicrographs were taken at various magnifications.

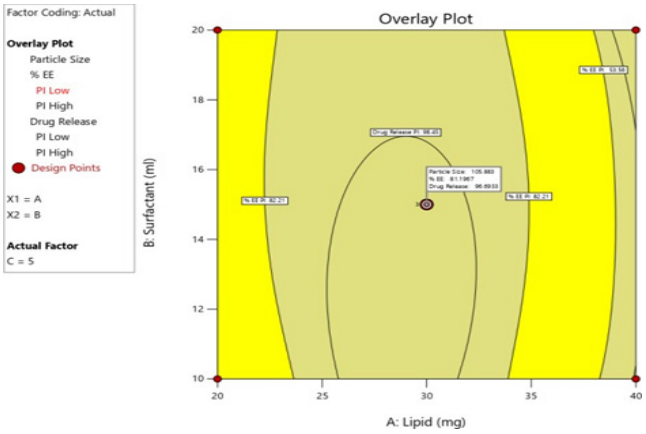

Figure 5: Point of confirmation for optimized formulation (OPT-LGP-SLN)

Table 3: Point of confirmation for optimized formulation (OPT-LGP-SLN)

Response variable	*Predicted values*	*Observed values*
Particle size (nm)	105.83	101.36
Entrapment Efficiency (%)	81.19	80.56
Drug Release (hr) (%)	96.69	96.31

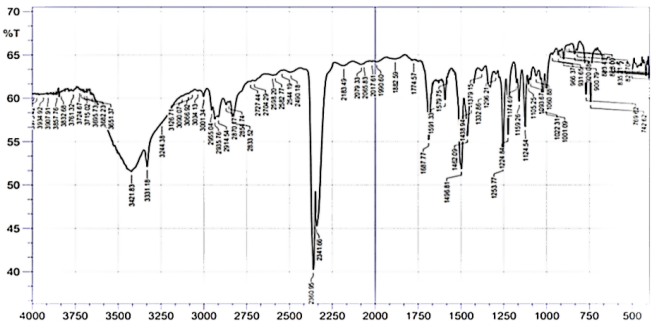

Figure 6: FTIR spectrum of linagliptin

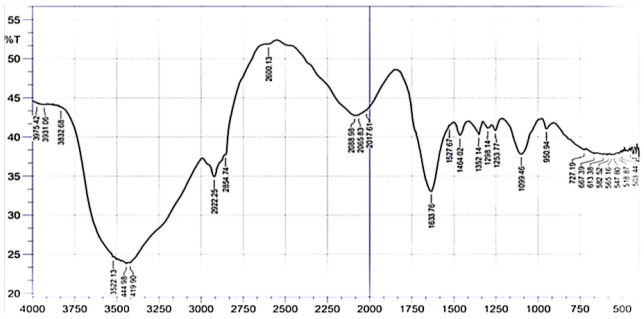

Figure 7: FTIR spectra of optimized formulation

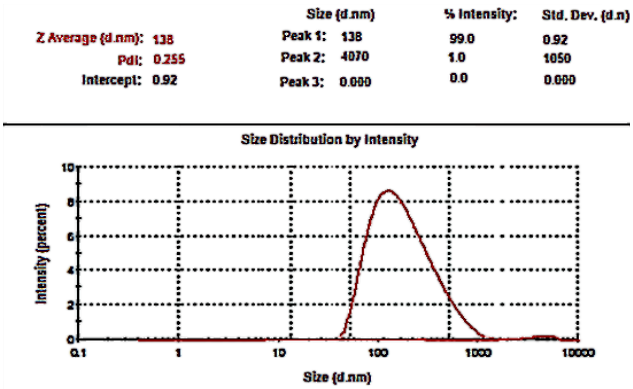

Figure 8: Particle size distribution of the optimized formulation

The SEM investigation revealed a dispersed distribution of SLN. Additionally, the study demonstrated that the surface and looks of optimized SLN are symmetrical. Figure 10 (resolution of 2.0 µm) shows that the optimized formulation's particle size is uniform.

Drug Release Profile

Using the dialysis-bag method, the SLN dispersion's *in-vitro* drug diffusion profile was obtained and compared to a simple drug dispersion. Compared to the release from simple drug dispersion, the SLN dispersion's drug release was observed to be more consistent, as depicted in Figure 11. Additional investigations revealed a persistent discharge after the first burst emission. With SLN dispersion, 96% drug release was seen in 24 hours, and 95% with a simple drug suspension in 24 hours.

Pharmacokinetics Study

Figure 12 displays a plot of the mean plasma drug concentration-time profile. The highest plasma concentration increased by approximately 5.5 times, suggesting a significant rise in drug absorption following oral delivery of SLNs loaded with LGP. When LGP-loaded SLNs were administered instead of raw LGP powder, statistical analysis revealed a highly substantial ($p < 0.001$) increase in Cmax.

AUC_{0-t} and $AUC_{0-\infty}$ are used to quantify the overall amount of drug-exposed and are regarded as bioavailability measurements. The drug bioavailability increased five times (a highly significant fivefold increase) in LGP-loaded SLNs ($AUC_{0-t} \approx 134.5$ µg*h/mL) compared to raw LGP ($AUC_{0-t} \approx$ 27.23 µg*h/mL). In comparison to raw LGP powder, LGP-loaded SLNs effectively increased the oral bioavailability of LGP by almost five times, as can be shown from the earlier data. There are several additional reasons why bioavailability may be rising. The primary factors that significantly enhanced the drug's absorption are the nanoscale size of the particles, the drug molecules' nearly molecular dispersion into the lipid carrier, and the presence of surfactants. Another explanation

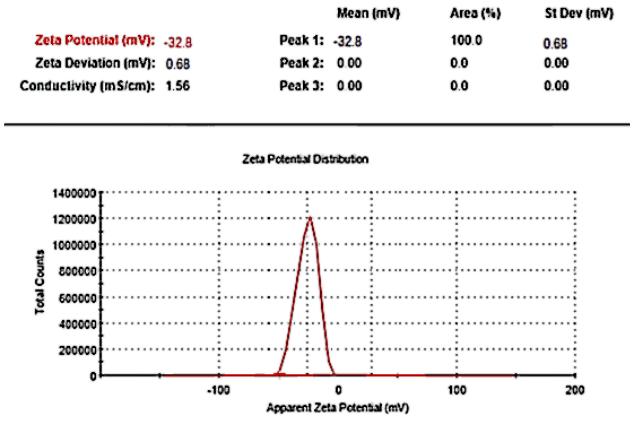

Figure 9: Optimized formulation's zeta potential

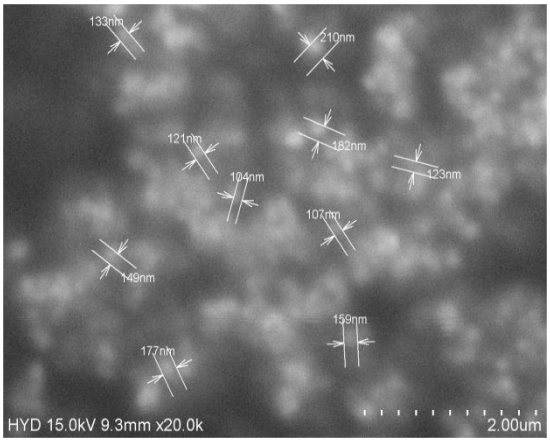

Figure 10: SEM study of optimized formulation

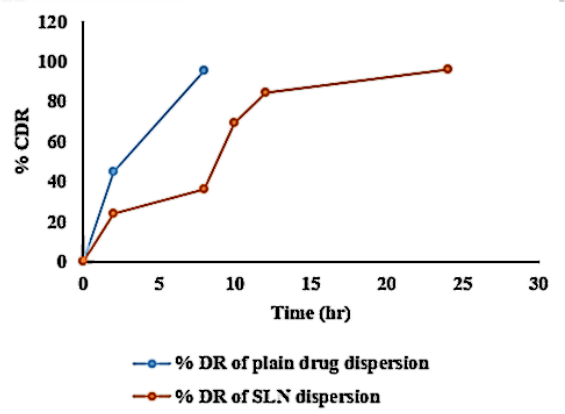

Figure 11: Comparison of drug release profile of plain drug dispersion and SLN dispersion

Table 4: Pharmacokinetic characteristics of OPT-LGP-SLN and LGP powder

Parameter	*LGP powder*	*OPT-LGP-SLN*
C_{max} (µg/mL)	3.40 ± 0.025	17.60 ± 0.035
T_{max} (hour)	1.13 ± 0.02	3.85 ± 0.025
AUC $_{0\text{-}24h}$ (µg*h/mL)	27.23 ± 0.036	134.53 ± 1.47
AUC $_{0\text{-}\infty}$ (µg*h/mL)	24.21± 1.86	136.01 ±1.49
Total clearance (L/h)	0.401± 0.002	0.072 ± 0.001

Data are expressed as Mean ±SD

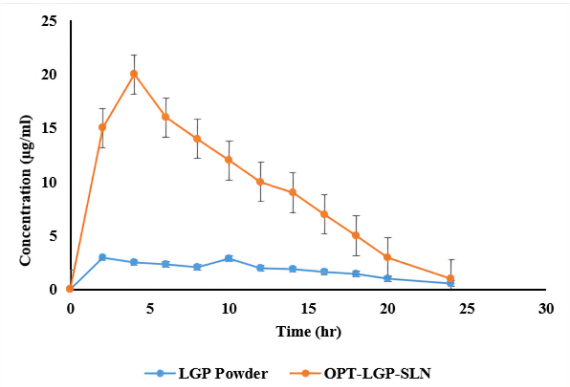

Figure 12: Mean plasma concentration-time profiles resulting in oral treatment at a dose equal to 10 mg/kg

proposed that SLNs' small particle size could allow them to infiltrate inter-villar gaps or show bio-adhesion to the gastrointestinal system wall, extending their stay there. Better bioavailability would follow from this greater adherence.

T_{max}, or time to achieve maximum plasma concentration, is a metric used to determine how quickly drugs are absorbed. The absorption of raw LGP powder varied greatly amongst subjects, with T_{max} values ranging from a half-hour to four hours. Its early breakdown in the stomach, which causes greater inconsistency in the absorption in the intestine, may be T_{max} the cause of this previously documented behavior. T_{max} of LGP-loaded SLNs and raw LGP powder were compared using statistical analysis. Compared to raw LGP powder (t_{max} = 1.1 h), LGP-loaded SLNs displayed a prolonged release with $t_{max} \approx$ 3.8 h, a significant difference ($P < 0.05$). Prolonging the drug's release was suggested by increasing t_{max} in the SLNs formula. After taking the drug orally as nanoparticles, the apparent total clearance from plasma was 0.072 L/h for linagliptin, compared to 0.401 L/h for raw powder. Consequently, after receiving nanoparticles, the body's overall linagliptin clearance dropped by a factor of 5.5. This prolonged circulation time and reduced drug clearance were caused by the drug's gradual release pattern, which was corroborated by these results from the produced nanoparticles, as illustrated in Figure 12.

Stability Study of Optimized Formulation

Table 5 displays the findings of a 6-month stability study conducted on the optimized formulation at room temperature as well as under refrigeration. Throughout the duration, the formulation was suitable.

Table 5: Stability studies of Optimized formulation

Time (Months)	*EE (%)*	*In-vitro drug release (%)*
Refrigeration temperature (i.e., 5 ± 2 °C)		
Initial	85.42	96.31
1	85.21	96.12
3	85.10	95.78
6	84.68	95.62
Ambient temp. (25 ± 2 °C/ 60 ± 5% RH)		
Initial	85.42	96.31
1	85.17	96.04
3	84.63	95.87
6	84.51	95.42

CONCLUSION

A DPP-4 inhibitor called linagliptin is used to treat type II diabetes mellitus. SLNs loaded with linagliptin were made using the solvent evaporation technique of emulsification and optimized by the Box Behken design. The optimized formulation's average size was 138 nm by the particle size analysis. Similarly, a value of 0.255 was found by the PDI. This suggests that the SLN in the formulation has a homogeneous size dispersion. With a zeta value of -32.8 mV, stability is indicated. The optimized formulation's homogeneous particle size was revealed by the SEM study. When compared to the release from a simple drug dispersion, the *in-vitro* drug diffusion profile of the SLN dispersion was found to be more consistent. A 6-month stability analysis of the optimized formulation at room temperature and under refrigeration produced excellent results every time. The linagliptin SLN formulations were evaluated *in-vivo* using Wistar rats. The drug bioavailability increased five times (a highly significant fivefold increase) in LGP-loaded SLNs ($AUC_{0\text{-}t} \approx$ 134.5 µg*h/mL) compared to raw LGP ($AUC_{0\text{-}t}$ = 27.23 µg*h/mL).

REFERENCES

1. Mishra V, Bansal KK, Verma A, Yadav N, Thakur S, Sudhakar K, Rosenholm JM. Solid lipid nanoparticles: Emerging colloidal nano drug delivery systems. Pharmaceutics. 2018;10(4):191. doi: 10.3390/pharmaceutics10040191. Available from: doi: 10.3390/pharmaceutics10040191.
2. Smith T, Affram K, Nottingham EL, Han B, Amissah F, Krishnan S, Trevino J, Agyare E. Application of smart solid lipid nanoparticles to enhance the efficacy of 5-fluorouracil in the treatment of colorectal cancer. Sci. Rep. 2020;12(1):16989. doi: 10.1038/s41598-020-73218-6.
3. Talarico L, Consumi M, Leone G, Tamasi G, Magnani A. Solid lipid nanoparticles produced via a coacervation method as promising carriers for controlled release of Quercetin. Molecules. 2021;26(9):2694. doi: 10.3390/molecules26092694.
4. Tan CP, Schwarz K. Development and characterization of solid lipid nanoparticles (SLNs) made of cocoa butter: A factorial design study. J. Food Eng. 2018;231:30–41. doi: 10.1016/j.jfoodeng.2018.03.006.
5. He J, Huang S, Sun X, Han L, Chang C, Zhang W, Zhong

Q. Carvacrol Loaded Solid Lipid Nanoparticles of Propylene Glycol Monopalmitate and Glyceryl Monostearate: Preparation, Characterization, and Synergistic Antimicrobial Activity. Nanomaterials. 2019;9(8):1162. doi: 10.3390/nano9081162.

6. Ban C, Jo M, Park YH, Kim JH, Han JY, Lee KW, Kweon DH, Choi YJ. Enhancing the oral bioavailability of curcumin using solid lipid nanoparticles. Food Chem. 2020;302:125328. doi: 10.1016/j.foodchem.2019.125328.
7. Borges A, De Freitas V, Mateus N, Fernandes I, Oliveira J. Solid lipid nanoparticles as carriers of natural phenolic compounds. Antioxidants. 2020;9(10):998. doi: 10.3390/antiox9100998.
8. Singh H, Singh M, Singh N, Jain H, Goel H, Chawla V. Unraveling the Therapeutic Prospects of Solid Lipid Nanoparticles for the Treatment of Parasitic Diseases. International Journal of Drug Delivery Technology. 2024;14(1):447-462. Available from: doi: 10.25258/ijddt.14.1.64.
9. Taghi HS, Abdulelah FM, Al-Karagully HJ. Solid Lipid Nanoparticle; A Potential Lipid-Based Drug Delivery System. International Journal of Drug Delivery Technology. 2021;11(2):582-586. Available from: doi: 10.25258/ijddt.11.2.61.
10. Kumar R, Singh A, Garg N. Acoustic cavitation assisted hot melt mixing technique for solid lipid nanoparticles formulation, characterization, and controlled delivery of poorly water soluble drugs. J. Drug Deliv. Sci. Technol. 2019;*54*:101277. doi: 10.1016/j.jddst.2019.101277.
11. Hassett KJ, Benenato KE, Jacquinet E, Lee A, Woods A, Yuzhakov O, Himansu S, Deterling J, Geilich BM, Ketova T. Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. Mol. Ther. Nucleic Acids. 2019;*15*:1–11. doi: 10.1016/j.omtn.2019.01.013.
12. Souto EB, Baldim I, Oliveira WP, Rao R, Yadav N, Gama FM, Mahant S. SLN, and NLC for topical, dermal, and transdermal drug delivery. Expert Opin. Drug Deliv. 2020;17(3):357–377. doi: 10.1080/17425247.2020.1727883.
13. Singh C, Yashwant, Gupta AK, Garg V. Formulation Development and Evaluation of Divalproex Sodium Extended-release Tablets. International Journal of Drug Delivery Technology. 2022;12(4):1769-1773.DOI: 10.25258/ijddt.12.4.46
14. Sgorla D, Lechanteur A, Almeida A, Sousa F, Melo E, Bunhak É, Mainardes R, Khalil, N, Cavalcanti O, Sarmento B. Development and characterization of lipid-polymeric nanoparticles for oral insulin delivery. Expert Opin. Drug Deliv. 2018;15:213–222. doi: 10.1080/17425247.2018.1420050.
15. Dudhipala N, Janga KY, Gorre T. Comparative study of nisoldipine-loaded nanostructured lipid carriers and solid lipid nanoparticles for oral delivery: Preparation, characterization, permeation, and pharmacokinetic evaluation. Artif. Cells Nanomed. Biotechnol. 2018;46(sup2):616-625. doi: 10.1080/21691401.2018.1465068.
16. Magalhães J, Chaves LL, Vieira AC, Santos SG, Pinheiro M, Reis S. Optimization of rifapentine-loaded lipid nanoparticles using a quality-by-design strategy. Pharmaceutics. 2020;12(1):75. doi: 10.3390/pharmaceutics12010075.