FORMULATION DESIGN AND EVALUATION OF POLYMERIC NANOPARTICLES OF LOVASTATIN BY 3^2 FACTORIAL DESIGN

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ABSTRACT

Lovastatin is an Oral Antilipidemic Drug Which inhibit the HMG- COA reductase used in the treatment of Hypercholesterolemia and Coronary Heart Disease. This study aim to design and develop Lovastatin loaded Nanoparticles using 3^2 factorial design to optimize formulation parameters for sustained drug release by using Nanoprecipitation Method. The 3^2 factorial design was employed to evaluate the effects of Eudragit L-100(X_1) and TPP(X_2) are independent variables Concentration on critical parameters like particle size(Y_1) and Percentage of drug release(Y_2) are dependent variables that the optimization techniques provides on efficient and economical method to give the essential information and help to understand the relation between controlled independent and dependent variable. The FTIR, SEM and Zetapotential studies are conducted. The result shows optimized formulation exhibited sustained release profile with 99.42% of drug being released over a 12hrs, following Non-fickian release kinetics. The study demostrates the potential of Lovastatin loaded Eudragit L-100 and Tpp Nanoparticles as a promising approach for Sustained drug delivery, offering advantages in terms of improved bioavailability, reducing dosing frequency and enhanced patient compliance.

KEYWORDS: Lovastatin, Antilipidemic agent, TPP, Eudragit L-100, FTIP, SEM, Non-fickian release kinetics.

INTRODUCTION

The aim of any drug delivery system is to provide the appropriate location in the body with a sufficient amount of medication to reach efficiently and then retain the required concentration of drug. That is, over a given duration of care the drug delivery system will deliver drugs at a pace determined by the body's needs. This idealized target points to the two most critical aspects of drug distribution, namely spatial positioning and the temporal distribution of a drug. Spatial placement refers to delivering the drug to a specific organ or tissue, while temporal delivery refers to regulating the rate of delivery of drugs to the target tissue. A properly built managed drug-delivery release system can be a big step towards solving these two problems. It is for this reason that in industrial and academic laboratories the science and technology responsible for the production of controlled-release pharmaceuticals has been, and continues to be, the subject of much attention.

CONVENTIONAL DRUG THERAPY[1]:

Reviewing certain basic aspects of traditional drug delivery is useful for gaining understanding for the importance of guided drug therapy. Consider single dosing of a hypothetical drug using a basic one-compartment pharmacokinetic dispensing model.A traditional dosage type of the drug, e.g.: a solution, suspension, capsule tablet etc., may produce a blood level versus time profile, depending on the route of administration. The word drug blood levels refers to the concentration of drug in blood or plasma, but the concentration may be plotted on the ordinate in any tissue. The administration of a drug by either intravenous injection or an extra vascular route, e.g. orally, intramuscularly or rectally, does not sustain drug blood levels for prolonged periods of time within the therapeutic range. The limited period of action is attributed to the

inability to control temporal distribution of traditional dosage types. When an effort is made to maintain medication blood levels within the therapeutic range for longer periods, e.g. by increasing the initial intravenous injection dosage, early toxic levels can be produced. Clearly the strategy is unacceptable and inappropriate. An alternative approach is to administer the drug repetitively using a constant dosing interval, as in multiple-dose therapy.In this case the blood level of the drug has reached and the time taken to reach that level depends on

the dosage and the period of dosing. Multiple-dose treatment poses many possible challenges.

1. 1. If the dosing period is sufficient for the drug's biological half-life, it may result in broad peaks and valleys at the level of the drug blood. Of example, drugs with short half-lives need regular designs to keep the dosage rates stable.
2. 2. The amount of the drug blood might not be within the therapeutic range at an early enough stage, which is an significant factor for some disease states.
3. The failure of this strategy can result in patient non-compliance with the multiple-dose regimens.

Potential issues associated with traditional drug treatment can be resolved in many cases. When this is the case, drugs administered by multiple dosing in conventional dosage forms can produce the desired blood level of the drug for an extended time period. However, often these problems are sufficiently significant to make drug therapy with conventional dosage forms less desirable than controlled releases.This reality, combined with the inherent inability to achieve spatial positioning of traditional dosage types, is a compelling reason for investigating controlled-release drug delivery systems.

MATERIALS AND METHODS

Preformulation Studies

Description of drugs

Physicochemical properties of drugs such as state, colour and odour were physically examined and compared with the reported description of drugs. The sample was taken in a clean glass slide. Colour and physical form were inspected visually and noted.

Melting point determination

The melting point of the drug was determined using capillary melting point method. The temperature range over which the drug melts was observed visually.

Solubility studies

The solubility of drugs was determined in different solvent systems. Small amounts of the drugs were added to 5 ml of each solvent in screw-capped glass tubes and shaken. The solutions were examined physically for the absence or presence of drug particles qualitatively. Quantitative solubility determined by UV-Spectrophotometer at 258nm for lovastatin.

Calibration Curve of Lovastatin

The primary Stock solution at a concentration of 1mg/mL was prepared by dissolving accurately weighed quantity of pure drug lovastatin (100 mg) in pH 6.8 phosphate buffer and made up to 100 mL with same buffer solution. Aliquots were prepared from the stock solution by pipetting 5, 10, 15, 20, 25 and 30mL to get concentrations of 5, 10, 15, 20, 25 and 30µg/mL by diluting with same buffer solution. The absorbance of prepared aliquots was measured at 246nm using UV- spectrophotometer against an appropriate blank. The standard calibration curve showed linearity, through that the drug obeys Beers and Lambert's law in the concentration range of 5–30 µg/mL. A standard graph was plotted by keeping the known concentration on X-axis and obtained absorbance on Y-axis.

Experimental Design

A 3^2 factorial Design with two factors at three levels was employed to systematically study the formulation of lovastatin nanoparticles. A total of nine trails were performed at all possible combinations. The Independent variables are Eudragit L-100(X1) and Sodium Tripolyphospahte (X2) were selected on the basis of trails taken during optimization of excipients which were varied at three levels (Low, Medium and High). The levels of the factors were studies were chosen so that their relative difference was adequate to have measurable effect on the response along, with the information that the selected levels are with in practical use. Particle Size (Y1) and *Invitro* drug release stdies (Y2) were used as dependent variables. Design Expert 11.0 Software (State /Ease., USA) was used for the generation and evaluation of statistical experimental design.

Table 6.3: Composition of the Lovastatin loaded Nanoparticles by 3^2 factorial design

SNO	Formulation Code	Eudragit RS-100	Sodium Tripolyphosphate
1	LN1	1	1
2	LN2	1	1.25
3	LN3	1.25	1.25
4	LN4	1.5	1.25
5	LN5	1.25	1
6	LN6	1	1.5
7	LN7	1.5	1
8	LN8	1.25	1.5
9	LN9	1.5	1.5

Method of preparation of Lovastatin nanoparticles:

The Lovastatin nanoparticle was prepared using TPP as a cross linker with a slight modification according to the nanoprecipitation technique. Through magnetic stirring lovastatin was applied to this solution after complete dissolution of Eudragit L- 100 in acetic acid. Sodium tripolyphosphate (STPP) was then added in dropwise at a uniform rate through a syringe. In this form, glacial acetic acid dissolved in distilled water by 1.6 percent, and more Eudragit L-100 used in different concentrations. It was further stirred at 13000 rpm for 2 hours followed by a 5 min centrifugation. The supernatant was discarded, and pH-6.8phosphate buffer nanoparticles were re-suspended

Table 6.4: Composition of the Lovastatin loaded Nanoparticles

Formulation Code	Lovastatin (mg)	Eudragit RS-100 (%)	Sodium Tripolyphosphate (%)	Acetic acid (v/v)
LN1	40	1	1	20
LN2	40	1	1.25	20
LN3	40	1.25	1.25	20
LN4	40	1.5	1.25	20
LN5	40	1.25	1	20
LN6	40	1	1.5	20
LN7	40	1.5	1	20
LN8	40	1.25	1.5	20
LN9	40	1.5	1.5	20

EVALUATION OF LOVASTATIN LOADED NANOPARTICLES[24-28]

Particle Size and PDI:

Particle size measurement was carried out using dynamic laser scattering. Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK) had calculated the particle size and PDI of NPs. To determine the particle size the NPS suspension was diluted with distilled water. Twelve measurements were taken, and determined their average.

Figure 6.1: MALVERN Particle size and Zeta potential Analyzer

Entrapment Efficiency (EE):

Entrapment Efficiency (EE) of the nanoparticles in Lovastatin was calculated by an indirect process. In short, the suspension of the NPs had been centrifuged for 10 min at 12000 rpm. The supernatant collected was diluted with methanol, and the volume of free Lovastatin current 238 nm spectrophotometer. Using of UV - visible was quantified in the supernatant. The EE was calculated using the formulation as follows:

% Entrapment Efficicency= (Amount of lovastatin added in formulation- Amount present in supernatant))/(Amount of lovastatin added in formulation)

×100

The percentage yield of nanoparticle:

The yield of nanoparticle was determined by comparing the whole of nanoparticle formed against the combined weight of the copolymer and drug

% Yield = (Amount of Drug)/ (Amount of drug+ polymer) ×100

RESULTS AND DISCUSSION

Description of Drugs

An identification of lovastatin based on physical examination and melting point results are mentioned in the following table 7.1.

Table 7.1: Identification of Lovastatin

Sr. No.	Test	Specification	Observation	Inference
1	State	Solid Crystalline	Solid Crystalline	Complies
2	Colour	White	White	Complies
3	Melting Point	174°C	173.5°C	Complies

From the tables 7.1, it was observed that the obtained value of melting point of all the drugs was found to be similar to the referenced value which proved that the received drug samples meet the reported properties.

Solubility Study

The solubility of both drugs was determined by dissolving the drug in different solvents such as Distilled Water, pH 6.8 phosphate buffer, methanol, ethanol, ether, chloroform, and acetone. The solubility of these drugs in various solvents was determined by visual examination as well as experimentally for quantitative solubility and results were shown in table 7.2 respectively.

Table 7.2: Solubility Profile of Lovastatin

Sr. No.	Solvent	Solubility	Solubility (gm/mL)
1	Distilled Water	-	0.01 ± 0.2
2	pH 1.2 HCl Buffer	++	12.26 ± 0.96
3	pH 6.8 Phosphate Buffer	++	25.32 ± 0.74
4	Methanol	+++	56 ± 0.68
5	Ethanol	+++	32.24 ± 0.79
6	Ether	+	0.51 ± 0.016
7	Chloroform	+	0.65 ± 0.033
8	Acetone	+	1898 ± 0.02

++++ Freely Soluble ++ Soluble + Slightly Soluble - Insoluble

From the solubility studies, it was revealed that lovastatin in insoluble in distilled water and freely soluble in methanol and ethanol, slightly soluble in ether, chloroform and acetone.

Calibration Curve of Lovastatin

The calibration curve of lovastatin was prepared in pH 6.8 phosphate buffer in 5- 30 µg/ml concentration range. The absorbance of the corresponding concentration was determined at λmax 248 nm by using UV spectrophotometer, and absorbances were shown in table 7.3. A curve was plotted between concentrations versus absorbance, as shown in figure 7.1respectively. Linearity was obtained within the 5-30 µg/ml concentration range. This indicated that beer's lamberts law was followed over this range. The R^2 value of the curve in pH 6.8 phosphate buffer was found to be 0.9991 respectively.

Table 7.3: Calibration data for lovastatin in pH 6.8 Phosphate buffer

Sr. No.	Concentration (µg/mL)	Absorbance
1	0	0
2	5	0.137
3	10	0.311
4	15	0.439
5	20	0.611
6	25	0.788
7	30	0.956

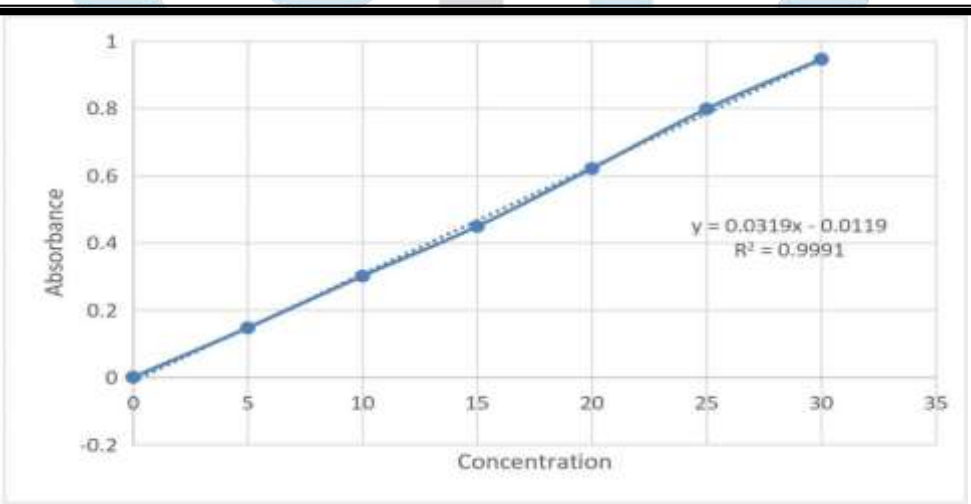


Figure 7.1: Calibration Curve of Lovastatin in pH 6.8 Phosphate buffer

EVALUATION PARAMETERS

Table 7.4: Evaluation Studies of Prepared Nanoparticles

Formulation Code	Drug Content (%)	Entrapment Efficiency (%)	Loading Efficiency	Percentage Yield (%)
LN1	75.24	84.48	56	75.28
LN2	71.54	64.87	52	70.48
LN3	60.78	80.24	54	84.78
LN4	64.87	92.38	48	96.28
LN5	62.98	84.89	49	85.14
LN6	65.21	86.29	48	83.89

LN7	59.34	77.57	67	82.74
LN8	91.98	85.78	49	88.12
LN9	84.89	78.47	78	85.42

Drug Content, Entrapment Efficiency, Drug Loading and Percentage yield were given in table 7.4. Practical yield of the prepared nanoparticles was in the range of 70.48 to 96.28. The yield of nanoparticles decreased with increasing the concentration of polymer ratio, which might be due to generation of stickiness by polymer Eudragit L-100.

It was found that with increasing the amount of polymer, the actual drug loading and EE increased. The EE was found to be in the range from 64.28 to 92.38%. The drug loading of nanoparticles was found to be in the range of 48 to 78 %. It was observed that the drug content and encapsulation efficiency depends on the concentration of polymer, solvent ratio and stirring rate.

Table 7.7: Variables in 3^2 factorial design for Lovastatin Loaded Nanoparticles

Independent Variables	**Levels**		
	Low	**Medium**	**High**
Eudragit L -100 (%)	1	1.25	1.5
TPP(%)	1	1.25	1.5
Dependent Variables			
Particle Size (nm) (Y1)			
Invitro drug release (%) (Y2)			

Table 7.8: Observed Responses for lovastatin loaded nanoparticles by 3^2 factorial design

Formulation Code	**Eudragit RL-100**	**TPP**	**Particle Size (nm)**	***Invitro* Drug release (%)**
LN1	1	1	210.51	91.18
LN2	1	1.25	167.8	95.47
LN3	1.25	1.25	233.33	96.41
LN4	1.5	1.25	198.4	97.35
LN5	1.25	1	252	94.59
LN6	1	1.5	168.9	99.75
LN7	1.5	1	195	97.99
LN8	1.25	1.5	268	98.23
LN9	1.5	1.5	267	96.71

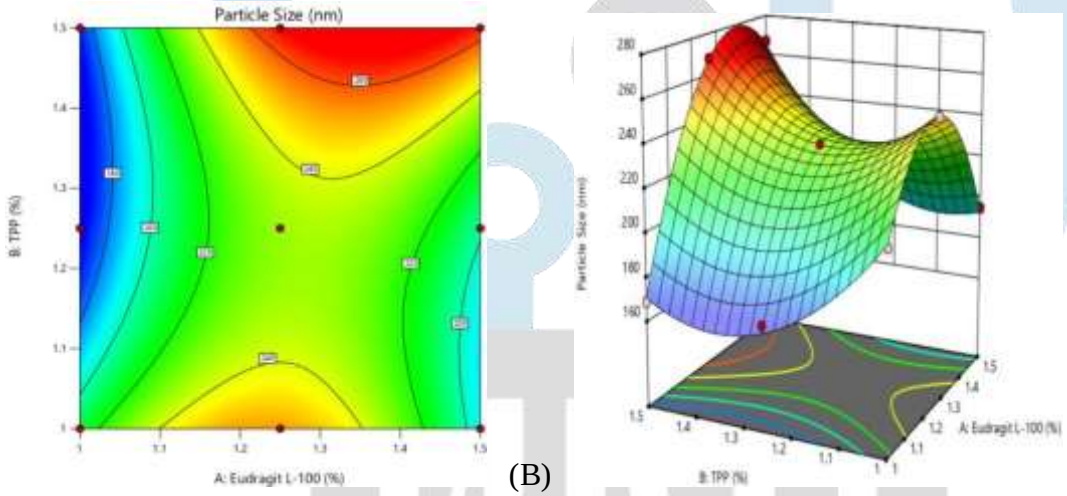
Results depicted in table 7.9 that the variables chosen have strong influence on the selected responses, as particle size and invitr drug release values were in the range of respectively.

Effect of particle size on drug loaded nanoparticles

The following polynomial equation was proposed by the model for Particle size (*Y1*) of the formulation

Particle Size (*Y1*) = 233.07 +18.86 A +7.73 B + 28.40 AB – 49.84 A^2 + 27.06 B^2 -- (1)

Where *Y1* is the particle size of lovastatin loaded formulations, A is the Eudragit RS-100 and B is the concentration of TPP. Among the independent factors, polymer concentration was observed to have significantly higher positive effect on the particle size of the nanoparticles and is evident from the very high positive value for its coefficient. The negative coefficients for the concentrations shown that the particle size of the nanoparticles was increased at higher concentrations. The interaction between the independent factors is also found to be significant. Overall, the model is significant (F- value = 189.43; $p < 0.0006$). Figure 7.3 shows that the contour plots and its 3D response plots which show the effect of different independent factors on pH of the gel.



(A) (B)

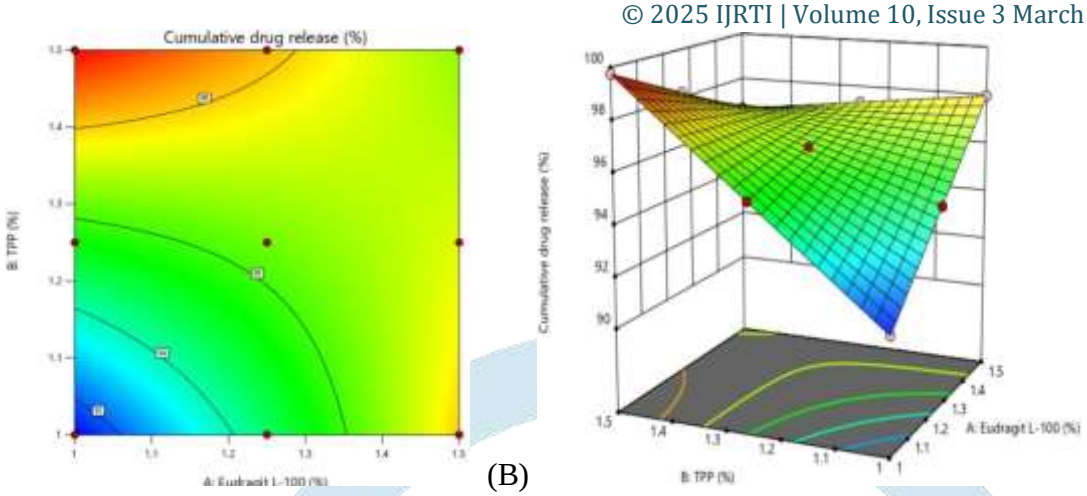
Figure 7.3: Counter Plot (A) and its Response surface plot (B) showing the effects of X1 and X2 on Particle size

Effect of % drug release on Lovastatin Loaded Nanoparticles

The following polynomial equation (2) was proposed by the model for % cumulative drug release (*Y2*) on formulation

Invitro drug release (*Y2*) = 96.41 + 0.94A + 5.76 B -2.46 AB---------- (2)

All independent factors were observed to have significantly higher positive effect of the invitro drug release of lovastatin loaded nanoparticle formulations and is evident from the very high positive value for its coefficient. The negative coefficients for the concentrations shown that nanoparticle formulations is decreased at higher concentrations. The interaction between the independent factors is also found to be significant. Overall, the model is significant (F-value = 269.37; $p < 0.0001$). The R squared values were in reasonable agreement. The signal to noise ratio was found to be satisfactory as the observed adequate precision ratio is above 4. Figure 7.4 represents the contour plots and its 3D response plots which show the effect of different independent factors on viscosity.



(A) (B)

Figure 7.4: Counter Plot (A) and its Response surface plot (B) showing the effects of X1 and X2 on *Invitro* drug release

Evaluation and Validation of the Optimized Formulation

Using 3^2 factorial design, all nine formulations were subjected to experimental trails, to obtain an optimized formula which is comparable to the predicted values. The required limits of the predicted values are clearly defined. Based on this, values for optimized formulation LN10 was obtained. The overlapping of the obtained values over the predicted values confirms the predictability and validation of the model.

Table 7.9: Summary of results of regression analysis for responses

	Value	F-Value	p-Value
Particle Size			
R^2	0.9968	189.43	0.0006
Adjusted R^2	0.9916		
Predicted R^2	0.9616		
Adequate Precision	35.38		
Invitro drug release			
R^2	0.9912	268.8	0.0001
Adjusted R^2	0.9948		
Predicted R^2	0.9812		
Adequate Precision	17.12		

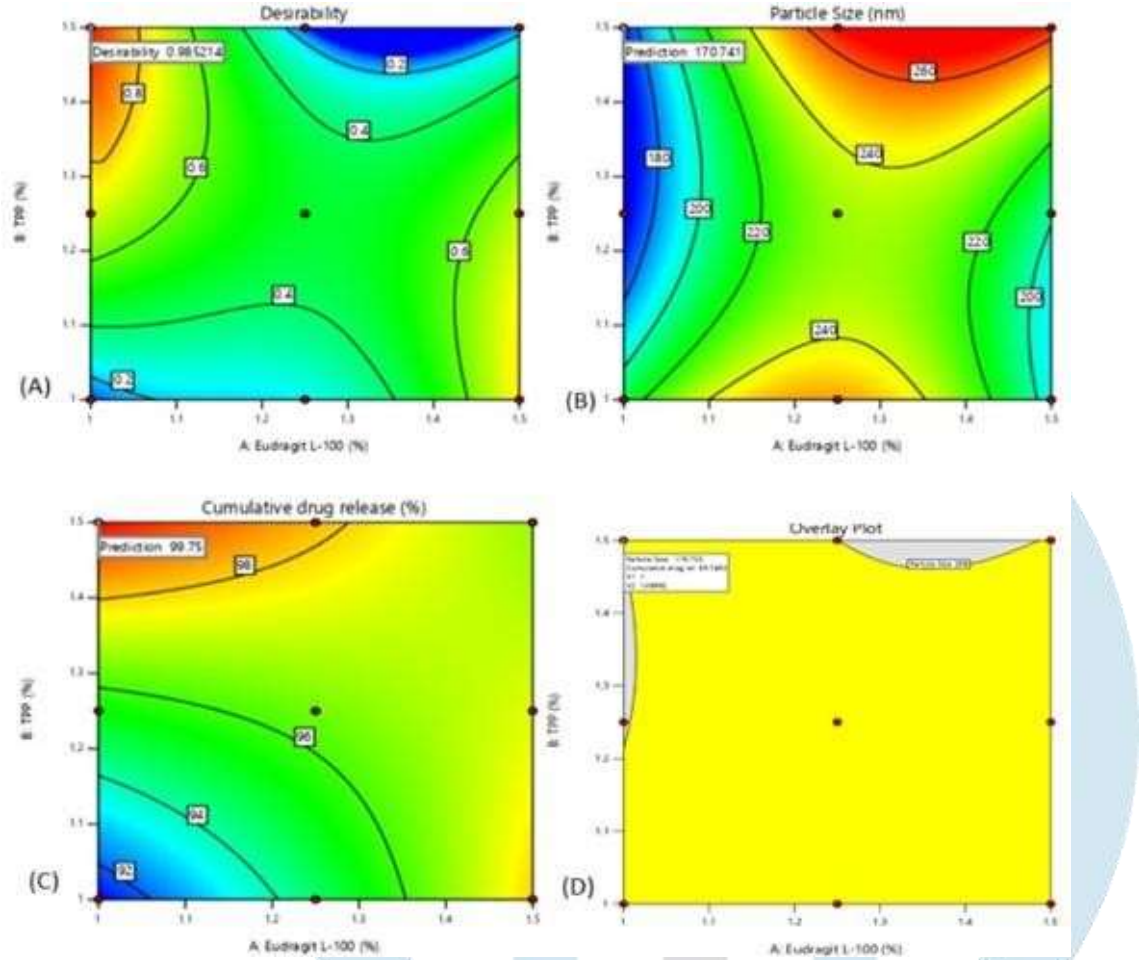


Figure 7.5: (a) Desirability value of Optimized Formulation and Counter plot of (b) Particle size (c) Invitro drug release (d) overlay plot of Lovastatin Loaded Nanoparticles

Table 7.10: Optimization of Lovastatin Loaded Nanoparticles

Independent Factors and responses		Check Point
X1: Eudragit L-100		1
X2: TPP		1.5
Y1 : Particle Size	Predicted	170.74
	Observed	172.28
Y2: Invitro drug release	Predicted	99.75
	Observed	99.42

Evaluation of Optimized Formulation

Based on the 3^2 factorial design, LN10 an optimized formulation was prepared and subjected to evaluation such as drug loading, Particle size, PDI and % Entrapment Efficiency.

Table 7.12: Evaluation of Optimized Formula LN10

Sr. No.	Evaluation Parameter	Result
1	Drug Loading	86.82
2	Entrapment Efficiency	95.23
3	Particle Size	172.28
4	Percentage Yield	98.42

Table 7.13: Invitro Drug Release studies of Optimized Formula

Time (hr)	Cumulative Percent Drug Release
0	0
0.5	12.28
1	19.32
2	27.23
3	37.12
4	42.78
6	54.13
8	75.28
10	89.13
12	99.42

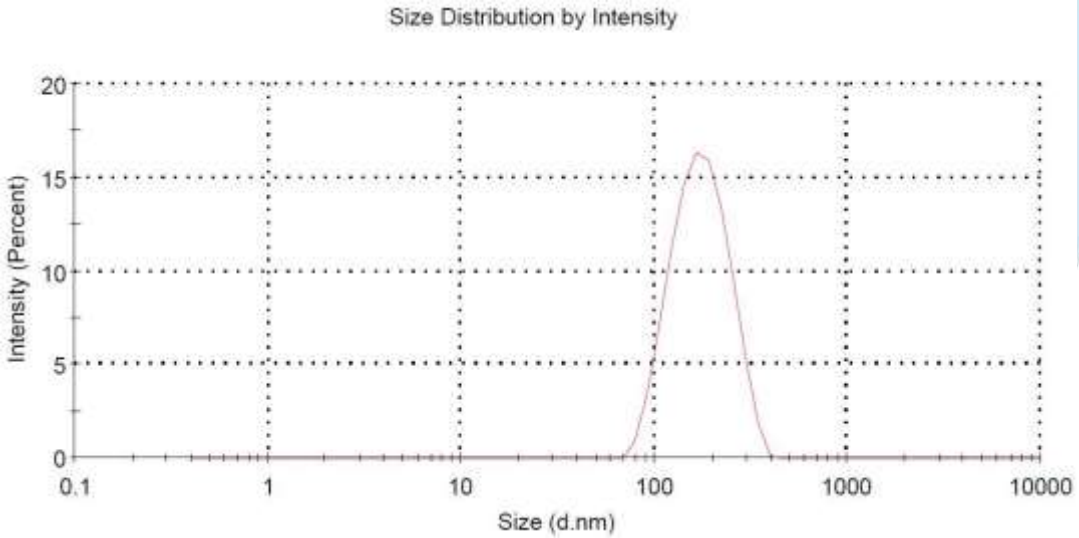


Figure 7.6: Particle size of Optimized Formulation

Characterization of Optimized Formula

Fourier Transform Infrared Spectroscopy (FTIR)

Pure drug, Excipients and Optimized formulation were subjected to FT-IR Studies. The obtained spectra are tabulated in 7.14 and shown in figure no. 7.10 and 7.11 Characteristics peaks of puredrug were comparedwith the peaksof optimized formulation. The characteristic bands of pure pure were identifiable and no major shifts were observed in optimized formulation which indicates that the drug is intact in the formulation has not reacted with the excipients.

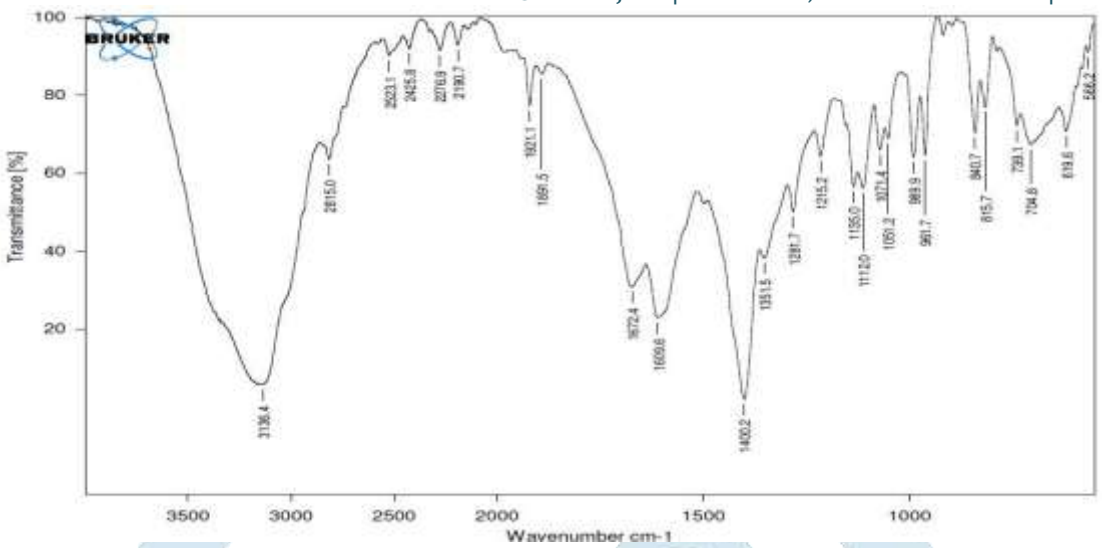


Figure 7.10: FTIR of lovastatin pure Drug

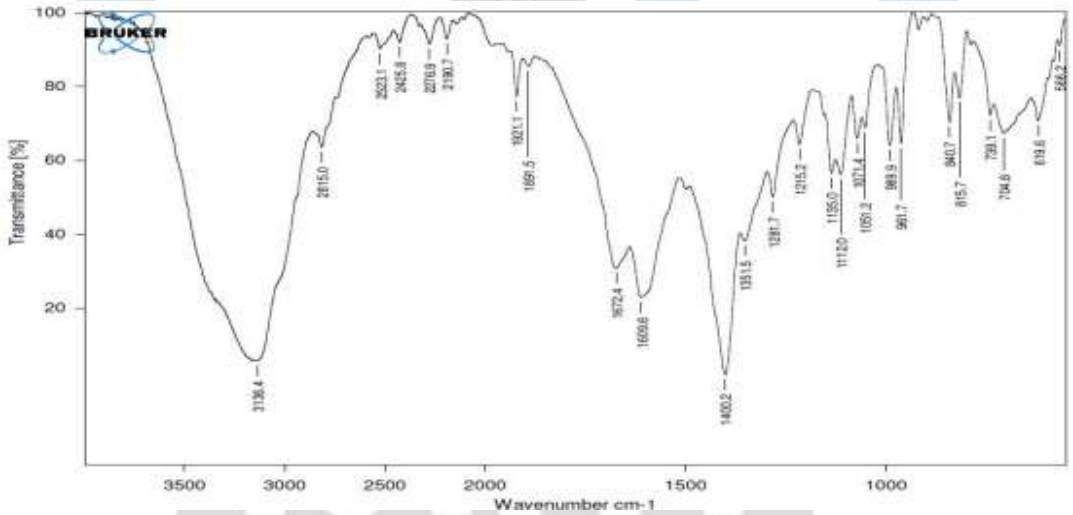


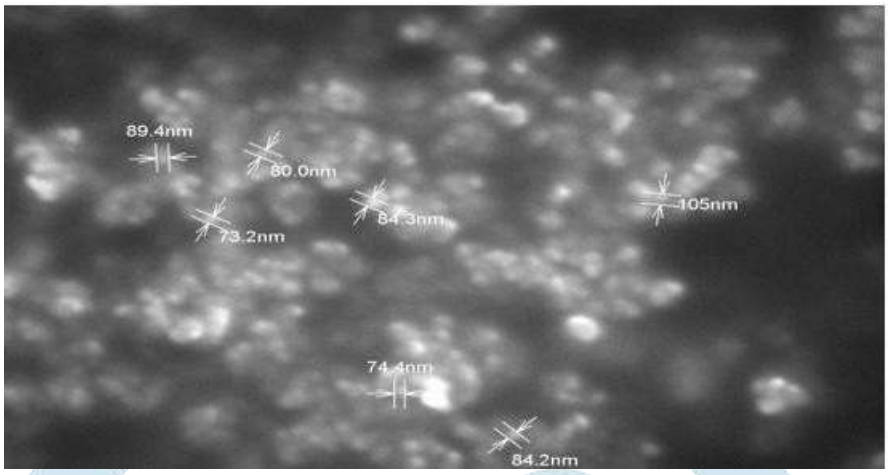
Figure 7.11: FTIR of Optimized Formulation

Table 7.13: FTIR Interpretation of Pure Drug and Optimized Formulation

S. No.	Functional Group	Observed Wave Number (cm^{-1})	
		Pure API	Optimized Formulation
1	C-H bending	740.0 cm^{-1}	755.96
2	C = C strength	1479.3 cm^{-1}	1502.8
3	C = O strength	1799.53 cm^{-1}	1819.25
4	C – H strength	3020.86 cm^{-1}	3122.45
5	O – H strength	3318.84 cm^{-1}	3489.48
6	N – H Strength	3444.0cm^{-1}	3448.0 cm^{-1}

7.7.3. Scanning Electron Microscope (SEM):

Figure 7.12 SEM has shown that the nanoparticles were small, spherical and porous in nature. The SEM image of optimized formulation appeared as smooth-surfaced particles indicating complete adsorption of lipids containing amorphous drug inside the pores of Eudragit L-100 polymer.



1. Figure 7.12: SEM Figure of Optimized Formulation

1.1. Summary

The present study focused on the formulation, optimization, and evaluation of lovastatin-loaded Eudragit nanoparticles aimed at achieving sustained drug release. Lovastatin, a lipid-lowering drug, was chosen for nanoparticle formulation to improve its bioavailability, prolong its therapeutic effect, and enhance patient compliance by reducing the frequency of dosing. The nanoparticles were prepared using the nanoprecipitation method, which is known for its simplicity, scalability, and ability to produce nanoparticles with controlled particle size and drug loading efficiency. The formulation was optimized using the 3^2 factorial design, a statistical approach that allows for the efficient exploration of multiple formulation variables and their interactions.

In this study, various concentrations of Eudragit L-100 and TPP, biocompatible and biodegradable polymer, were used to prepare Lovastatin nanoparticles. Eudragit L-100 was selected due to its pH-sensitive release properties, which are particularly useful for targeted drug delivery in the gastrointestinal tract. The nanoprecipitation method was employed to formulate the nanoparticles, where the drug and polymer are dissolved in a suitable organic solvent and then precipitated in an aqueous medium, leading to the formation of nanoparticles.

A total of nine formulations were prepared, each with varying concentrations of Eudragit L-100 and TPP. These formulations were systematically evaluated based on critical parameters, including drug loading, percentage entrapment efficiency, percent yield, and particle size. The 3^2 factorial design, a response surface methodology, was utilized to optimize these parameters. This design is advantageous because it requires fewer experimental runs compared to other designs, while still providing reliable data on the interaction between variables.

The compatibility between Lovastatin and Eudragit L-100 was assessed using Fourier Transform Infrared (FTIR) spectroscopy study. FTIR analysis revealed that there were no significant interactions between the drug and the polymer, indicating that the drug's chemical stability was maintained during the formulation process.

The morphology of the nanoparticles was examined using Scanning Electron Microscopy (SEM). The SEM images showed that the nanoparticles were spherical in shape with a smooth surface, which is desirable for controlled drug release and stability. The particle size of the prepared nanoparticles was found to range between 167.8 nm and 268 nm. The variation in particle size among the formulations was attributed to the differences in polymer concentration and the conditions used during the nanoprecipitation process.

Drug loading and entrapment efficiency are critical parameters for nanoparticle-based drug delivery systems. High drug loading indicates that a significant amount of drug is encapsulated within the nanoparticles, which is essential for achieving the desired therapeutic effect. The formulations showed satisfactory drug loading and entrapment efficiency, suggesting that the nanoprecipitation method was effective in incorporating Lovastatin into the Eudragit nanoparticles.

The *in vitro* drug release study of the optimized formulation was conducted to evaluate the release profile of Lovastatin from the nanoparticles. The results showed that the optimized formulation exhibited a sustained release profile, with 99.42% of the drug being released at the end of 12 hours. This sustained release is beneficial for maintaining a consistent drug concentration in the bloodstream, reducing the need for frequent dosing, and minimizing potential side effects.

The release kinetics of the optimized nanoparticles were analyzed to determine the mechanism of drug release. The results indicated that the drug release followed Non- Fickian kinetics, which suggests that the release mechanism is governed by both diffusion and polymer relaxation. Non-Fickian release kinetics is often observed in polymeric drug delivery systems, where the drug release rate is controlled by a combination of factors, including the diffusion of the drug through the polymer matrix and the erosion or swelling of the polymer.

CONCLUSION

In conclusion, the study successfully developed and optimized Lovastatin-loaded Eudragit nanoparticles using the nanoprecipitation method. The use of the 3^2 factorial design allowed for the efficient optimization of the formulation parameters, resulting in nanoparticles with desirable characteristics such as high drug loading, efficient drug entrapment, and controlled particle size. The FTIR study confirmed the compatibility between Lovastatin, Eudragit L-100 and TPP, while the SEM analysis demonstrated that the nanoparticles were spherical with a smooth surface, which is favorable for drug delivery applications.

The *in vitro* drug release study revealed that the optimized nanoparticles provided a sustained release of Lovastatin over 12 hours, following Non-Fickian release kinetics. This sustained release profile is advantageous for maintaining consistent therapeutic levels of the drug, potentially improving patient adherence and outcomes.

Overall, the study highlights the potential of lovastatin-loaded Eudragit nanoparticles as a promising approach for sustained drug delivery, offering advantages in terms of improved bioavailability, reduced dosing frequency, and enhanced patient compliance. Further studies, including Stability studies, *in-vivo* evaluation and clinical trials, are recommended to fully explore the therapeutic potential of this formulation.

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