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Development Of A Box–Behnken Design Optimized Linagliptin Liposphere Formulation For Improved Bioavailability And Sustained Release

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ABSTRACT

The present study aimed to develop and optimize Linagliptin-loaded lipospheres for sustained drug delivery using a Box–Behnken Design (BBD). Stearic acid, cetyl alcohol, and Tween 80 were selected as independent variables, while entrapment efficiency and particle size were taken as response variables. Fifteen experimental runs were performed, and the formulations were evaluated for percentage yield, entrapment efficiency, particle size, zeta potential, polydispersity index (PDI), and flow properties. Among the prepared formulations, F15 was identified as the optimized batch, exhibiting the highest percentage yield (86.65%), maximum entrapment efficiency (81.15%), and minimum particle size (220.12 nm). The zeta potential (–36.25 mV) and PDI (0.336) confirmed good physical stability and narrow size distribution. Scanning electron microscopy revealed spherical and smooth-surfaced lipospheres. The in vitro drug release study of F15 displayed a biphasic pattern with 97.45% cumulative release at 10 hours, following zero-order kinetics ($R^2 = 0.9957$) and non-Fickian diffusion as per the Korsmeyer–Peppas model ($R^2 = 0.9869$). Stability studies indicated that refrigerated storage (4 ± 2°C) maintained particle size, PDI, and entrapment efficiency more effectively compared to ambient conditions (25 ± 3°C / 65 ± 5% RH). The optimized Linagliptin lipospheres demonstrated promising potential as a controlled-release oral drug delivery system with improved drug encapsulation, stability, and sustained release characteristics. These findings suggest that liposphere-based delivery of Linagliptin could enhance therapeutic efficacy and patient compliance in the management of type 2 diabetes mellitus.

Keywords: *Linagliptin, Lipospheres , Box–Behnken Design, Entrapment Efficiency, Particle Size, Zeta Potential, In Vitro Drug Release, Sustained Release, Stability Studies, Diabetes Mellitus.*

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1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia due to insulin resistance and/or reduced insulin secretion [1]. Effective long-term treatment requires not only potent antidiabetic agents, but also drug delivery systems that can maintain therapeutic concentrations, improve bioavailability, and reduce dosing frequency to enhance patient compliance [2]. Linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, has become an important therapeutic agent for T2DM owing to its prolonged half-life and potent inhibitory action. However, its oral bioavailability is relatively modest (~30%) largely because of P-glycoprotein (P-gp) efflux and first-pass metabolism in the intestine and liver, which limits its therapeutic efficacy [3].

Lipid-based delivery systems, such as lipospheres, solid lipid nanoparticles (SLNs), and self-nanoemulsifying systems, have shown promise in enhancing the bioavailability of poorly absorbed or efflux-susceptible drugs [4]. Lipospheres are solid lipid particles with a hydrophobic core stabilized by a lipid coat or surfactant layer; they can protect the drug from degradation, modulate release, and improve cellular uptake [5]. Studies have demonstrated that lipospheres and related lipid systems can significantly improve bioavailability, reduce drug dosing, and provide sustained release profiles [6]. The optimization of such delivery systems is critical because formulation variables (lipid type and concentration, surfactant types and concentration, process parameters) can profoundly affect particle size, entrapment efficiency, release kinetics, and stability [7].

Design of Experiments (DoE) approaches, particularly Response Surface Methodology (RSM) and designs such as Box–Behnken Design (BBD), provide efficient strategies for systematically studying multiple formulation parameters and their interactions, thereby reducing the number of experiments while optimizing critical quality attributes. These methods have been successfully applied in optimizing SLNs, liposomes, microspheres, and other novel delivery systems for both lipophilic and hydrophilic drugs [8]. In previous research, linagliptin-loaded SLNs have been developed to mitigate P-gp mediated efflux and improve oral absorption, using factorial designs. These studies reported improved pharmacokinetic parameters and enhanced bioavailability in animal models [9]. Additionally, other formulation approaches such as SNEDDS (Self-Nanoemulsifying Drug Delivery Systems) have been explored to tackle linagliptin's poor water solubility and absorption limitations [10].

Given this background, the present study is aimed to develop and optimize lipospheres of Linagliptin using a 3-factor, 3-level Box–Behnken Design. The focus is on maximizing entrapment efficiency and sustaining release while controlling particle size, to eventually enhance the oral bioavailability and therapeutic efficacy of Linagliptin.

2. MATERIAL AND METHODS

Material

The materials used for the preparation of linagliptin-loaded lipospheres included Linagliptin API (gift sample from a pharmaceutical company), stearic acid (Loba Chemie Pvt. Ltd., Mumbai), and cetyl alcohol (Shreeji Pharma International, Gujarat) as lipid components. Analytical-grade chemicals such as disodium hydrogen phosphate, dipotassium hydrogen phosphate, and sodium chloride (S. D. Fine Chem. Ltd., Mumbai) were employed for buffer preparation. Methanol, ethanol, and chloroform (Qualigens Fine Chemicals, Mumbai) were used as solvents, while Tween 80 (Alkem Laboratories Ltd., Mumbai) served as the surfactant. Sodium hydroxide (Loba Chemie Pvt. Ltd., Mumbai) and hydrochloric acid (Shreeji Pharma International, Gujarat) were utilized for pH adjustment during formulation development.

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 [11-12].

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

Table 2: Design matrix in Box-Behnken design coded values for lipospheres containing Linagliptin

Std	Run	Factors		
		Coded Values		
		Factor A	Factor B	Factor C
12	1	0	1	1
17	2	0	0	0
4	3	1	1	0
6	4	1	0	-1
1	5	-1	-1	0
7	6	-1	0	1
11	7	0	-1	1
10	8	0	1	-1
16	9	0	0	0
2	10	1	-1	0
3	11	-1	1	0
5	12	-1	0	-1
13	13	0	0	0
9	14	0	-1	-1
8	15	1	0	1

Table 3: Design matrix in Box-Behnken design coded values for lipospheres containing Linagliptin

Std	Run	Actual Values		
		Factor A : Stearic acid	Factor B: Cetyl alcohol	Factor C: Tween 80
12	1	750	150	4
17	2	750	100	2.5
4	3	1000	150	2.5
6	4	1000	100	1
1	5	500	50	2.5
7	6	500	100	4
11	7	750	50	4
10	8	750	150	1
16	9	750	100	3
2	10	1000	50	2.5
3	11	500	150	2.5
5	12	500	100	1

13	13	750	100	2
9	14	750	50	1
8	15	1000	100	4

Final Equation in Terms of Coded Factors

Entrapment Efficiency = +64.19+5.71 A+0.6888B+0.3016C-3.02 AB+3.29 AC-0.0375 BC+4.98 A^2+0.6343 B^2+3.00 C^2

Final Equation in Terms of Actual Factors

Entrapment Efficiency = +99.10465-0.094487 Stearic acid+0.145185 Cetyl alcohol-13.00069 Tween 80-0.000241 Stearic acid * Cetyl alcohol+0.008780 Stearic acid * Tween 80+0.008780 Stearic acid * Tween 80+0.000080 Stearic acid ^{2}+0.000254 Cetyl alcohol ^{2}+1.33335 Tween 80 ^{2}

Final Equation in Terms of Coded Factors

Particle Size = +335.00-32.36 A-8.75 B-3.47 C+9.57 AB-29.98 Ac+7.17 Bc-59.69 A^2+0.8395 B^2+4.21 C^2

Particle Size = -135.34109+1.42639 Stearic acid-1.05565 Cetyl alcohol+38.72552 Tween 80+0.000766 Stearic acid * Cetyl alcohol-0.079933 Stearic acid * Tween 80+0.095667 Cetyl alcohol * Tween 80-0.000955 Stearic acid ^{2}+0.000336 Cetyl alcohol ^{2}+1.86908 Tween 80 ^{2}

Linagliptin-encapsulated liposphere characterization

Liposphere yield as a percentage

The following formula was used to determine the yield of lipospheres as a percentage of weight [13]:

$$\% \text{ Yield} = \frac{\text{Weight of lipospheres}}{\text{Wt. of drug} + \text{Wt. of excipients}} \text{X100}$$

Entrapment efficiency

By consuming a known quantity of lipospheres, which theoretically should contain 10 mg of the medicine, the amount of linagliptin present in the lipospheres was ascertained [14]. After crushing the lipospheres, the powdered microspheres were removed and dissolved in 10 millilitres of methanol. They were then agitated for 15 minutes at 5-minute intervals and left for a full day. Whatmann filter paper was then used to filter the mixture. After the proper dilution, the absorbance was then measured spectrophotometrically at 294 nm using a UV-visible spectrophotometer.

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Experimental drug content}}{\text{Initial drug content in the formulation}} \text{X100}$$

Measurement of mean particle size

Photo Correlation Spectroscopy (PCS) on a submicron particle size analyser (Malvern Instruments) at a scattering angle of 90° was used to calculate the lipospheres' mean size. For the measurement, 0.5 mg of the lipospheres was suspended in 5 ml of distilled water [15].

Determination of zeta potential

By measuring the electrophoretic mobility in a micro electrophoresis flow cell, the zeta potential of the drug-loaded lipospheres was determined using a zeta sizer (Malvern zetasizer instruments) [16]. Water was used to measure each sample.

Surface morphology (Scanning electron microscopy)

Scanning electron microscopy was used to analyse the lipospheres' morphology and surface topography [17]. Using a double-sided sticky tape, the lipospheres from the optimised batch were placed on the SEM sample stab. They were then coated with gold (~200 nm) using an ion sputtering apparatus for five minutes at a reduced pressure of 0.133 Pa. The scanning electron microscope was used to view the gold-coated lipospheres, and appropriate magnification photomicrographs were taken.

Flow property determination of the Lipospheres

Bulk density: Both the tapped bulk density (TBD) and the loose bulk density (LBD) were calculated (Mohammed and Bhise, 2013). The LBD and TBD were estimated using the following formulas after a precisely weighed quantity of granules was placed in a 50 ml measuring container and tapped 100 times on a level, hard wooden surface [18].

$$\textbf{LBD (Loose bulk density)} = \frac{\text{Mass of powder}}{\text{Volume of Packing}}$$

$$\textbf{TBD (Tapped bulk density)} = \frac{\text{Mass of powder}}{\text{Tapped Volume of Packing}}$$

Compressibility index: Carr's compressibility index, which was computed using the following formula, was used to assess the powder mix's percentage compressibility: -

$$\textbf{Carr's Index} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \text{X100}$$

Hausners ratio: The following formula is used to compare the tapped density to the bulk density:

$$\textbf{Housner's ratio} = \frac{\text{Tapped bulk density}}{\text{Loose Bulk density}}$$

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

Linagliptin's dissolution from the produced lipospheres was tracked with the USP XXV paddle II machine (Labindia DS – 8000). A cellulose dialysis tube containing 5 mL of dissolving liquid was filled with 10 mg of lipospheres. In order to keep the sink condition in the dissolution vessel, this tube was connected with a paddle. The dissolution medium consisted of 900 millilitres of pH 1.2 buffer that was rotated at 50 ±1 rpm and kept at 37±0.5ºC. At prearranged intervals, the 5 ml aliquots were removed, and new dissolving medium was added in their stead. The amount of linagliptin in the samples was then determined using spectrophotometry at 294.0 nm [19].

Stability Studies

To determine the impact of environmental conditions on the drug contents, size, and PDI of Lipospheres, the optimised formulation F15 was put through a three-month stability study. During three months, the optimised Lipospheres were kept in a stability chamber at 25 ± 3°C and 4 ± 2°C, with a relative humidity of 65 ± 5%, in sealed amber glass bottles. Drug contents, size, and PDI were assessed three times at 30-day intervals to assess stability [20].

3. RESULTS AND DISCUSSION

The present study focused on the design and optimization of Linagliptin-loaded lipospheres using a Box–Behnken Design (BBD) approach to achieve maximum entrapment efficiency, minimum particle size, and sustained drug release characteristics.

The Box–Behnken design was employed with three independent variables stearic acid concentration (A), cetyl alcohol concentration (B), and Tween 80 concentration (C) each at three levels (low, medium, high). The selected levels are shown in Table 1, while the design matrix with coded and actual values is presented in Table 2 and Table 3, respectively. This design was chosen because it allows efficient optimization with a reduced number of experiments and avoids extreme combinations, thereby improving reliability.

The percentage yield, entrapment efficiency, and particle size of all 15 formulations are summarized in Table 4**.** The yield of lipospheres ranged between 68.41–86.65%, with the highest yield observed for formulation LF15 (86.65%), which also exhibited the highest entrapment efficiency (81.15%) and the smallest particle size (220.12 nm). These findings suggest that optimized lipid and surfactant concentrations improved drug encapsulation and promoted the formation of more uniform particles. In contrast, formulations with lower lipid concentrations (LF5, LF6, LF9) showed reduced entrapment efficiency (60–63%) and larger particle sizes (>325 nm), likely due to insufficient lipid matrix availability for drug entrapment.

The effect of formulation variables on entrapment efficiency and particle size is further explained by the 3D response surface plots shown in Figure 1 and Figure 2, respectively. An increase in stearic acid concentration was found to enhance entrapment efficiency due to improved matrix density. However, excessive lipid levels increased particle size, possibly because of aggregation during emulsification. Tween 80 concentration played a crucial role in reducing particle size by lowering interfacial tension, leading to better droplet dispersion.

The optimized formulation (F15) was selected based on the desirability function, which considered maximum entrapment efficiency and minimum particle size. The experimental and predicted values of particle size and entrapment efficiency are presented in Table 5, showing a close agreement between actual and predicted responses (desirability = 0.993). The particle size distribution of F15 is illustrated in Figure 3, confirming its narrow particle size range.

The zeta potential (–36.25 mV) and PDI (0.336) of F15, presented in Figure 4, indicate excellent electrostatic stabilization and a narrow size distribution, ensuring good physical stability of the lipospheres. The overlay plot between cetyl alcohol and Tween 80, shown in Figure 5, highlights the design space for achieving the optimal balance between particle size reduction and maximum drug entrapment.

The flow properties of the optimized lipospheres are summarized in Table 6. The Hausner ratio (1.38) and Carr's index (27.46%) indicate fair flowability, which is acceptable for further pharmaceutical processing. The surface morphology observed by SEM (Figure 6) confirmed spherical particles with smooth surfaces, suggesting successful encapsulation of Linagliptin.

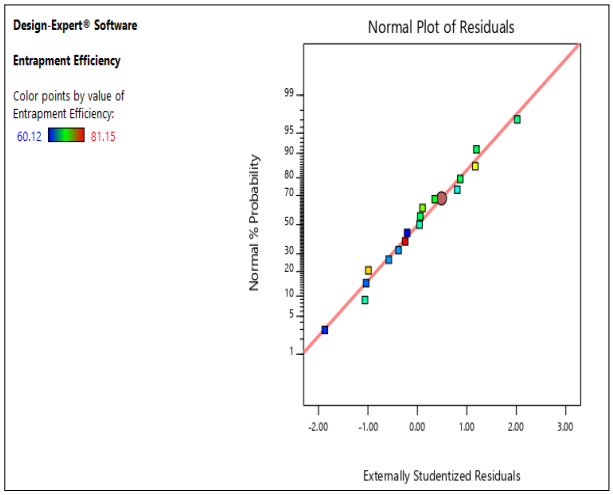
The in-vitro drug release profile of F15 is presented in Table 7, showing a biphasic release pattern with initial slow release followed by sustained release, reaching 97.45% cumulative drug release at 10 hours. Regression analysis (Table 8) revealed that the release data followed Zero-Order kinetics (R^2 = 0.9957), suggesting concentration-independent release. Additionally, the Korsmeyer–Peppas model (R^2 = 0.9869) confirmed non-Fickian diffusion-controlled release, indicating both diffusion and erosion mechanisms were involved in drug release.

Stability studies conducted under accelerated and refrigerated conditions are summarized in Table 9. Samples stored at 4 ± 2°C retained entrapment efficiency, particle size, and PDI with minimal changes over 90 days, whereas those stored at 25 ± 3°C / 65 ± 5% RH showed a slight increase in particle size and PDI, along with a decrease in entrapment efficiency, indicating some aggregation under stress conditions.

The combination of response surface methodology, statistical validation, and physicochemical characterization confirmed that F15 was the optimized formulation with desirable properties, excellent stability under refrigerated conditions, and a prolonged release profile suitable for sustained drug delivery.

Table 4: Results of percentage yield, entrapment efficiency and particle size of Linagliptin loaded lipospheres

Run order	F. Code	Yield (%)	Entrapment efficiency (%)	Particle size (nm)
12	LF1	75.65	68.85	315.65
17	LF2	70.32	65.95	365.58
4	LF3	73.32	73.32	255.65
6	LF4	81.15	75.66	269.98
1	LF5	68.85	60.12	314.44
7	LF6	70.12	62.22	350.36
11	LF7	73.32	68.87	336.65
10	LF8	69.98	66.98	330.45
16	LF9	68.74	63.32	325.45
2	LF10	83.32	76.65	236.15
3	LF11	70.12	68.85	295.65
5	LF12	68.41	69.90	280.32
13	LF13	68.85	60.98	296.65
9	LF14	68.88	66.85	380.15
8	LF15	86.65	81.15	220.12



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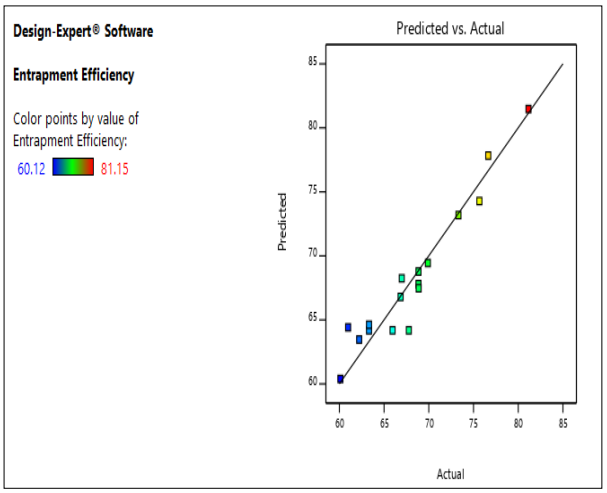

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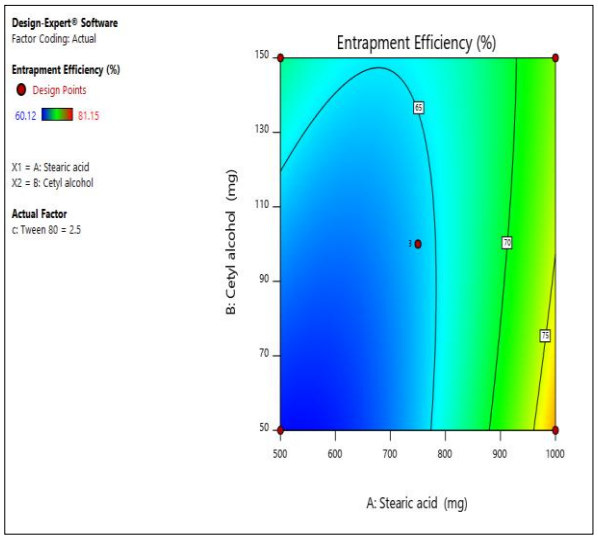

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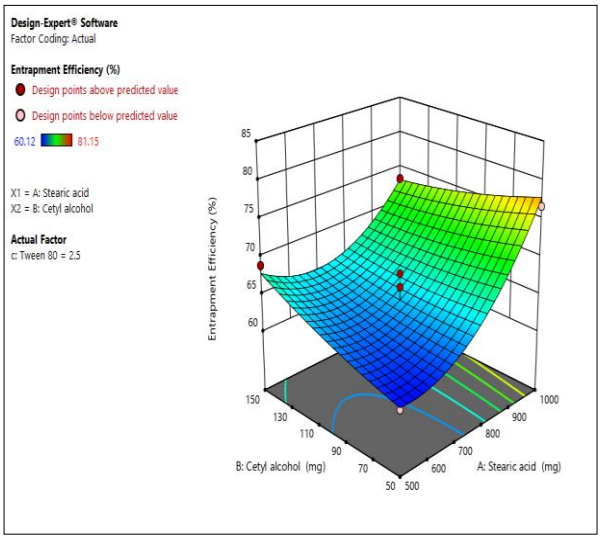

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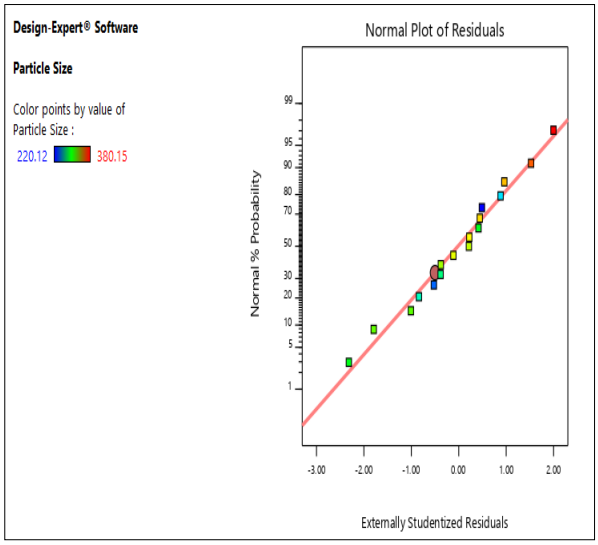
Figure 1: Different Graphs of Entrapment Efficiency Obtained By DOE



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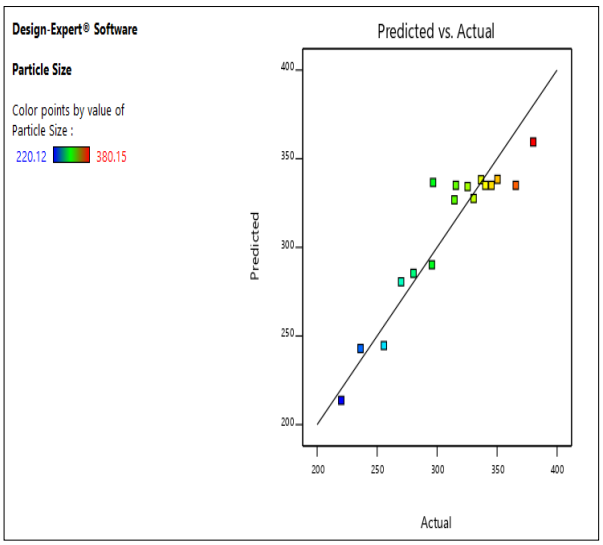

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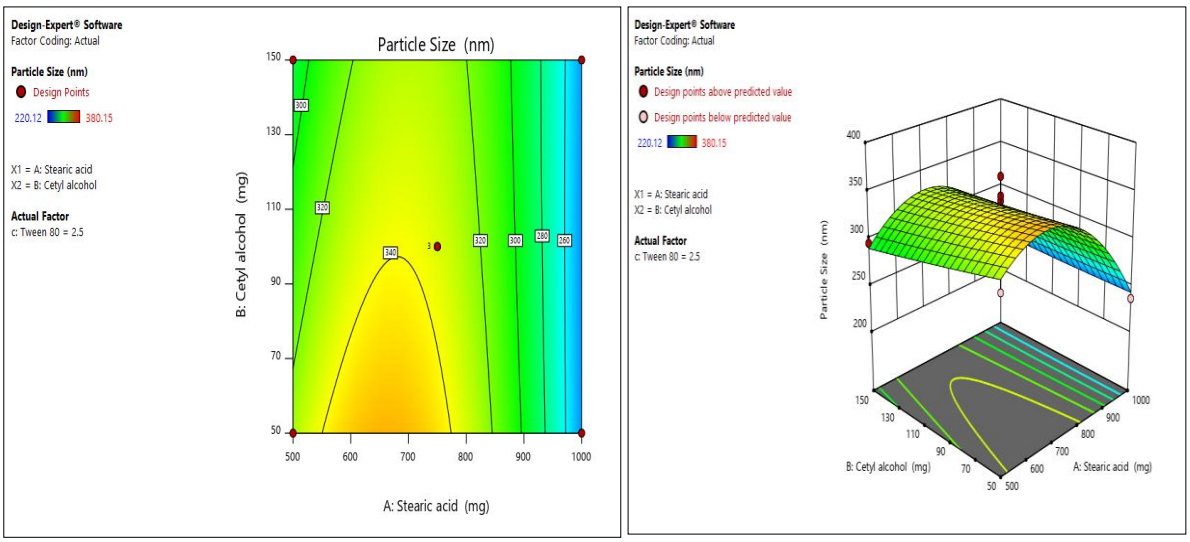


C D

Figure 2: Different Graphs of Particle Size Obtained By DOE

Table 5: Experimental data with predicted response

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	
Stearic acid	**Cetyl alcohol**	**Tween 80**	**Entrapment Efficiency**	**Particle Size**	**Desirability**
991.083	100.000	4.000	80.802	220.120	0.993

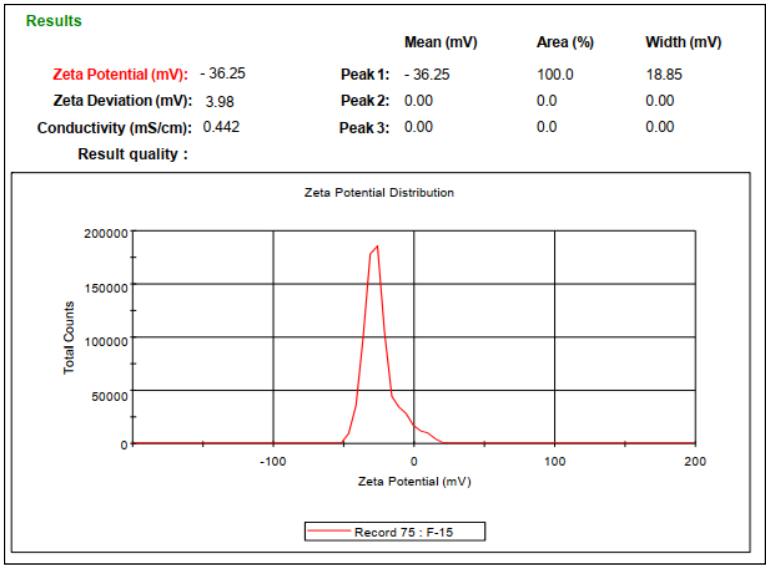


Figure 3: Graph of Particle Size of optimized formulation F15

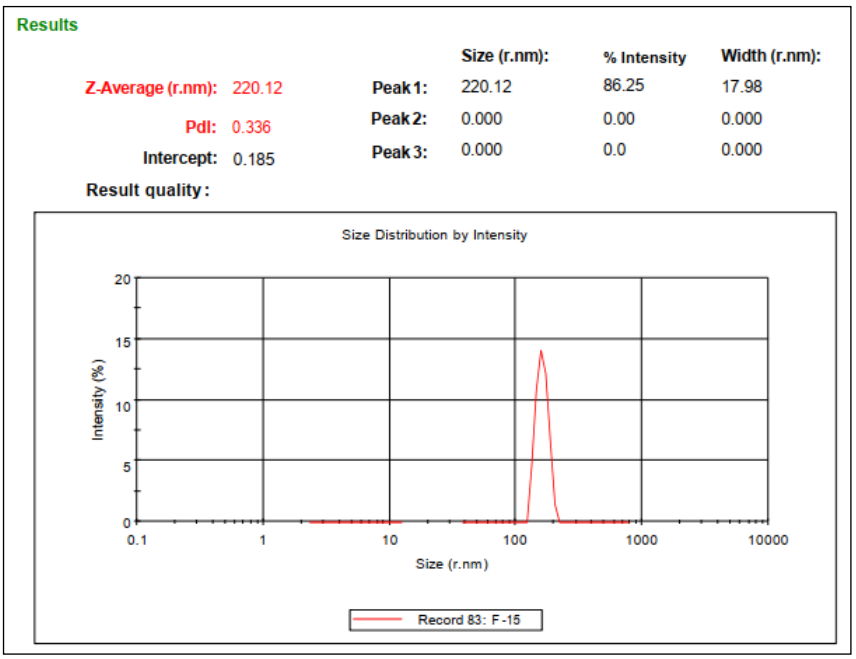
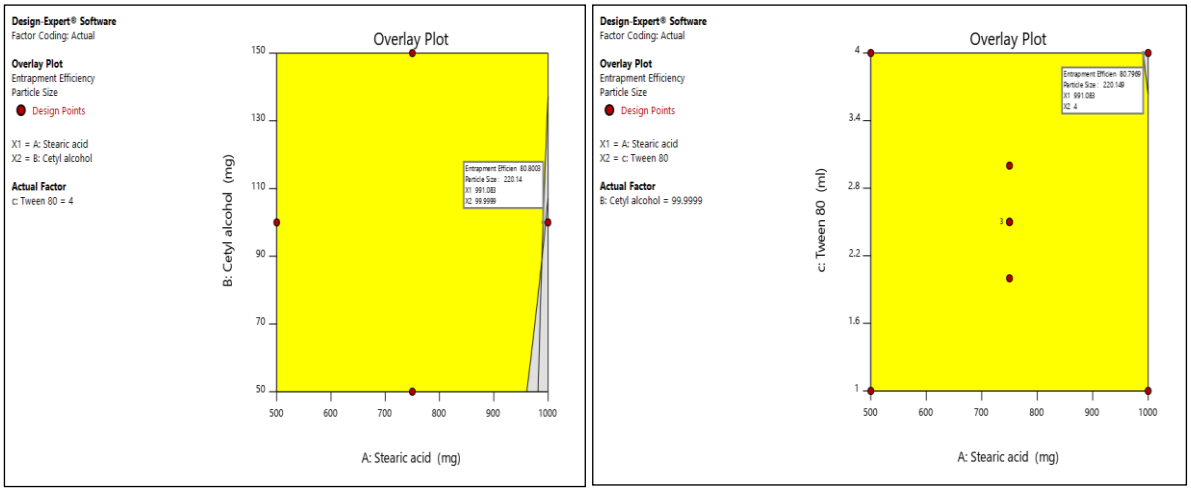


Figure 4: Graph of Zeta potential and PDI of optimized formulation F15



A **B**

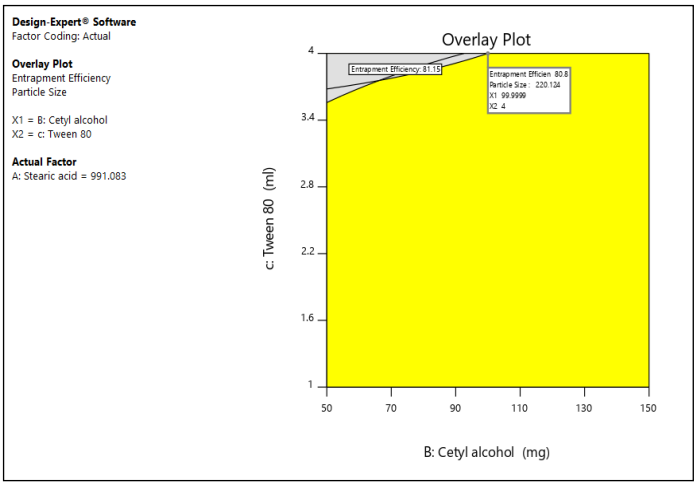


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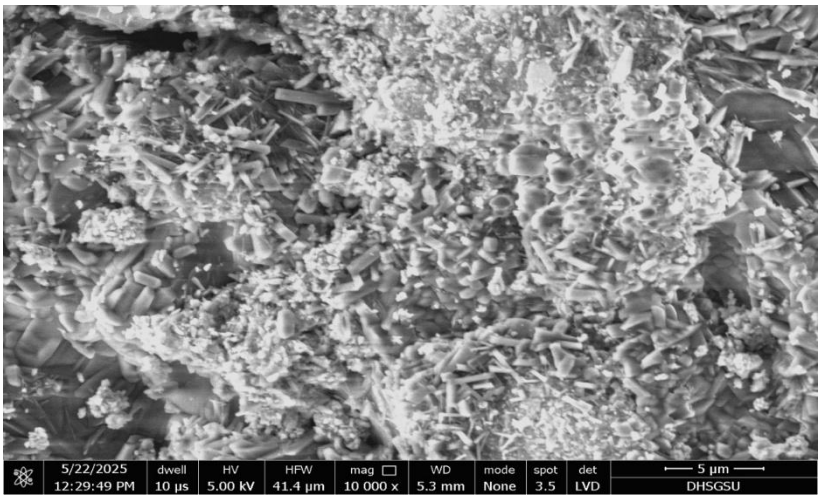
Figure 5: Overlay plots between cetyl alcohol and tween 80

Table 6: Results of flow properties of optimized liposphere formulation F15

F. Code	Bulk Desity	Tapped Density	Hausner Ratio	compressibility index
F15	0.412	0.568	1.38	27.46



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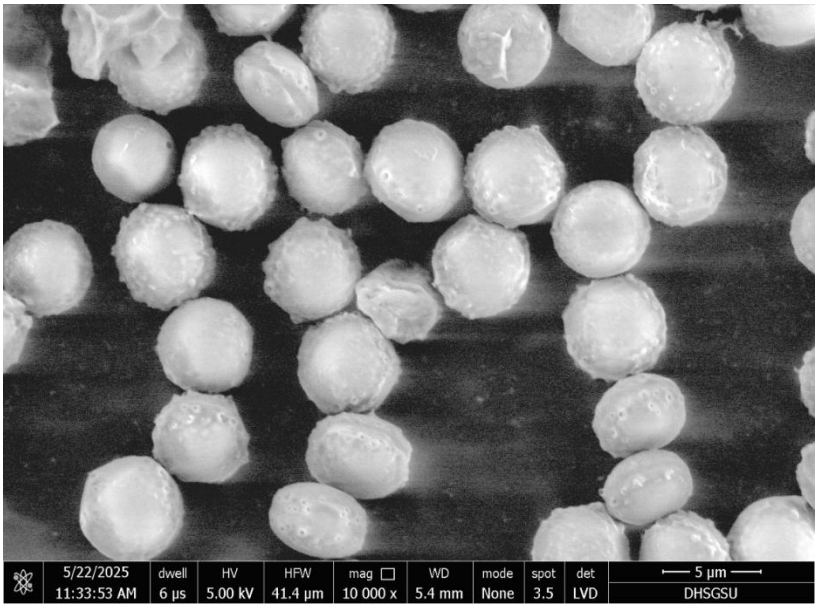

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Figure 6: SEM image of A, pure drug and B, optimized liposphere formulation F15

Table 7: *In vitro* drug release kinetics study of optimized formulation F15

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1.000	0.000	14.45	1.160	85.55	1.932
2	1.414	0.301	22.23	1.347	77.77	1.891
3	1.732	0.477	30.32	1.482	69.68	1.843

4	2.000	0.602	38.85	1.589	61.15	1.786
5	2.236	0.699	46.65	1.669	53.35	1.727
6	2.449	0.778	59.98	1.778	40.02	1.602
7	2.646	0.845	66.69	1.824	33.31	1.523
8	2.828	0.903	79.85	1.902	20.15	1.304
10	3.162	1.000	97.45	1.989	2.55	0.407

Table 8: Regression analysis data of optimized formulation F15

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F15	0.9957	0.7778	0.9579	0.9869

Table 9: Results of Stability studies at 25 ± 3°C / 65 ± 5% RH, of optimized formulation F15

Storage Condition	Time (Days)	Entrapment Efficiency (%)	Particle Size (nm)	PDI
25 ± 3°C / 65 ± 5% RH	0	81.15 ± 0.35	220.12 ± 1.52	0.336 ± 0.02
	30	79.45 ± 0.50	228.35 ± 2.10	0.352 ± 0.03
	60	76.85 ± 0.60	236.45 ± 2.36	0.368 ± 0.04
	90	73.95 ± 0.75	245.15 ± 2.84	0.385 ± 0.05
4 ± 2°C (Refrigerated)	0	81.15 ± 0.35	220.12 ± 1.52	0.336 ± 0.02
	30	80.85 ± 0.40	221.85 ± 1.68	0.340 ± 0.02
	60	80.25 ± 0.45	223.65 ± 1.85	0.344 ± 0.02
	90	79.85 ± 0.50	224.85 ± 2.00	0.348 ± 0.02

4. CONCLUSION

The present study successfully developed and optimized a linagliptin-loaded liposphere formulation using the Box–Behnken Design approach. The systematic application of Response Surface Methodology allowed identification of the optimal combination of formulation variables that maximized entrapment efficiency, minimized particle size, and provided a controlled drug release profile. The optimized liposphere formulation demonstrated sustained release over an extended period, which is desirable for reducing dosing frequency and improving patient compliance in the management of type 2 diabetes mellitus. Moreover, the stability studies indicated that the formulation maintained its physicochemical characteristics and drug content during the storage period, confirming its robustness and suitability for scale-up. The results suggest that lipid-based lipospheres can effectively enhance the oral bioavailability of linagliptin by improving its solubilization and potentially overcoming P-gp mediated efflux and first-pass metabolism.

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