RESEARCH PAPER

Optimization and Characterization of Lovastatin Loaded Ufasomes by Definitive Screening Design

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ABSTRACT:

Lovastatin is a lipid-lowering agent belonging to the class of HMG-CoA reductase inhibitors, widely used in the treatment of Hyperlipidemia. Lovastatin was loaded into Ufasomes to enhance its solubility and bioavailability. Reverse phase evaporation method was employed to formulate Lovastatin-loaded ufasomes using a systematic optimization approach. The independent variables considered were oleic acid (mg) (A), cholesterol (mg) (B), stirring speed (rpm) (C), and Surfactant concentration (v/v) (D), and their effects were evaluated on entrapment efficiency (EE%), particle size (nm), and zeta potential (mV).The ufasomal suspension prepared by reverse phase evaporation was subjected to lyophilization, and the obtained powder was further characterized for physicochemical properties. Among all the formulations, the optimized formulation (e.g., UF-4) showed a particle size of 185 nm, zeta potential of −35 mV, and entrapment efficiency of 88%. The optimized formulation exhibited enhanced drug release of 93.50% compared to the pure drug (20.40%). Kinetic studies of the optimized formulation showed the highest R^2 value for the Higuchi model ($R^2 = 0.9998$), indicating that the drug release follows a diffusion-controlled mechanism.

Key words: Lovatsatin, Ufasomes, Definitive screening, Reverse Phase Evaporation.

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INTRODUCTION:

Unsaturated fatty acids, like oleic acid, spontaneously form bilayer vesicles in aqueous media, especially in alkaline circumstances, to form ufasomes, innovative vesicular drug delivery methods. These vesicles are adaptable carriers for a variety of medicinal treatments because of their special amphiphilic character, which enables them to encapsulate both hydrophilic medications in their internal water core and lipophilic drugs within the lipid bilayer. By promoting improved absorption across biological membranes, ufasomes increase drug stability, shield medications from deterioration, and boost bioavailability. They are particularly helpful for enhancing patient compliance, lowering the frequency of doses, and controlling and sustaining drug release. Ufasomes are less expensive, simpler to make, and have minimal toxicity and superior biocompatibility when compared to traditional carriers like liposomes. However, the pH of the surrounding environment affects their stability, and the presence of unsaturated bonds makes them vulnerable to oxidative destruction, which may restrict long-term storage. Ufasomes have been used extensively in topical, oral, and transdermal drug delivery systems, especially for medications that improve solubility, permeability, and overall therapeutic efficacy, such as antifungals, anti-inflammatory agents, and lipid- lowering medications like lovastatin.[1-5]

MATERIALS:

Lovastatin was obtained from Yarrow Chemicals (Mumbai, India). Cholesterol, isopropyl alcohol, oleic acid, and arachidonic acid were procured from Lobe Chemie (Mumbai, India). Sodium oleate was purchased from Ibuy chemicals (Mumbai, India). All reagents were of analytical grade and used as received without further purification.[6-10]

METHOD OF PREPARATION:

Reverse Phase Evaporation Method:

Reverse phase evaporation method involves dissolving fatty acids such as oleic acid, arachidonic acid, and sodium oleate along with cholesterol in an organic solvent, isopropyl alcohol, to form the organic phase. Lovastatin is dissolved in distilled water to prepare the aqueous phase. The aqueous phase is added slowly into the organic phase with continuous stirring on a magnetic stirrer to form a water-in-oil emulsion. The mixture is then subjected to probe sonication to obtain a stable emulsion with reduced droplet size. The organic solvent is removed under reduced pressure using a rotary evaporator at controlled temperature, leading to the formation of a gel-like intermediate which upon further evaporation results in the formation of Ufasomal vesicles. The obtained dispersion is further sonicated to reduce particle size and obtain uniform vesicles. The prepared Ufasomes are observed under a binocular microscope for confirmation of vesicle formation and subsequently

subjected to Lyophilization for drying and further characterization.[11-15]

EXPERIMENTAL DESIGN:

In this current Research work Definitive Screening Design under Response Surface Methodology was used by taking Independent variables and Dependent variables. The Independent variables are surfactant concentration, cholesterol, organic phase, stirring speed, oleic acid: Arachidonic acid and dependent variables are particle size, entrapment efficiency, zeta potential.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Response 1	Response 2	Response 3
Run	A: Surfactant concentration	B:Cholesterol	C: Organic Phase	D: Stirring speed	E: Oleic acid: Arachidonic acid	Particle Size	Entrapment Efficiency	Zeta potential
	w/v	w/w	v	RPM	w/w	nm	%	mV
1	1	10	30	1250	3:1	195	83	-38
2	5	10	17.5	500	3:1	241	73	-30
3	5	50	5	1250	1:1	280	68	-32
4	1	30	30	500	1:1	190	86	-33
5	5	30	5	2000	3:1	285	66	-37
6	3	30	17.5	1250	3:1	243	74	-37
7	5	50	30	500	3:1	281	64	-31
8	3	50	30	2000	3:1	276	67	-39
9	1	10	5	2000	1:1	193	88	-38
10	3	30	17.5	1250	1:1	232	78	-33
11	1	50	17.5	2000	1:1	233	79	-38
12	5	10	30	2000	1:1	259	72	-30
13	1	50	5	500	3:1	216	80	-40
14	3	10	5	500	1:1	198	85	-31

Calibration curve:

Prepare a series of standard solutions of known concentrations and measure their absorbance using a suitable analytical instrument UV– vis spectrophotometer Plot absorbance versus concentration to obtain a calibration curve and use it to determine unknown sample concentrations.[16,17]

FT-IR:

The sample is prepared (e.g., mixed with KBr and compressed into a pellet or placed directly on the ATR crystal) and exposed to infrared radiation in an FTIR spectrophotometer. The instrument records the absorption spectrum, which is used to identify functional groups and characterize the sample.[18-20]

Particle Size:

The particle size of ufasomes is determined by diluting the vesicle dispersion with distilled water and analyzing it using dynamic light scattering (DLS) in a particle size analyzer. The instrument measures the mean vesicle diameter and size distribution based on light scattering patterns.[21-24]

Zeta potential:

The zeta potential of ufasomes is measured by diluting the vesicle dispersion with distilled water and analyzing it using a zeta potential analyzer based on electrophoretic mobility. It indicates the surface charge of vesicles, which helps predict stability and aggregation behavior.[25,26]

Entrapment Efficiency:

Entrapment efficiency of Ufasomes is determined by separating the unentrapped (free) drug from vesicles using centrifugation or dialysis. The amount of entrapped drug is quantified by UV–Vis Spectro photometry and expressed as a percentage of the total drug used.[26-28]

X-Ray Diffraction:

The Ufasome sample is Lyophilized, finely powdered, and placed in the sample holder of an X-ray diffractometer. The diffraction pattern is recorded over a suitable 2θ range to determine crystallinity and possible drug–excipient interactions. [28]

Diffraction Scanning Calorimetry (DSC):

The lyophilized Ufasomes sample is accurately weighed, sealed in an aluminum pan, and placed in a Differential Scanning Calorimeter. The sample is heated at a controlled rate to record thermograms, which reveal phase transitions and drug–excipient interactions. [28,29]

Transmission electron microscopy (TEM):

A drop of diluted Ufasomes dispersion is placed on a carbon-coated copper grid, stained with phospho tungstic acid, and allowed to dry. The sample is then observed under a transmission electron microscope to study vesicle size, shape, and morphology. [28-30]

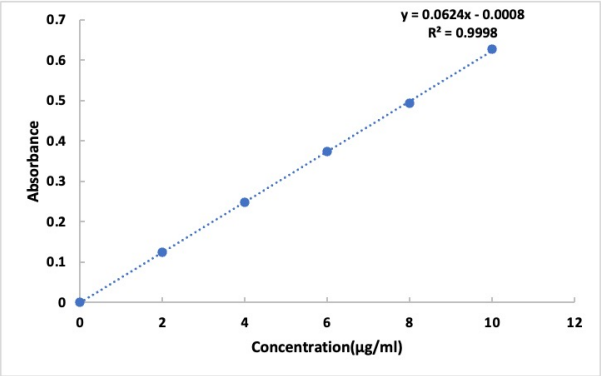


Fig: No.1Standard Calibration Curve of Lovastatin

The calibration curve of Lovastatin shows a linear increase in absorbance with concentration, following the Beer–Lambert law. The high R^2 value (0.9998) indicates excellent accuracy and reliability for quantitative analysis.

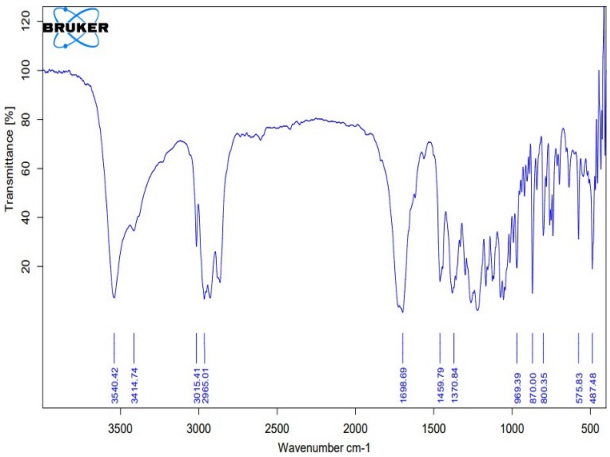


Fig: No.2 FT-IR Spectra of Optimized Lovastatin loaded Ufasomes

The FT-IR spectrum of lovastatin shows characteristic peaks confirming its functional groups and structural integrity. The broad band around 3015 cm^{-1} indicates O–H stretching, while peaks at 2950–2850 cm^{-1} correspond to aliphatic C–H stretching. A strong peak near 1700–1735 cm^{-1} represents C=O stretching of ester/lactone groups, and bands in the range of 1600–1450 cm^{-1} indicate C=C vibrations. Peaks between 1250–1050 cm^{-1} are due to C–O stretching. The absence of significant peak shifts suggests that the drug is stable and shows no chemical incompatibility.

Optimization And Characterization Of Lovastatin Loaded Ufasomes By Definitive Screening Design

Table 1: ANOVA results of the response variable of Experimental Design

	Particle Size (R1)				Entrapment Efficiency (R2)				
Source	Sum of Squares	Mean Square	F-value	p-value	Sum of Squares	Mean Square	F-value	p-value	
	16056.36	1784.04	14219.	< 0.0001	820.79	91.20	2672.45	< 0.0001	
Model		21							significant
A-Surfactant concentration	9207.28	9207.28	73384.	< 0.0001	458.64	458.64	13439.69	< 0.0001	
B-Cholesterol	3471.11	3471.11	27665.	< 0.0001	145.89	145.89	4275.03	< 0.0001	
C-Organic Phase	32.41	32.41	258.30	< 0.0001	11.31	11.31	331.51	< 0.0001	
D-Stirring speed	1655.11	1655.11	13191.	< 0.0001	39.50	39.50	1157.59	< 0.0001	
E-Oleic acid: Arachidonic acid	355.56	355.56	2833.9	< 0.0001	54.52	54.52	1597.51	< 0.0001	
A^2	0.0806	0.0806	0.6423	0.4678	0.0411	0.0411	1.20	0.3340	
B^2	0.2368	0.2368	1.89	0.2414	0.0411	0.0411	1.20	0.3340	
C^2	0.0806	0.0806	0.6423	0.4678	0.0411	0.0411	1.20	0.3340	
D^2	0.2368	0.2368	1.89	0.2414	0.3224	0.3224	9.45	0.0372	
Residual	0.5019	0.1255			0.1365	0.0341			
Cor Total	16056.86				820.93				

Zeta potential (R3)

Source	Sum of Squares	Mean Square	F-value	p-value
Model	174.00	19.33	214.85	< 0.0001
A-Surfactant concentration	92.90	92.90	1032.45	< 0.0001
B-Cholesterol	7.90	7.90	87.83	0.0007
C-Organic Phase	11.65	11.65	129.50	0.0003
D-Stirring speed	42.59	42.59	473.32	< 0.0001
E-Oleic acid: Arachidonic acid	48.80	48.80	542.36	< 0.0001
A^2	0.2368	0.2368	2.63	0.1800
B^2	0.2368	0.2368	2.63	0.1800
C^2	1.11	1.11	12.36	0.0246
D^2	0.2368	0.2368	2.63	0.1800
Residual	0.3599	0.0900		
Cor Total	174.36			

Equation

$Y_1 = 237.47+30.83A+18.93B+1.83C+13.07D+5.35E+0.1842A^2-0.3158B^2+0.1842C^2-0.3158D^2$

$Y_2 = 75.95-6.88A-3.88B-1.08C-2.02D-2.10E-0.1316A^2-0.1316B^2-0.1316C^2+0.3684D^2$

$Y_3 = 34.97+3.10A-0.9032B+1.10C-2.10D-1.98E+0.3158A^2+0.3158B^2$

From ANOVA table the model is significant

Effect of Independent Varibles Vs Response Y1(Particle size)

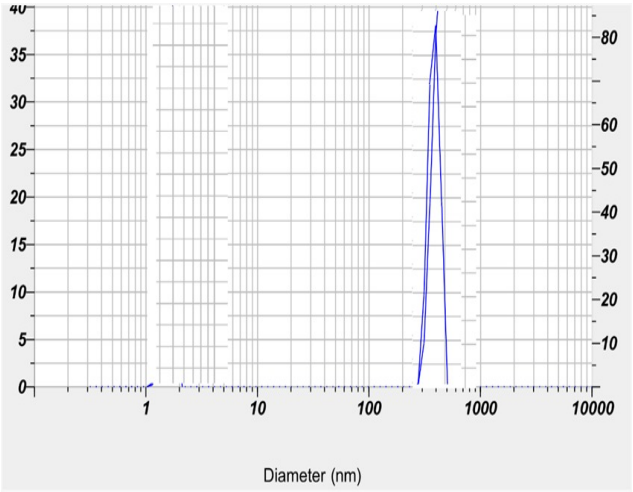
The given equation represents a polynomial regression model used to predict particle size based on formulation variables (A, B, C, D, and E).The constant (237.47) indicates the baseline particle size, while coefficients of A–E show how each factor influences particle size (positive values increase size, negative values decrease it).The quadratic terms (A^2, B^2, C^2, D^2) indicate non-linear effects, meaning changes in these variables can have curvature impact on particle size rather than a simple linear relationship.

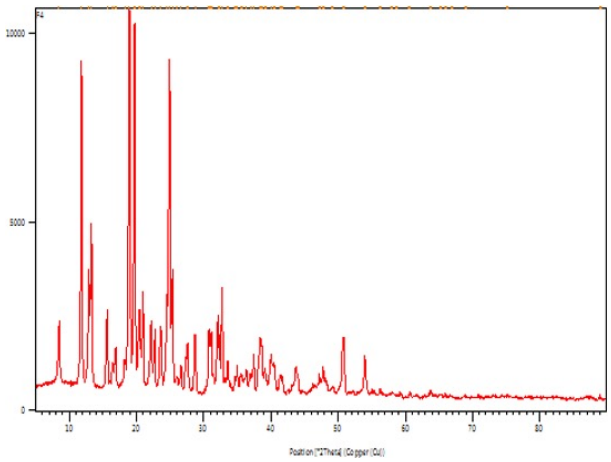
Effect of Independent varibles Vs Response Y2(Entrapment Efficiency)

The equation represents a quadratic regression model where the baseline entrapment efficiency is 75.95, and negative coefficients of A–E indicate that increasing these factors decreases entrapment efficiency. Quadratic terms show non-linear effects, with A^2, B^2, and C^2 reducing efficiency at higher levels, while D^2 has a positive influence, indicating improvement at higher concentrations.

Effect of Independent varibles Vs Response Y3(Zeta Potential)

The equation represents a quadratic regression model for predicting zeta potential based on formulation variables (A–E). The constant (−34.97) indicates the baseline zeta potential, while A and C (positive coefficients) increase zeta potential, whereas B, D, and E (negative coefficients) decrease it. The quadratic terms show non-linear effects: A^2, B^2, and D^2 (positive) enhance zeta potential at higher levels, while C^2 (negative) reduces it due to curvature influence.

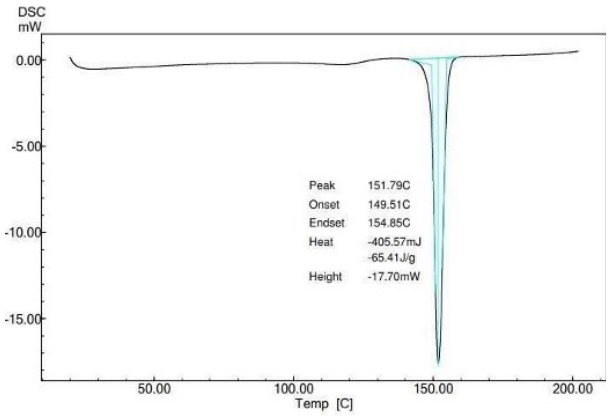




-35mV

Particle size 185nm

Fig: No.3 Zeta Potential and Particle Size of Optimized Formulation



A)
B)

Fig: No.4 DSC STUDIES of A) Pure drug, B) Optimized formulation (OPLU1)

DSC Studies: Reduction in Melting points from 151ºC to 127 ºC from pure Drug to Optimized Formulation in DSC peaks indicates decrease in crystallinity and increase in solubility.

A)
B)

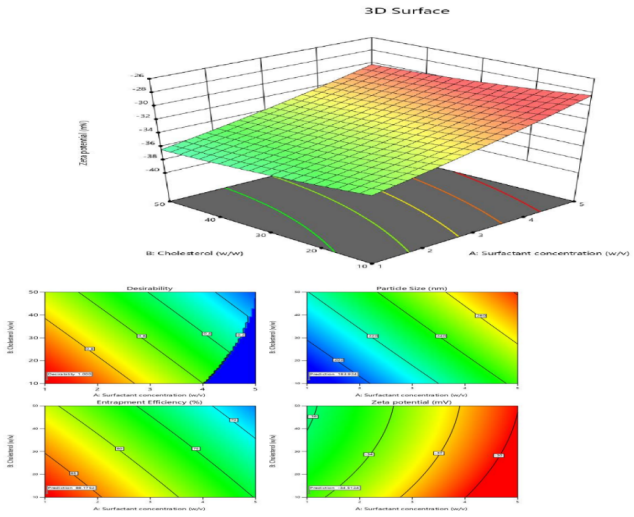


Fig: No.5 XRD STUDIES Of A) Pure Drug, B) Optimized Formulation

XRD Studies: Reduction in Peak Intensities in XRD peaks from pure Drug to Optimized Formulation indicates decrease in crystallinity and increase in solubility.

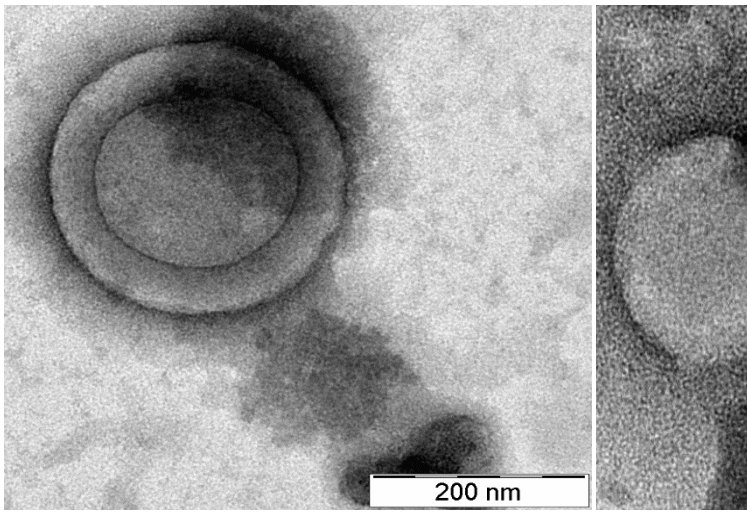
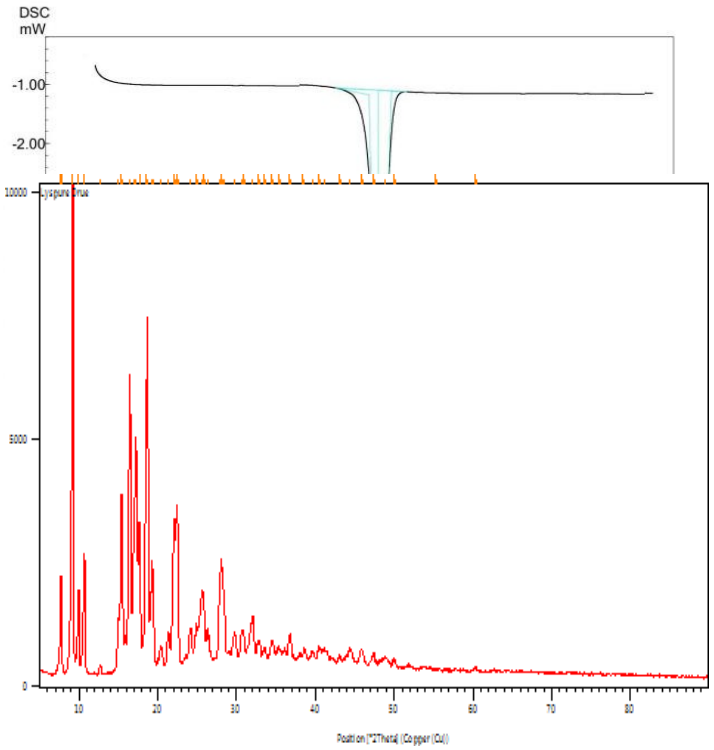
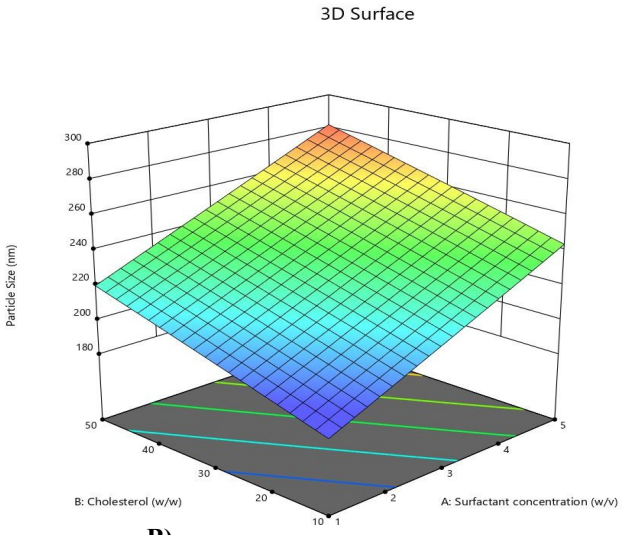


Fig: No. 6 TEM Images of Optimized Formulation (OPLU1)

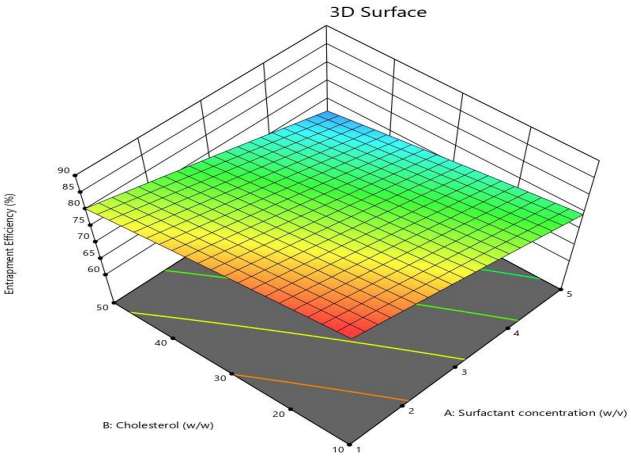


Optimization And Characterization Of Lovastatin Loaded Ufasomes By Definitive Screening Design

A)



B)



C)

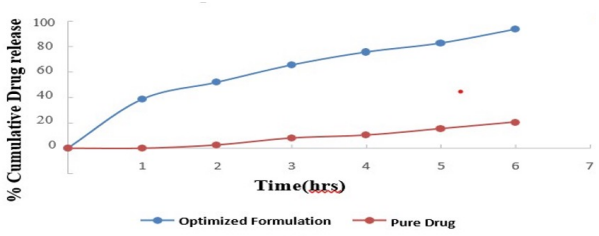


Fig: No. 7 3D SURFACE PLOT OF RESPONSE OF A) Particle size (Y1), B) Entrapment Efficiency(Y2), C) Zeta Potential (Y3).

The data shows that the optimized formulation releases the drug much faster and in higher amounts compared to the pure drug over 6 hours. While the optimized formulation reaches about 93% drug release, the pure drug releases only around 20%. This means the optimized formulation improves drug release and works more effectively, showing a steady and controlled release pattern.

Kinetic Study: To Know drug release kinetics of the optimized formulation of the dissolution were subjected to different Kinetic models such as Zero order, First order, Higuchi, Korsmeyer peppasetc…

Table.NO.2Kinetic Studies of Optimized Formulation OPLU1

Formulation code	Zero order R^2	First order R^2	Higuchi model R^2	Korsmeyerpeppas model	
				R^2	n
OPLU1	0.924	0.964	0.997	0.503	1.5

From the above observations Kinetic analysis (R^2) of release data based on best curve fitting method for Lovastatin loaded Ufasomes, R^2 value is more for Higuchi model i.e., $\mathbf{R^2}$= 0.9979 indicating that the drug release from Ufasomal carrier is based on Fickian diffusion, predicting that the rate of drug release is proportional to the square root of time.

CONCLUSION:

Lovastatin, a BCS class II medication used to treat hyperlipidemia, has a low oral bioavailability of 5%. To get over its solubility problems, it acts on an HMG CoA reductase inhibitor. Ufasomes were loaded with lovastatin as nanocarriers. The Definitive screening design was used to optimize the Lovastatin-loaded Ufasomes. Oleic acid, Arachidonic acid, stirring speed, and cholesterol were considered independent factors, while particle size, Zeta potential, and entrapment efficiency were

considered dependent variables. The Lovastatin-loaded Ufasomes were prepared using the reverse phase evaporation method, and then they were characterized using DSC, XRD, and TEM analysis. Among fourteen formulations obtained through Definitive screening design, OPLU-1 was chosen as an optimized formulation with less particle size of 185 nm, and maximum entrapment efficiency of 88 %. In vitro dissolution studies were conducted for both the optimized formulation and the pure drug (lovastatin). A comparison of the dissolution profiles of the optimized formulation and the pure drug. The optimized formulation revealed amaximumdrugreleaseof93.35% within 6 hours, which is greater solubility and drug release. For the optimized formulation (OPLU- 1), kinetic studies such as zero order, first order, Higuchi, and korsmeyer pepas were conducted. The results showed that the Higuchi model had a maximum R square value of 0.997, indicating that the drug release from the carrier is based on Fickian diffusion, predicting that the rate of drug release is proportional to the square root of time.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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