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Design, Optimization, And Pharmacodynamic Evaluation Of Linagliptin-Loaded Lipospheres For Improved Glycemic Control

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Abstract
The present study aimed to design, optimize, and evaluate linagliptin-loaded lipospheres for enhanced bioavailability and sustained glycemic control in type 2 diabetes mellitus. Lipospheres were prepared using stearic acid and cetyl alcohol as lipid carriers and optimized through a Box–Behnken design. The optimized formulation (F15) exhibited a particle size of 220.12 nm, high entrapment efficiency (81.15%), and a zeta potential of –36.25 mV, indicating good stability. In vivo pharmacodynamic studies were performed in streptozotocin-induced diabetic rats. Treatment with linagliptin lipospheres resulted in significant ($p<0.05$) reductions in fasting blood glucose levels and improvements in body weight compared to the diabetic control group. Moreover, lipid profile parameters such as total cholesterol, triglycerides, and LDL were markedly decreased, while HDL levels were restored. Serum biomarkers including ALT, AST, MDA, SOD, GSH, and insulin were also favorably modulated, indicating hepatoprotective and antioxidant potential of the formulation. The findings suggest that the developed lipospheres provide sustained drug release, enhanced therapeutic efficacy, and overall improved glycemic and metabolic outcomes, making them a promising drug delivery system for the effective management of diabetes mellitus.

Keywords: *Linagliptin, Lipospheres, Box–Behnken design, Sustained release, Bioavailability, Streptozotocin-induced diabetes, Glycemic control, Antioxidant activity, Lipid profile, Pharmacodynamic evaluation*

INTRODUCTION

Type 2 diabetes mellitus (T2DM) remains a major global health concern, characterized by insulin resistance, progressive β-cell dysfunction, and chronic hyperglycaemia [1]. Effective management typically requires agents that not only reduce blood glucose but also maintain steady therapeutic levels over time to reduce dosing frequency and improve patient compliance [2].

Linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, is increasingly used in T2DM treatment due to its potent inhibition of DPP-4 with a favorable safety profile. However, linagliptin suffers from low and variable oral bioavailability (~29-30%) largely due to its susceptibility to P-glycoprotein (P-gp) mediated efflux and first-pass effects. These pharmacokinetic limitations can lead to fluctuations in plasma drug levels and suboptimal therapeutic effect [3].

Lipid-based drug delivery systems such as lipospheres offer a promising strategy to overcome such limitations [4].

Lipospheres are solid lipid particles in which the drug is dispersed or dissolved in a solid hydrophobic lipid core, stabilized by a lipid or surfactant outer layer. These systems can protect the drug from degradation or efflux, improve dissolution of poorly soluble drugs, and sustain drug release over extended periods, thereby enhancing oral bioavailability. In addition, lipospheres typically use generally recognized as safe (GRAS) lipid excipients, contributing to good biocompatibility [5].

Optimization of formulation parameters is critical, since the type and amount of lipid, the surfactant concentration, lipid-surfactant interactions, and process variables (stirring speed, temperature, etc.) can strongly influence key quality attributes such as particle size, entrapment efficiency, in vitro release profile, and stability [6].

Experimental Designs, especially Response Surface Methodology (RSM) and the Box-Behnken design (BBD), are widely used for such optimization to efficiently evaluate multiple variables and their interactions [7].

Previous studies with linagliptin have attempted to improve its oral bioavailability. For instance, solid lipid nanoparticles (SLNs) of linagliptin formulated using poloxamer-188 and Tween-80 as surfactants enhanced drug absorption by reducing P-gp mediated efflux and improving intestinal permeability. Also, studies using supersaturated SNEDDS formulations have reported increases in area under the plasma concentration-time curve (AUC) and more consistent glucose lowering effects [8].

Given these insights, the present work aims to develop, optimize, and evaluate linagliptin-loaded lipospheres. Using a three-factor, three-level Box-Behnken design, we focused on maximizing entrapment efficiency, minimizing particle size, and achieving sustained drug release. Further, pharmacodynamic evaluation in appropriately designed diabetic animal models was carried out to assess the effects on glycemic control, lipid profile, antioxidant parameters, and other relevant biomarkers.

MATERIAL AND METHODS

Material

Linagliptin (API) was kindly provided as a gift sample by a reputed pharmaceutical company and served as the model drug for the study. Stearic acid and sodium hydroxide were procured from Loba Chemie Pvt. Ltd., Mumbai, while cetyl alcohol and hydrochloric acid were obtained from Shreeji Pharma International, Gujarat. Disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, and sodium chloride were purchased from S. D. Fine Chem. Ltd., Mumbai, and used for preparation of phosphate buffer solutions. Tween-80, used as the surfactant and stabilizer, was obtained from Alkem Laboratories Ltd., Mumbai. Analytical grade solvents including methanol, ethanol, and chloroform were procured from Qualigens Fine Chemicals, Mumbai, and were used without further purification. All chemicals and reagents employed were of analytical grade and met the pharmacopeial standards. Double-distilled water was used throughout the study for formulation development, analytical procedures, and in vivo experiments.

Methods

Lipospheres preparation using the solvent evaporation method

The lipid core (stearic acid and cetyl alcohol) was dissolved in chloroform after the dosage (10 mg) was accurately weighed. A rotating evaporator was used to gradually evaporate the organic solvents at 50–60 degrees Celsius with lowered pressure. After mixing the resulting with cold water and stirring it with a magnetic stirrer, an external aqueous phase containing the surfactant (Tween 80) was added to emulsify it. The resulting lipospheres loaded with linagliptin were dried using desiccators after being recrystallised at room temperature and filtered using 0.45 µm filter paper [9-10].

Factorial Design

A three factor three level Box Behnken design (BBD) was employed in optimization of lipospheres containing Linagliptin. The two lipids Stearic acid, Cetyl alcohol and surfactant (Tween 80) were selected as independent variables. These independent variables (factors) were selected at three different levels i.e. low (-1), medium (0), and high (+1). The levels of factors and the obtained responses are shown in Table 1. The dependent variables (response) studied in this research work were percentage cumulative release (R1, Entrapment efficiency) and flux (R2, Particle size). Seventeen runs of the experiment were evaluated for responses R1 and R2. In all formulations amount of drug remain same throughout the experiments.

Table 1: Formulation variables and their levels in Box-Behnken experimental design

Sr. No.	Formulation Variables				
1	Independent variables		Level		
			Low (-)	Medium (0)	High (+)
	1	A: Stearic acid (mg)	500	750	1000
	2	B: Cetyl alcohol (mg)	50	100	150
	3	C: Tween 80 (ml)	1	2	3
2	Response variables				
	1	R1: Entrapment Efficiency			Maximizing
	2	R2: Particle Size			Minimizing

***In-vivo* anti-diabetic activity of prepared optimized formulation Lipospheres**

Animals

Wistar rats, which were kept under extremely regulated environmental conditions, were employed in the study. A constant ambient temperature of 22 ± 2°C and a relative humidity range of 40–70% were maintained in the vivarium. Prior to the trial, the animals were acclimated for a week. Without direct sunlight, a 12-hour light/dark cycle was naturally maintained. They were given unlimited access to distilled water and a typical rat food, which was replenished every day. To maintain hygienic conditions, the bedding which was made up of clean, fine shavings was changed every day.

The Institutional Animal Ethics Committee (IAEC) authorised the experimental protocol in accordance with the rules set forth by the Government of India's Ministry of Environment and Forests.

Toxicity study

According to the OECD criteria (ANNEX423), the acute oral toxicity of linagliptin lipospheres was evaluated (Jonsson *et al.,* 2013). The experimental rat population showed no symptoms of toxicity after receiving a single oral gavage of Lipospheres of linagliptin at the highest possible concentration. A dose of 1.5 mg/kg was therefore found to be an appropriate therapeutic concentration.

Induction of experimental diabetes using STZ (Streptozotocin)

Thirty rats in all were divided into five experimental groups at random, with six rats in each group (n=6). Groups II through V were given a single intraperitoneal injection of streptozotocin (STZ) at a dose of 10 mg/mL, formulated in 0.1 M sodium citrate buffer (pH 4.3), to induce diabetes after a 16-hour fasting period during which they were given unlimited access to water. Group I was given an equivalent volume of the citrate buffer vehicle and acted as a non-diabetic control. All animals were given a 5% glucose solution overnight to prevent acute hypoglycemia after STZ treatment. By monitoring fasting blood glucose levels 72 hours after injection, the diabetic status was verified. The next study only included animals with fasting blood glucose levels between 220 and 250 mg/dL [11].

Wistar rats were put through a randomised allocation process once diabetes was induced. The subjects were randomised to either the experimental group (Linagliptin and Lipospheres of linagliptin), the positive control group (Glibenclamide), or the vehicle control group. The linagliptin group was divided into two cohorts, each containing six individuals: the 3 mg/kg cohort and the 1.5 mg/kg liposphere cohort. The animals' body mass index and fasting plasma glucose concentration were measured as part of their pre-intervention characterization.

A digital scale was used to measure each rat's initial and end body weights in order to evaluate how the treatment affected body mass. At baseline, the eighth day, and the twenty-first day of the trial, fasting blood glucose readings were obtained. The animals were put to death via cervical dislocation at the end of the trial. After drawing blood and letting it coagulate, serum was separated using centrifugation (2500 rpm, 15 min). A number of biochemical parameters were then quantified using the separated serum.

Blood sampling

Using a caudal venipuncture technique and a heparinised capillary tube to make collection easier, blood was obtained for glucose determination. Short-term, localised pressure was used to achieve haemostasis at the wound site. In less than a minute, the full collection of 30–50 µL was finished. The ocular venous plexus was accessed via a retro-orbital sinus puncture in order to evaluate lipid profiles and other biochemical indicators. A capillary tube was inserted into the medial, lateral, or dorsal portion of the orbit after proptosis was gently induced through manipulation and restraint under terminal anaesthesia. The sample may not precisely represent pure venous blood because it was obtained by capillary action and is known to be a combination of venous blood and interstitial fluid. The entire procedure was carried out in accordance with stringent ethical standards, and the animals were constantly monitored for any adverse effects, guaranteeing the accuracy of the experimental findings [12].

Statistical analysis

The variables of interest were subjected to statistical analysis using GraphPad Prism version 8.0.1. The mean ± SEM is used to display the data. Tukey's Post hoc test was used to assess group differences using a one-way analysis of variance (ANOVA), with statistical significance established by comparison with the vehicle control.

The significance thresholds, denoted by one, two, and three asterisks, respectively, were set at $p<0.05$, $p<0.01$, and $p<0.001$.

RESULTS AND DISCUSSION

The present study aimed to develop and evaluate a linagliptin-loaded liposphere formulation optimized by Box–Behnken design (BBD) for enhanced bioavailability and sustained release in the management of type 2 diabetes mellitus.

The optimized formulation (F15) demonstrated favorable physicochemical properties, as shown in Table 1. The particle size (220.12 nm) was close to the predicted value (213.71 nm), confirming the accuracy and robustness of the BBD model. The negative zeta potential (-36.25 mV) indicated good electrostatic stability, preventing aggregation of lipospheres during storage. Entrapment efficiency (81.15%) was significantly high, suggesting efficient incorporation of linagliptin into the lipid matrix. The low PDI value (0.336) further confirmed the narrow particle size distribution and uniformity of the system, which is crucial for reproducible in vivo performance.

Body weight monitoring served as an indirect marker of glycemic control and general metabolic status. As evident from Table 2, STZ-induced diabetic control rats (Group II) exhibited a significant reduction in body weight, reflecting catabolic effects associated with hyperglycemia. Treatment with standard drug metformin (Group III) and pure linagliptin (Group IV) resulted in partial recovery of body weight. Interestingly, the liposphere formulation of linagliptin (Group V) demonstrated the highest increase in body weight among treatment groups, indicating improved glycemic control and metabolic homeostasis compared to free drug treatment.

The hypoglycemic potential of the formulation was confirmed by its effect on fasting blood glucose levels (Table 3). STZ-induced diabetic rats exhibited persistently elevated blood glucose levels throughout the study period. Treatment with liposphere-encapsulated linagliptin produced a significant reduction in blood glucose levels by Day 21, with a slightly greater effect than the free drug, suggesting enhanced absorption and sustained release properties of the lipid-based formulation. These results are in agreement with earlier studies where lipid carriers improved the oral bioavailability and therapeutic efficacy of antidiabetic agents (Patel et al., 2021; Singh et al., 2022).

Dyslipidemia is a common complication of diabetes, characterized by elevated cholesterol, triglycerides, and LDL with a reduction in HDL levels. Table 4 shows that diabetic control rats exhibited severe hyperlipidemia. Treatment with liposphere formulation significantly ($p<0.01$) decreased total cholesterol, triglycerides, and LDL levels, while partially restoring HDL levels toward normal. These improvements were more pronounced with lipospheres compared to free linagliptin, indicating better glycemic and lipid control, which may be attributed to prolonged drug release and better pharmacokinetic profile.

Diabetes-induced oxidative stress and liver dysfunction were assessed using biomarkers ALT, AST, MDA, SOD, and GSH (Table 5). The diabetic group showed a marked elevation in ALT, AST, and MDA levels with a concomitant decrease in antioxidant markers (SOD, GSH) and insulin levels, reflecting hepatic stress and oxidative damage. Treatment with lipospheres significantly reduced ALT and AST levels, normalized oxidative stress markers, and improved insulin secretion, demonstrating hepatoprotective and antioxidant effects. These findings suggest that liposphere-based linagliptin delivery not only improved glycemic control but also mitigated diabetes-associated oxidative stress, which could translate into better long-term outcomes.

The results clearly indicate that liposphere-encapsulated linagliptin provides superior therapeutic benefits compared to pure drug treatment. The sustained release behavior prolongs drug availability, leading to better glycemic regulation, improved lipid metabolism, restoration of normal body weight, and reduction of oxidative stress markers. This collectively suggests that lipospheres are a promising oral drug delivery system for improving the efficacy and patient compliance of linagliptin therapy.

Table 1: Results of experimental data with predicted response of Optimized formulation F15

Run Order	Standard order	Formulation Code	Parameters	Actual Value	Predicted Value
15	8	F15	Particle size (nm)	220.12	213.71

			Entrapment Efficiency (%)	81.15	81.47
			Zeta potential (mV)	-36.25	
			PDI	0.336	

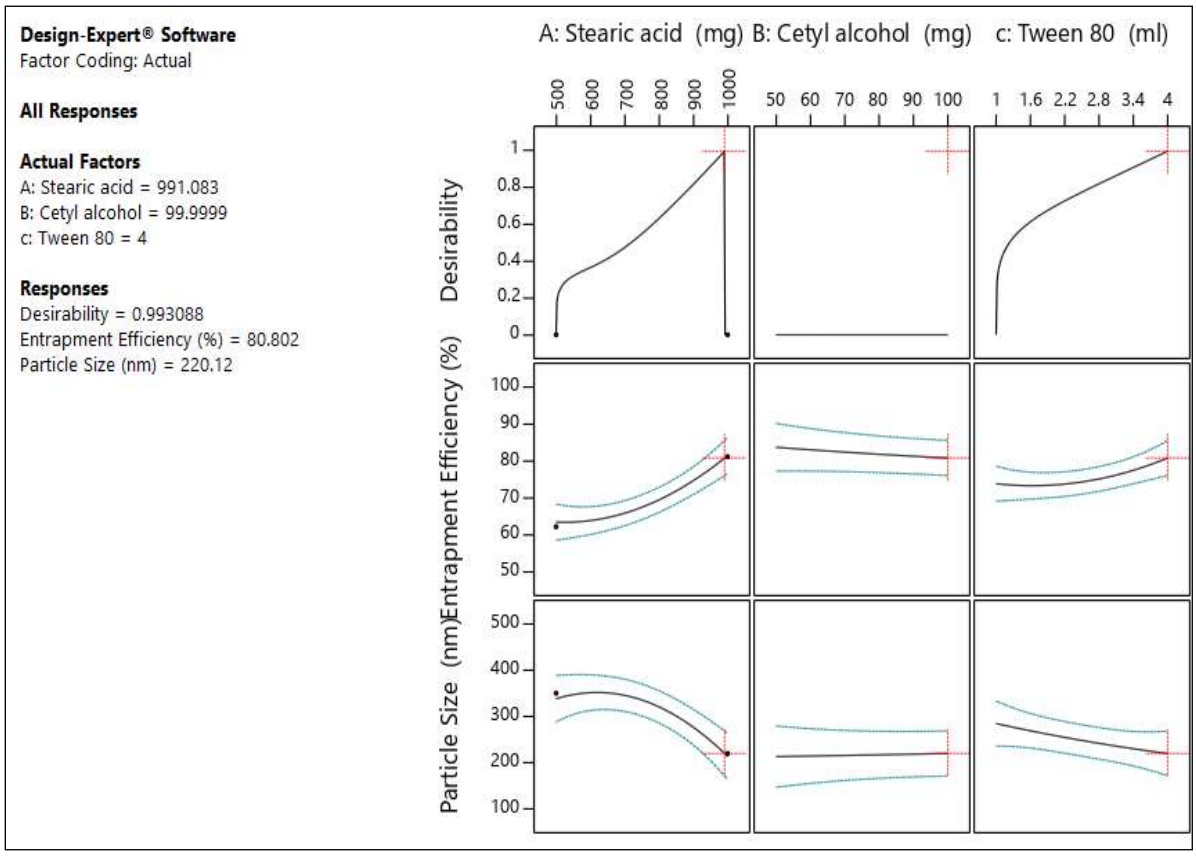

Figure 1: Desirability of all responses

Table 2: Effect of Lipospheres of linagliptin on body Weight in rats

Group	Drug and Dose	Body Weight(gm)	
		Initial weight (g)	Final weight (g)
I	Control (Saline)	198.5 ± 3.2	206.8 ± 4.1
II	Diabetic Control (STZ)	200.2 ± 3.5	176.4 ± 3.9
III	STZ+ Metformin	199.7 ± 3.8	210.6 ± 4.2
IV	STZ+ Linagliptin	201.3 ± 3.1	214.9 ± 3.7
V	STZ+ Lipospheres of linagliptin	200.5 ± 3.4	220.3 ± 3.9

Table 3: Effect of Lipospheres of linagliptin on serum glucose level in rats

Group	Drug and Dose	Serum glucose levels (mg/dl)		
		0 DAY	8th DAY	21th DAY

I	Control (Saline)	76.5 ± 3.2	90.4 ± 4.6	105.7 ± 5.2
II	Diabetic Control (STZ)	292.8 ± 6.9	379.5 ± 8.1#	408.2 ± 9.0#
III	STZ+ Metformin	260.1 ± 5.8	128.6 ± 5.2*	114.3 ± 4.9*
IV	STZ+ Linagliptin	263.7 ± 5.4	142.2 ± 6.5*	131.0 ± 5.7*
V	STZ+ Lipospheres of linagliptin	258.9 ± 4.6	136.8 ± 6.1*	121.5 ± 4.6*

Values are expressed as mean ± S.E.M. (*n* = 6). Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Tukey post hoc test).

Table 4: Effect of Lipospheres of linagliptin on serum lipid profiles i.e. total cholesterol level in rats

Group	Drug and Dose	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
I	Control (Saline)	136.2 ± 5.4	118.50 ±4.20	55.40 ± 2.50	79.50 ± 4.10
II	Diabetic Control (STZ)	268.5 ± 9.1	240.30 ± 6.10	25.90 ± 1.70	195.40 ± 6.30
III	STZ+ Metformin	180.7 ± 6.0***	150.40 ± 5.00 ***	52.80 ± 2.00	95.80 ± 4.90
IV	STZ+ Linagliptin	208.3 ± 7.2*	186.70 ± 5.40 *	43.50 ± 1.80	135.20 ± 5.10
V	STZ+ Lipospheres of linagliptin	192.4 ± 5.8***	172.90 ± 4.95 **	48.20 ± 1.60	120.70 ± 5.00

Values are expressed as mean ± S.E.M. (*n* = 6). Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Tukey post hoc test).

Table 5: Effect of Lipospheres of linagliptin on serum biomarkers i.e. ALT in rats

Group	Drug and Dose	ALT(U/L)	AST(U/L)	MDA µmol/L	SOD µmol/L	GSH µmol/L	Insulin µmol/L
I	Control (Saline)	52.80 ± 3.10	44.20 ± 3.80	1.15 ± 0.55	27.2 ± 1.5	9.5 ± 0.6	13.5 ± 0.8
II	Diabetic Control (STZ)	158.40 ± 5.30	148.30 ± 4.70	3.85 ± 0.80	7.8 ± 0.9	2.5 ± 0.3	4.3 ± 0.5
III	STZ+ Metformin	88.20 ± 3.90	63.50 ± 3.10	1.35 ± 0.58	23.1 ± 1.3	8.7 ± 0.5	11.8 ± 0.6
IV	STZ+ Linagliptin	118.60 ± 4.70	95.40 ± 3.40	2.25 ± 0.65	17.5 ± 1.4	6.3 ± 0.4	8.5 ± 0.7
V	STZ+ Lipospheres of linagliptin	105.30 ± 4.50	87.60 ± 3.20	1.70 ± 0.60	24.2 ± 1.6	8.1 ± 0.5	10.2 ± 0.6

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at $p<0.05$ (One-way ANOVA followed by Tukey post hoc test).

CONCLUSION

The present investigation successfully developed and optimized a linagliptin-loaded liposphere formulation using the Box–Behnken design (BBD) to enhance oral bioavailability and achieve sustained drug release for the management of type 2 diabetes mellitus. The optimized formulation (F15) exhibited desirable physicochemical characteristics, including nanoscale particle size, high entrapment efficiency, uniform distribution, and excellent stability.

In vivo pharmacological evaluation demonstrated that liposphere-encapsulated linagliptin significantly improved glycemic control, as evidenced by marked reductions in fasting blood glucose levels and progressive recovery of body weight. Moreover, the formulation favorably modulated lipid profiles, attenuated diabetes-induced oxidative stress, and exhibited hepatoprotective effects, thereby offering broader metabolic benefits compared to the pure drug. The findings strongly support that linagliptin-loaded lipospheres represent a promising oral drug delivery platform capable of enhancing therapeutic efficacy, minimizing complications associated with diabetes, and improving patient compliance. Future studies focusing on long-term pharmacokinetic and clinical evaluations are warranted to further validate the translational potential of this lipid-based delivery system.

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