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DEVELOPMENT AND EVALUATION OF SOLID LIPID NANOPARTICLES CONTAINING RASAGILINE MESYLATE BY CENTRAL COMPOSITE DESIGN

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ABSTRACT

This study aimed to create and optimize the production of SLNs loaded with Rasagiline Mesylate that were composed of Kolliwax S Fine as the lipid matrix using the homogenization procedure. Rasagiline (N-propargyl-1-R-aminoindan) mesylate (RM) is a propargylamine-based medication that is a strong, selective, and irreversible inhibitor of monoamine oxidase-B. It has been investigated for Parkinson disease treatment. The carrier system that is utilized for regulated, targeted drug delivery is called SLNs. For the purpose of optimizing SLN containing Rasagiline mesylate, the CCD was effectively utilized. Lipid, surfactant, and stabilizer concentration were the independent variables that were investigated for their influence. The constructed Rasagiline mesylate loaded SLNs' dependent variables include average Entrapment Efficiency, drug content, and drug release. using a CCD design, 13 repetitions of the tests were conducted with three independent variables (greater and lower levels). The average mean entrapment efficiency, drug content, and drug release of the solid lipid nanoparticles loaded with rasagiline mesylate were 80.02%, 90.02%, and 97.99% respectively. a dense spherical pattern of optimum solid lipid nanoparticles was shown by the morphological evaluation of optimal Rasagiline mesylate loaded SLNs. Since they are more easily able to cross the blood-brain barrier in all three symmetrical dimensions SEM pictures revealed spherically shaped SLNs (SEM) The anticipated data analyzed by DOE showed a strong correlation with the experimental results.

Keywords: Central Composite Design, Rasagiline mesylate, Scanning electronmicroscopy, Solid lipid nanoparticles, Design Expert.

1.INTRODUCTION

A propargylamine-based medication called rasagiline (N-propargyl-1-R-aminoindan) mesylate (RM) is a strong, irreversible inhibitor of monoamine oxidase-B. It has been investigated for Parkinson disease treatment. [1] Current studies on the effects of rasagiline mesylate and its equivalents on Alzheimer disease are being conducted. The oral drug has a short elimination half-life of 0.6-2 hours and a 36% bioavailability, despite its quick absorption and hourly peak plasma concentration. [2]The primary metabolite of RM is aminoindan. [3]

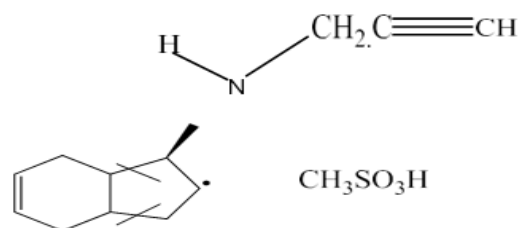


Figure 1.1: Chemical structure of Rasagiline mesylate

Parkinson disease (PD) is the second most prevalent neurodegenerative illness in the world. Clinically, PD is characterized by motor symptoms in the limbs, such as bradykinesia, resting tremor, and rigidity, which are followed by postural instability.[4] Within the ventral midbrain region's substantia nigra (pars compacta), melanin-containing dopaminergic neurons are selectively lost in Parkinson's disease (PD).[5]The subthalamic nucleus (STN) becomes disinhibited and hyperactive as a result of a dopamine shortage in the striatum.[6] The inability to adequately distribute therapeutic medications into the brain limits the treatment options for Parkinson disease. The blood brain barrier is the main obstacle to medication delivery into the brain (BBB). The only medications that can pass the blood-brain barrier are tiny compounds with low molecular weights (400–500 Da) and strong lipophilicity. [7] Numerous cutting-edge strategies, such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, biological drug delivery, chemical drug delivery systems, magnetic drug targeting, and disruption of the blood-brain barrier, have been used to address this issue. [8]SLN has emerged as a cutting-edge and quickly developing field of nanotechnology with numerous applications in medicinal science. Solid lipid nanoparticles were developed in 1991 to offer stability, storage, biocompatibility, and anti-drug degradation. These colloidal carriers, which range in size from 50 to 1000 nm, are stabilized by the surfactant and consist of a high-melting lipid matrix. [9–10] The typical methods include spray drying, solvent injection technique, high pressure homogenization, hot/cold homogenization, microemulsion, solvent emulsification, solvent evaporation, solvent diffusion, double emulsion, ultrasonication technique, and solvent emulsification-diffusion method. [11–13] To create SLN, we have chosen high pressure homogenization, which lowers viscosity, results in smaller particle sizes, and is a cost-effective method. [14–16] This study's goals were to create SLNs using high pressure homogenization and assess how various formulation and processing parameters affected the properties of RM-SLNs. Particle size and polydispersity index, Zeta potential, SEM, and other significant factors influencing SLN features were identified using a statistical model called CCD.

2 MATERIALS AND METHODS

2.1 Materials

Rasagiline mesylate was procured from Yarrow Chem Pvt Ltd, Mumbai, India. Kolliphor P188, Kolliphor RH 40, and Kolliwax S Fine were obtained as a gift sample from Aster Analytics Research Institute in Pune. All of the tests were conducted using analytic grade chemicals and reagents. Double distilled water was filtered through a 0.45 μm membrane.

2.2 Methods

2.2.1 Design of Experiments

Product should be designed to meet patients' needs and the intended product performance. Pharmaceutical development should include defining of quality target product profile (QTPP), identifying and determining potential critical quality attributes (CQAs), selecting an appropriate manufacturing process, defining a control strategy, and identifying through, for example, prior knowledge, experimentation, and risk assessment, the material attributes, and process parameters that can have an effect on product CQAs. The systematic approach could facilitate product development and continual improvement and innovation throughout the product lifecycle [17,18]. Investigations were done by factorial design to minimize particle size and maximize the encapsulation efficiency of the drug in SLN.

2.2.2 Selection of Lipid

Different lipids were selected based on solubility experiments. The drug's solubility was tested in several lipids. A digital shaker water bath (NOVA Instruments Pvt. Ltd., Ahmedabad, India) was used to determine the amount of drug dissolved in each lipid at a temperature of 5° C above the respective lipid's melting point. The lipid with the highest drug solubility was chosen for further investigation [19].

2.2.3 Selection of Surfactant

With the selected lipid, SLN were prepared using different surfactants and were evaluated with respect to the particle size, PDI, and entrapment efficiency. The particle size and PDI were determined using Malvern Zetasizer Nano series Nano-ZS, UK. Selection of the surfactant was made based on minimum particle size and PDI with maximum entrapment efficiency.[24]

2.2.4 Drug-Excipient Compatibility Study

FT-IR spectra of pure drug and the physical mixtures of drug and selected excipients stored at $25 \pm 2^\circ \text{C}$, $60\% \pm 5\%$ relative humidity for a period of 7 days were recorded using FT-IR spectrophotometer (JASCO, Japan (Model: V-4600)) in the range of $3000\text{--}500 \text{ cm}^{-1}$ and compared for any significant change [20, 21].

2.2.5 Selection of Formulation Technique

Various techniques for SLN formulation are high shear homogenization and ultrasound, high pressure homogenization, solvent emulsification and evaporation technique, microemulsion based SLN preparation technique, and so forth [22, 23–24]. The selection of the technique was made based on the evaluation of particle size, PDI, and entrapment efficiency of the nanoparticles obtained with the trial batches using the commonly used and reported to be reliable and powerful techniques. [1] High Pressure Homogenization. There are two general homogenization techniques (hot homogenization and cold homogenization) which can be used for the production of SLN [12]. In the present study, hot homogenization technique was investigated. The drug was incorporated into the melted lipid.

2.2.6 Preparation of SLNs

RM loaded SLNs were prepared by hot homogenization. Lipid (Kolliwax S Fine) was weighed and heated to 75°C until the entire lipid melts completely on water bath. To this lipid melt the drug (Rasagiline Mesylate) was added. Rasagiline Mesylate was dissolved in molten lipid mixed. Accurate quantity of surfactant (Kolliphor P 188) and Permeability enhancer (kolliphor RH 40) was dissolved in acetone this was the Lipidic phase. Accurate quantity of stabilizer (Tween 80), as per the Box Behnken Design (BBD), was dissolved in distilled water this was aqueous phase. The organic phase was added to aqueous phase slowly. After addition stirred at 1200 rpm for 30 minutes and visualized for clarity of solution. the dispersion of SLNs was mixed gently by slow magnetic stirring for one hour at room temperature until cooling the above dispersion SLNs was prepared added mannitol (5%) as a cryoprotectant. 50 ml aliquot of different batches of the optimized solid lipid nanoparticles were freeze dried mannitol (5% w/v) added as a cryoprotectant to 50 mL aliquot of sample, which were frozen in liquid nitrogen and lyophilized for 48h at 70°C , at a 0.05 mm Hg pressure. Freeze dried sample stored at room temperature. [18–25]

2.2.7 Optimization of Process Variables

On the basis of literature survey and a few trial batches, various critical process variables which may have significant effect on the critical quality attributes were identified for each step involved in the formulation and were subjected to optimization. Preliminary optimization of stirring time, RPM, and temperature was done by conducting the experiments at three levels of each process variables involved during stirring of the hot aqueous surfactant solution while adding the drug incorporated lipidic phase for the formation of coarse emulsion. Critical process variables involved during the high-pressure homogenization were optimized using design with Design Expert 13.0 software. The Lipid, Surfactant, Stabilizer were selected as independent variables and the response on EE%, Drug content%, Drug Release % were investigated.[27]

2.2.8 Optimization of Formulation Variables

Optimization of formulation variables and Design Expert 13.0 software was used for statistical analysis by ANOVA, generating model equations and constructing contour plots and 3D surface plots, Predicted Vs Actual for each response. Amount of drug with respect to lipid and concentration of surfactant, Stabilizer were investigated as independent variables at three levels and the critical quality attributes selected entrapment efficiency and drug content, drug release as responses.[28]

2.2.9 Evaluation of Optimized Formulation

Particle Size, Polydispersity Index (PDI), and Zeta Potential

The average particle size, PDI, and zeta potential of solid lipid nanoparticles were assessed using a Zetasizer Nano series Nano-ZS (Malvern Instruments, Malvern, UK). Dynamic light scattering, DLS, and Laser Doppler Electrophoresis were employed to determine particle size and zeta potential. The samples were placed in "folded capillary cells" and the findings for size, PDI, and zeta potential were recorded.

Differential Scanning Calorimetry

The sample's thermal analysis was carried out using differential scanning calorimetry (Hitachi DSC7020, Japan). The pure drug was held in a 40 μ L aluminium pan at 10°C/min with a heating rate ranging from 5 to 300°C under nitrogen flow.

Surface morphological studies by Scanning electron microscopy(SEM)

Surface morphology is useful for studying the three-dimensional structure of particles. Scanning electron microscopy was used in this work. The sample was fixed and dehydrated using acetone. Following drying, the sample was coated with gold for imaging. The SEM was run at 25°C. [29-32]

Entrapment Efficiency

To test the entrapment efficacy of rasagiline mesylate SLNs, centrifuge the SLN at 3500 rpm for 30 minutes and separate the pellet of settled SLN from the supernatant. The supernatant was then analyzed spectrophotometrically at 266nm using methanol as a solvent. The entrapment efficiency was calculated using the equation provided below.

Equation 1:

$$\%EQ = \frac{W_{total} - W_{free}}{W_{total}} \times 100.$$

Where,

W total is the amount of medication added to the formulation.

W free equals the amount of drug identified in the supernatant.

Drug content

The drug content of the resultant SLN dispersion was determined using a JASCO ultraviolet (UV) detector (Model: V-730). It was used to determine the mesylate of rasagiline. We accurately weighed the number of RM-loaded SLNs. The SLN dispersion in methanol was dissolved. A UV detector was used to examine the solution after it had been filtered with filter paper. The wavelength at which 266 was detected was used to calculate the concentration relative to a calibration curve. According to IP, the drug content limit ranges between 95% and 100%. [33-36]

Determination of Drug Release (in vitro study): -

The USP Type II Dissolution test device (paddle) (Electro lab, Mumbai, India) was utilized to perform in-vitro medicine release from Rasagiline mesylate-loaded SLNs. At 37°C $\pm$ 0.5°C, the paddle speed was set to 1500 rpm. For 12 hours, 900 mL of pH 7.4 phosphate buffer solution was used as the dissolving media.

At predefined intervals of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours, 5 ml samples were extracted and filtered through Whatman filter paper. To maintain volume consistency throughout the test, an equivalent volume of fresh medium prewarmed at 37°C is added to the dissolution medium after each measurement. A UV spectrophotometer was used to evaluate the samples at 266 nm.

A cumulative percent release against time (h) curve was constructed by dissolving the medication at the required intervals.

Stability Studies

The stability of the rasagiline mesylate-SLNs dispersion was tested over a ninety-day period while stored in glass vials. The samples were stored at 75% relative humidity $\pm$ 5% and 40°C $\pm$ 2°C. After 0, 30, 60, and 90 days, the Rasagiline Mesylate SLN formulation's practical size, PDI, Zeta potential, and % drug release were evaluated.

Data Analysis

The data was examined statistically with *t*-test and ANOVA. The data collected for the optimization of process and formulation factors were statistically examined using the Design Expert 13.0 software's built-in analysis of variance (ANOVA) function. It was used to assess the importance and degree of the effects of various factors and their interactions. Probability values of less than 0.0500 were considered statistically significant. [29-32]

3. RESULTS AND DISCUSSIONS

3.1 Design of Experiments

A literature survey was conducted to find and determine the QTPP, CQAs, manufacturing procedures for SLN, various processes, and formulation factors that influence product CQAs. For the planned solid lipid nanoparticulate drug delivery system, practically all routes of administration—oral, parenteral, cutaneous, ophthalmic, nasal, and so on—have been documented for topical, systemic, or central nervous system activities. For enhanced drug absorption, nanoparticles with a minimum average size are desired. Minimizing particle size, PDI (monodispersed), entrapment

efficiency, and zeta potential (± 20 mV) are critical CQAs for stability. [26], There is no leftover solvent to prevent toxicity and ensure safety. A CQA is a physical, chemical, biological, or microbiological property or characteristic that must fall within a specified limit, range, or distribution to assure the intended product quality [43, 44]. In the current study, various preliminary experiments were carried out to select appropriate excipients/materials that may directly and/or indirectly influence essential quality features. The medicine excipient's compatibility was verified prior to optimizing different process and formulation variables. Using the specified excipients, different essential process variables discovered in the literature were optimized successively as per the stages.[18]

3.2 Selection of Lipid

The solubility of the drug in the lipid components is critical for achieving optimal drug payload and high drug entrapment efficiency in the synthesis of solid liquid nanoparticles. Higher drug solubility in lipids allows for the creation of a smaller, lipophilic carrier with a high potential to pass the blood-brain barrier for CNS targeting of drugs. Table 3.1 and Figure 3.1 show the solubility results of RM in Kolliwax S Fine.

Table 3.1: Solubility result of RM in various lipids

Sr.no.	Name of Lipid	Solubility
1	KolliwaxSFine	100%
2	GMS	75%
3	Compritol888ATO	50%
4	PrecirolATO5	50%
5	Dynasan114	50%

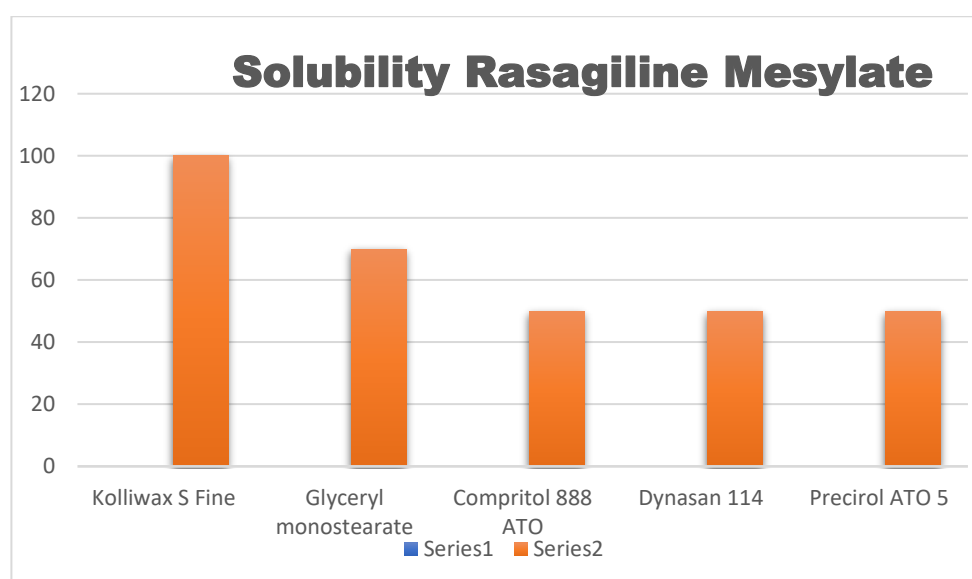


Figure 3.1: Solubility of RM in different lipid

3.3 Selection of Surfactant

SLN are a colloidal system of nanoparticles composed of solid lipids as a matrix medium and stabilized in aqueous environments using surfactants [15]. Surfactant-induced lipid solubilization and membrane fluidization increase endothelial cell permeability [46]. Nanoparticles were produced with four different surfactants using Kolliwax S Fine as a lipid and tested for particle size, PDI, and entrapment efficiency. The results are presented in Table 3.1. Kolliphor P 188 was determined to have the smallest particle size and PDI while also providing the highest entrapment efficiency. Particle size variations were statistically significant ($p < 0.05$), although differences in PDI and entrapment efficiency were non-significant. Kolliphor P 188 are nonionic triblock copolymers made up of a central hydrophobic chain of polyoxypropylene (polypropylene oxide) and two hydrophilic chains of polyoxyethylene. Poloxamers have also been shown to sterically stabilize nanoparticles and reduce the adsorption of plasma proteins or opsonin on the surface of nanoparticles by providing hydrophilic properties to the nanoparticles' surface, thereby preventing the clearance of drug-containing SLN from circulation [15]. Bulky and nonionic surfactants have lower toxicity compared to single-chain and ionic surfactants [45]. Hence, Kolliphor P 188[18-22]

Table 3.2: shows surfactant selection based on particle size, PDI, and zeta potential. Data is expressed as mean $\pm$ SD ($n=3$), with # indicating statistical significance ($p < 0.05$). PDI stands for Polydispersity Index.

Sr. No.	Lipid	Surfactant	Practical Size	PDI	Zeta potential
1	Kolliwax S Fine	Polysorbate 80	289.7nm	0.229	-21.7mV
2	Kolliwax S Fine	Kolliphor P 188	21.7nm	0.223	-11.9mV
3	Kolliwax S Fine	Polysorbate 20	234.1nm	0.228	-20.1mV
4	Kolliwax S Fine	Polysorbate 60	245.8nm	0.225	-17.9mV

3.4 Drug-Excipient Compatibility Study

Figure 3.2,3.3 depicts the FT-IR spectra of pure RM as well as physical mixes of Kolliphor P 188, Kolliwax S Fine, Kolliphor RH 40, Tween 80 (Optimization batch). Figure 3.2(a) shows that all major peaks of the drug were acquired in the FT-IR spectra as described in Table 3.3, and no significant change was found in the IR spectra of the Optimization batch mixture (Figure 3.3(b)), showing the drug's compatibility with the selected excipients[7]

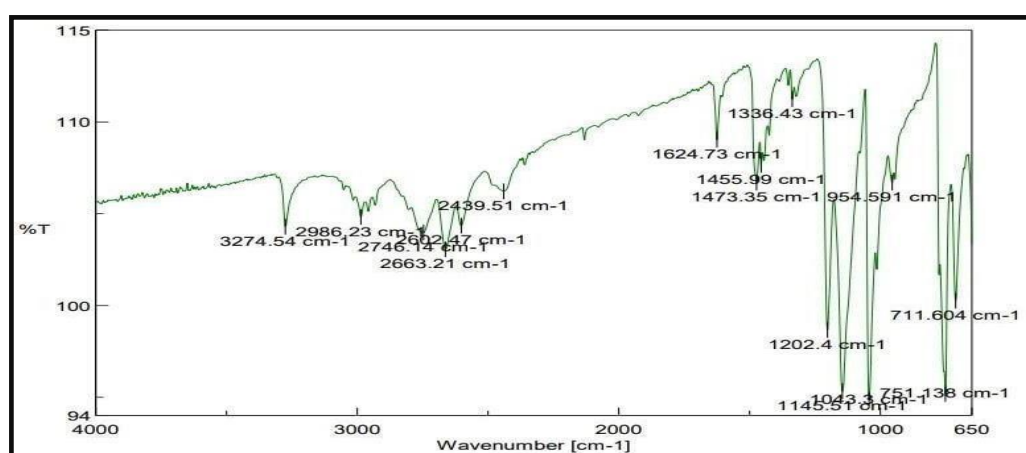


Figure 3.2:- FTIR Spectra of Rasagiline Mesylate

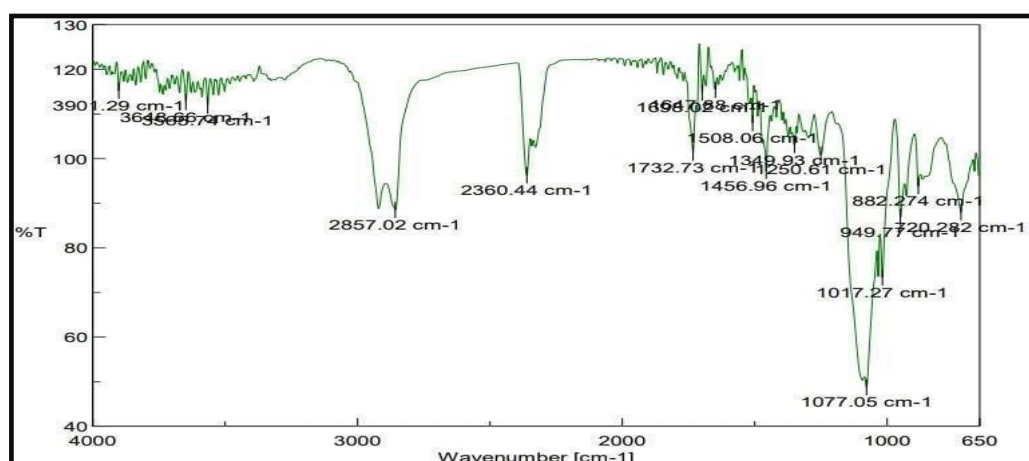


Figure 3.3: Interpretation of FTIR Spectra of SLNs

Table 3.3: Interpretation of FTIR Spectra of Rasagiline Mesylate

Functional Group	Peak Range	Observed Peak
Aromatic-C-Hstretching	2850-3000	2986.23
Aliphatic-C-Hstretching	2800-2900	2746.14

Secondary amine N-H	3300-3400	3274.54
Aliphatic amine C-N	1000-1350	1145.51
$\equiv$ C-H stretching vibrations of propargyl group	3300-3600	3569.98
Asymmetric S-O stretching	1000-1100	1023.89
C-S stretching	600-800	711.60

3.5. Optimization of Process Variables

The experiments were designed to investigate the effect of three independent variables, namely drug lipid ratio (lipid content), surfactant concentration, and stabilizer at three levels, on drug content, entrapment efficiency, and release. Traditionally, formula development entailed modifying one variable at a time. This approach makes it difficult to construct an optimum formulation since it provides little insight into how the factors interact. Of all the Response surface methods designs, the Box-Behnken design decreases the number of tests in a three-factor experimental design and requires fewer runs. A 3-factor, 3- response design would necessitate a total of 13 distinct runs with no repetitions and 3 runs with three repetitions. A Box Behnken experimental design decreases the number of experiment participants to 13. The design is also rotatable and has statistical missing corners, which might be advantageous when the experimenter wants to avoid combination factor extremes. This characteristic prevents potential data loss in such instances. Another advantage of the Box-Behnken design is that it excludes combinations in which all components are simultaneously tested at their maximum or lowest values. So, these designs are valuable in avoiding experiments performed under extreme designs, as shown in Table 3.4.[18]

Experimental design of SLNs as per BBDRun

Table 3.4: Formulation Table of SLN

Run	A-Lipid(mg)	B-Surfactant(mg)	C-Stabilizer %(w/w)
1	350	375	1.75
2	450	450	1.75
3	350	300	2
4	450	300	1.75
5	250	300	1.75
6	250	450	1.75
7	450	375	2
8	450	375	1.5
9	250	375	1.5
10	350	300	1.5
11	350	450	1.5
12	250	375	2
13	350	450	2

3.6 Drug content Statistical Analysis

The Design-Expert software [version 13.0.5.0: Stat-Ease] was used for statistical analysis and graph plotting. The results of the one-way analysis of variance [ANOVA] for the dependent variable were used to select a model that was statistically significant for both responses.[34]

3.7 Validation of Response Surface Methodology

A single optimized formulation based on maximum desirability was selected as a checkpoint to evaluation

for Entrapment Efficiency (%), Drug Release % and Drug Content %.

3.8 Drug content

Table 3.4 shows the percent drug content analysis results for the 13 RM-loaded solid lipid nanoparticle (SLN) formulations. The percent drug content data were calculated to quantify the amount of RM present in each formulation. The percentage drug content varied from 57.06% to 99.02%. These figures show that the amount of RM in the formulations is relatively consistent, approaching 100%.

The average % drug content of all 13 formulations was estimated, along with the standard deviation and coefficient of variation. The limited range of percent drug content results and small standard deviation indicate that RM is evenly distributed across the lipid core and vesicular dispersion of the SLNs. This homogeneous distribution is an important feature of drug-loaded nanoparticles because it assures consistent medication delivery and effectiveness. Furthermore, the near-100 percent drug content figures show that negligible drug loss occurs during the SLN production procedure. This is a significant finding since it shows that the formulation process is efficient and that the majority of the medicine is successfully encapsulated within the SLNs.[22-28]

Response 1: Drug Content

Table 3.5: ANOVA result for drug content as the response (Y₂)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1550.54	6	258.42	4.65	0.0417	significant
A-KolliwaxS Fine	284.41	1	284.41	5.12	0.0643	
B-KolliphorP 188	3.38	1	3.38	0.0608	0.8134	
C-Tween80	90.45	1	90.45	1.63	0.2491	
AB	4.62	1	4.62	0.0832	0.7827	
AC	1017.61	1	1017.61	18.32	0.0052	
BC	150.06	1	150.06	2.70	0.1514	
Residual	333.31	6	55.55			
Cor Total	1883.85	12				

Factor coding is Coded

Sum of square is Type III – Partial

The Model F-value of 4.65 implies the model is significant. There is only a 4.17% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case AC is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

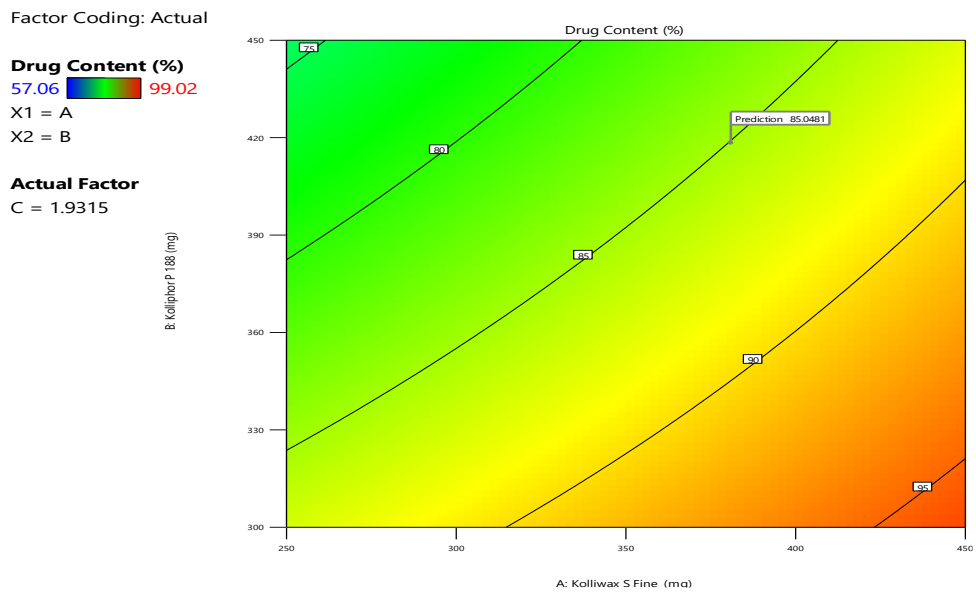


Figure 3.4: Counter Plot of Drug Content (%)

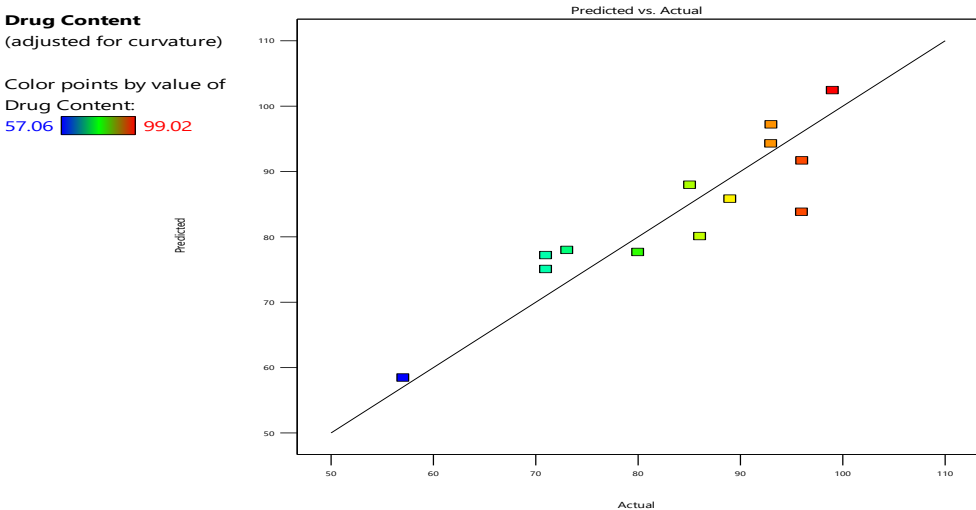


Figure 3.5: Predicted Vs Actual of Drug Content(%)

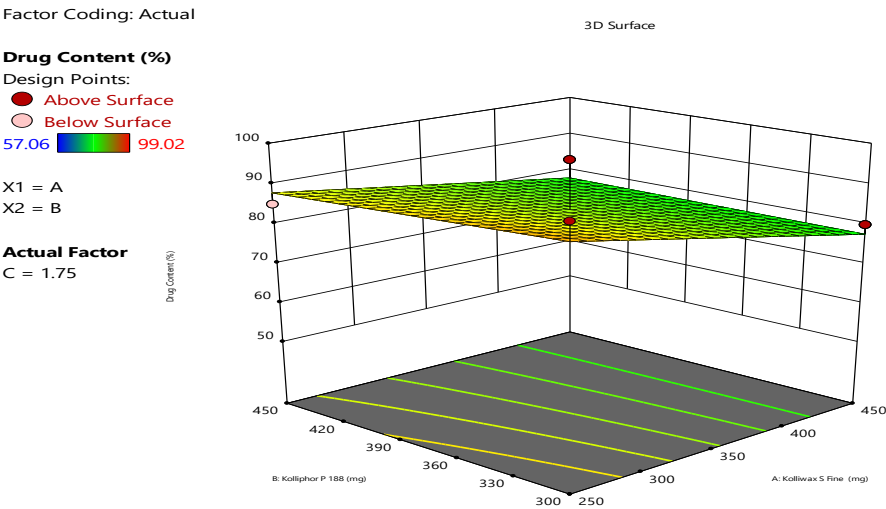


Figure 3.6: 3D Surface plot of Drug Content (%)

3.9 Entrapment Efficiency

The percentage entrapment efficiency is an important statistic for formulation and large-scale production. Among all SLN formulations, SLN-9 had the highest %EE, at 80.12%. SLN-9 was created with a lipid-to-drug ratio of 250 mg, 375 mg, and a 1.5% concentration. This high entrapment efficiency implies that a large amount of Rasagiline mesylate was successfully encapsulated within the SLNs; the results of the entrapment efficiency assessment are presented in the table. The solid lipid nanoparticles' entrapment effectiveness ranged from 53.9 to 71.4%. Creating an efficient medicine delivery system.[25]

Response 2: Entrapment efficiency

Table 3.6: ANOVA result for Entrapment Efficiency as the response (Y₂)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	688.06	3	229.35	4.24	0.0399	significant
A-Kolliwax S Fine	465.28	1	465.28	8.60	0.0167	
B-Kolliphor P 188	64.98	1	64.98	1.20	0.3016	
C-Tween 80	157.80	1	157.80	2.92	0.1219	
Residual	486.95	9	54.11			
Cor Total	1175.01	12				

Factor coding is Coded

Sum of square is Type III – Partial

The Model F-value of 4.24 implies the model is significant. There is only a 3.99% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

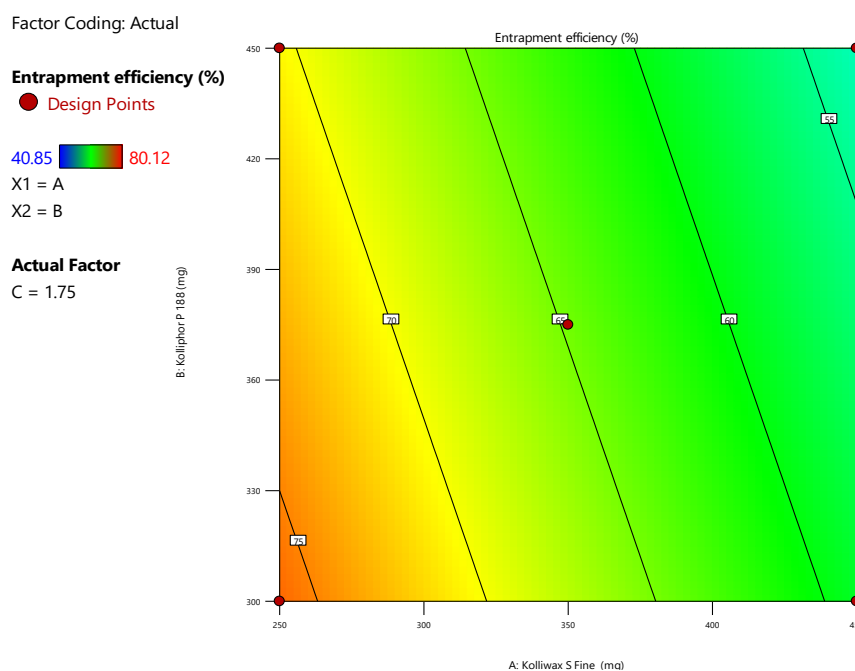


Figure 3.7: Counter Plot of Entrapment Efficiency(%).

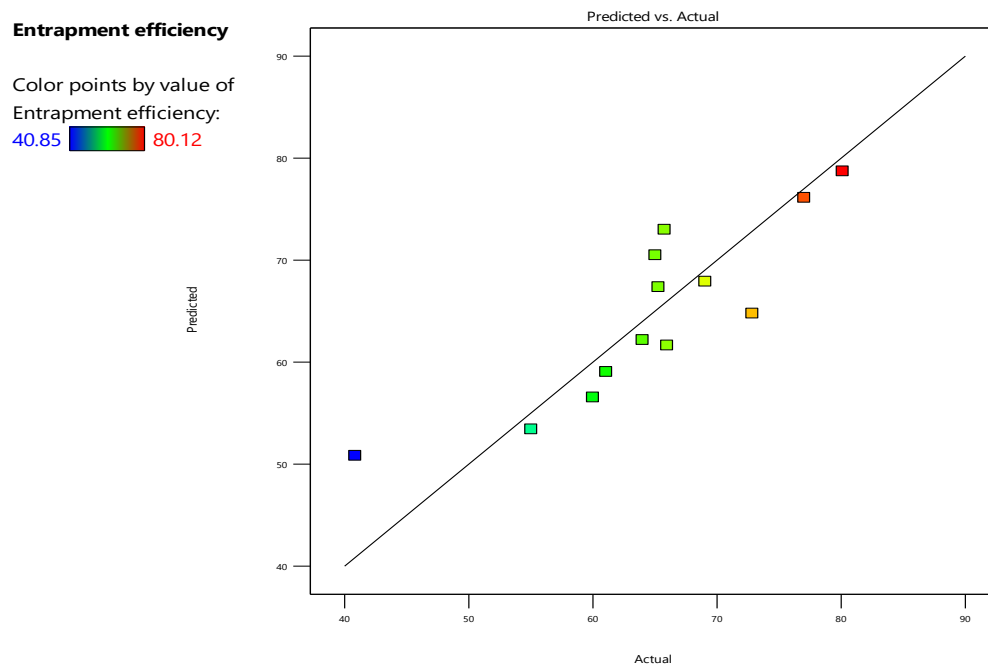


Figure 3.8: Predicted Vs Actual of Entrapment Efficiency(%)

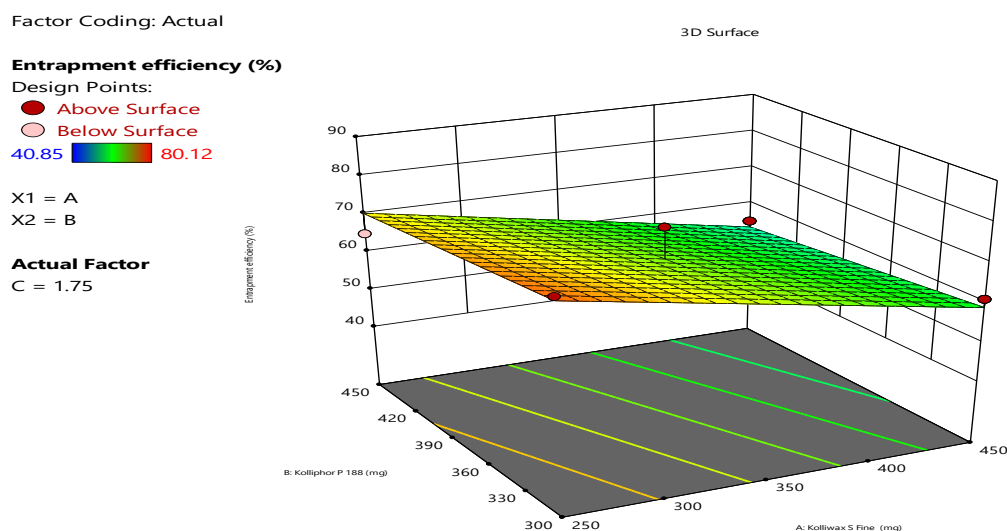


Figure 3.9: 3D Surface plot of Entrapment Efficiency(%)

3.10 DrugRelease

The drug release of the produced SLNs ranged between 60.43% and 97.99% (M1 to M13 batches). The formulation SLN-M9 had the maximum drug release (97.99%) and was manufactured with a lipid-to-drug ratio of 250:375:1.5%. The least drug release (60.43%) was found in SLN-M8, which was designed with a lipid-to-drug ratio of 450:375 mg and a concentration of 1.5%. The highest drug release (97.99%) was seen in SLN-9, produced with a lipid-to-drug ratio of 250:375 mg and a concentration of 1.5%. The % drug release numbers were calculated to quantify the amount of RM present in each formulation. The percentage medication release varied from 60.43% to 97.99%. These figures show that the amount of RM in the formulations is relatively consistent, approaching 100%[19]

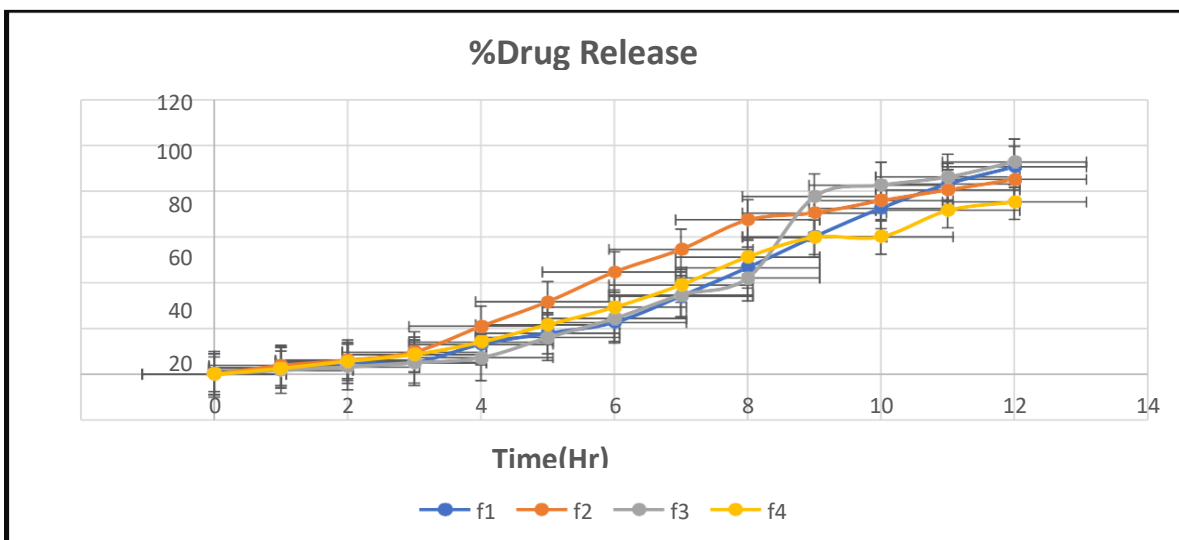


Figure 3.10: Cumulative % drug release of F1-F4 formulations

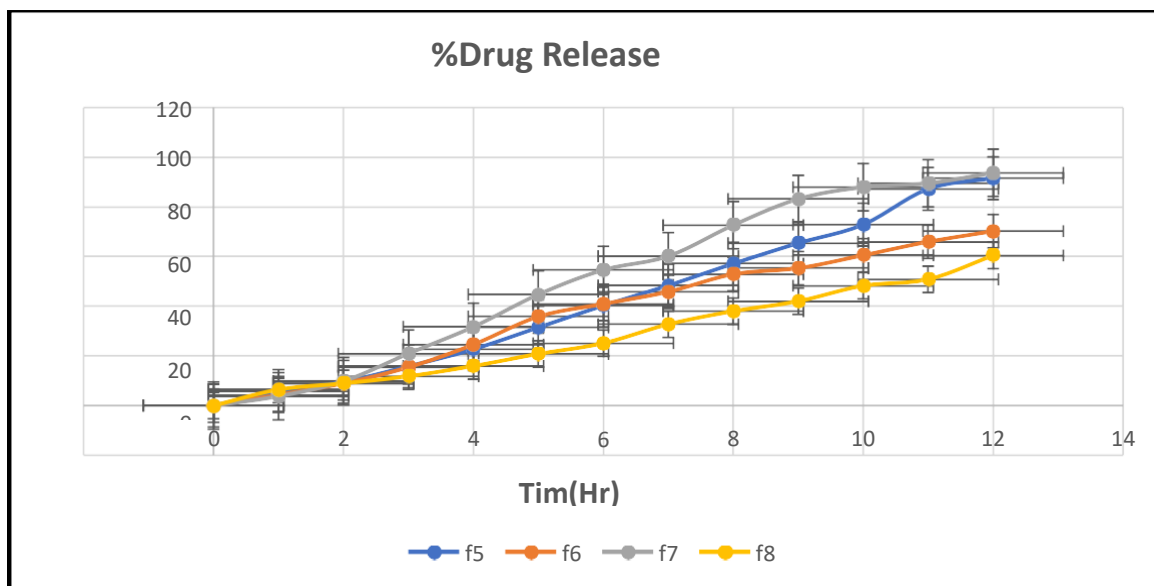


Figure 3.11: Cumulative % drug release of F5-F8 formulations.

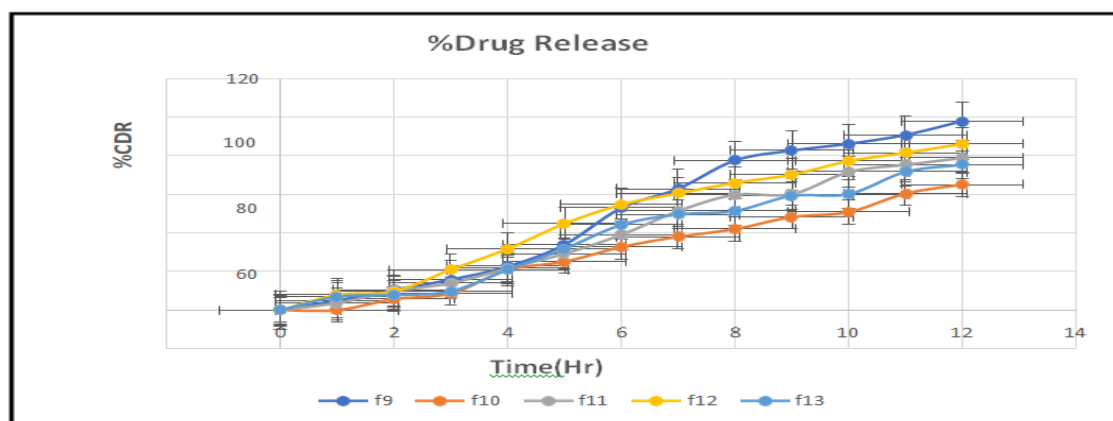


Figure 3.12: Cumulative % drug release of F9-F13 formulations

In a comparative study, M9 formulation gives a higher percentage of drug release compared to the other 13 formulations at the end of 12 hours, as shown in figure (3.10,3.11,3.12). As a result, it was concluded that the best optimized batch was M9 (97.99) because it had the highest percentage of drug release at 12 hours among all formulations.

Response 3: Drug Release

Table 3.7: ANOVA result for Drug Release as the response (Y_2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1342.02	6	223.67	5.07	0.0343	significant
A-KolliwaxS Fine	118.58	1	118.58	2.69	0.1521	
B-Kolliphor P188	14.58	1	14.58	0.3308	0.5861	
C-Tween80	312.50	1	312.50	7.09	0.0374	
AB	243.36	1	243.36	5.52	0.0571	
AC	484.00	1	484.00	10.98	0.0161	
BC	169.00	1	169.00	3.83	0.0979	
Residual	264.45	6	44.07			
CorTotal	1606.47	12				

Factor coding is **Coded**

Sum of square is **Type III – Partial**

The **Model F-value** of 5.07 implies the model is significant. There is only a 3.43% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case C, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Factor Coding: Actual

Drug Release (%)

● Design Points

60.43 97.99

X1 = A

X2 = B

Actual Factor

C = 1.75

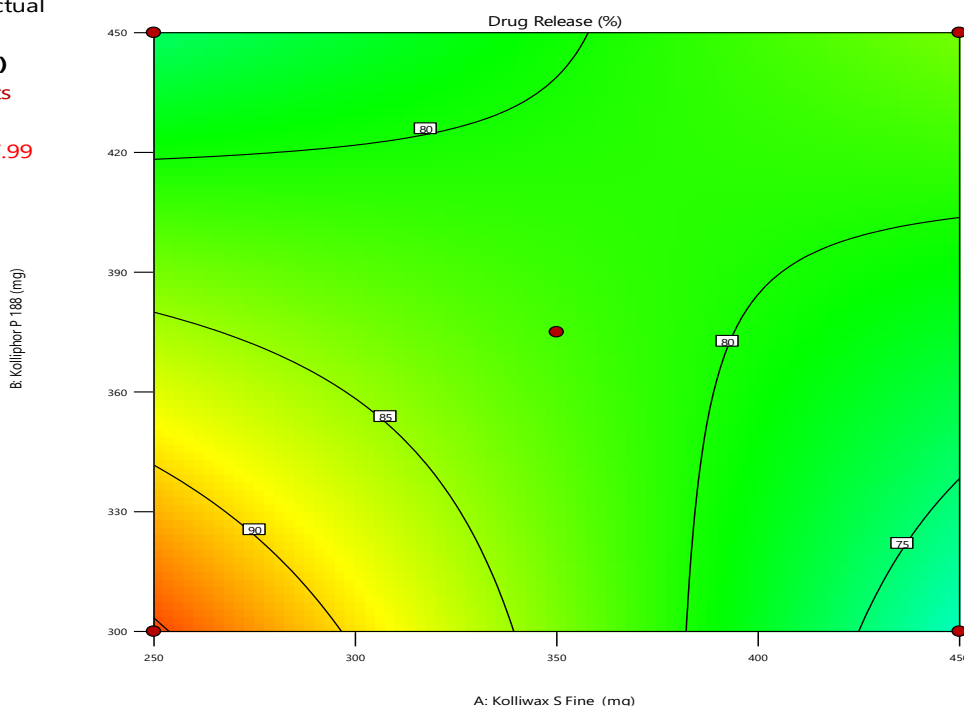


Figure 3.13: Counter Plot of Drug Release (%).

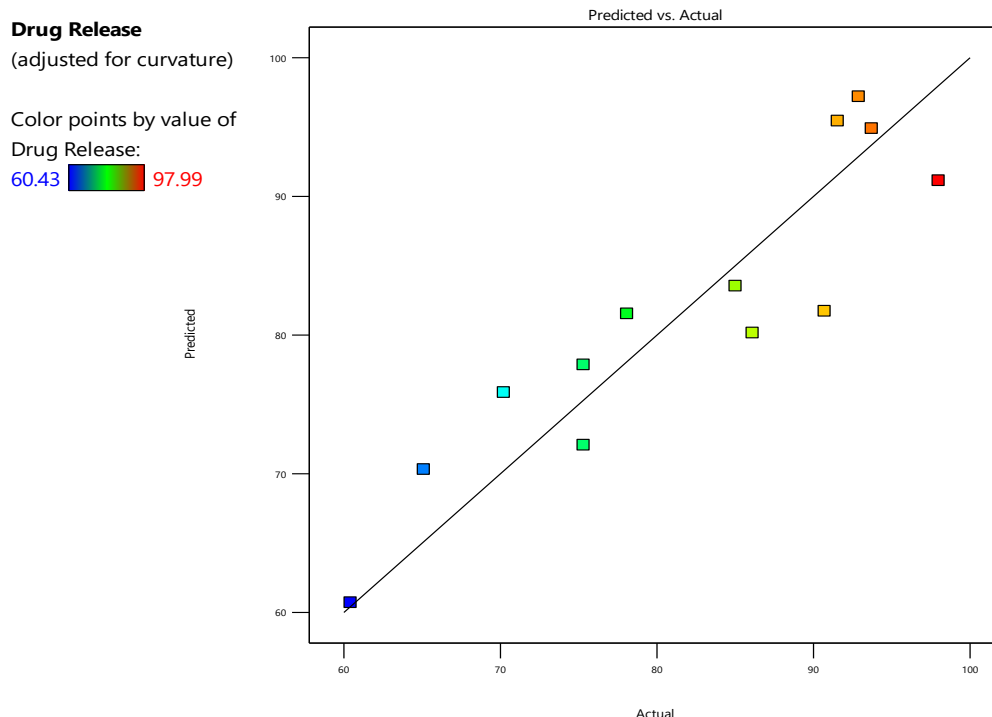


Figure 3.14: Predicted Vs Actual of Drug Release(%)

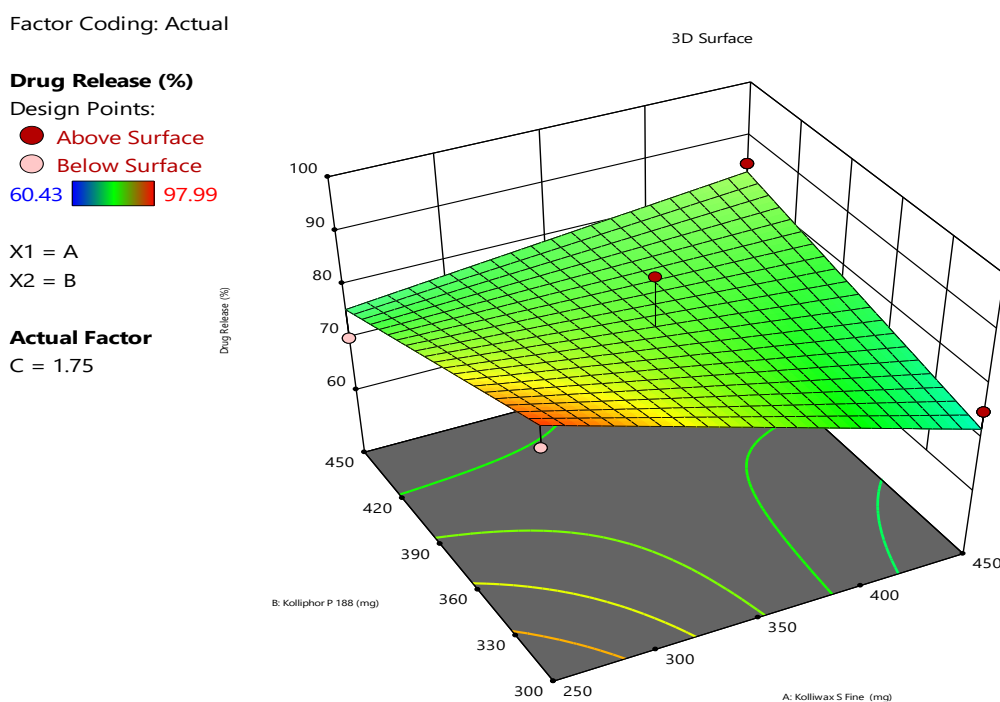


Figure 3.15: 3D Surface plot of Drug Release (%)

3.11 Stability Studies

According to ICH guidelines, the drug stability of the formulations was assessed under storage conditions. SLS stability was calculated using particle size, PDI, and Zeta potential.

The results showed that none of the investigated parameters had changed considerably. Table 3.8 presents the results of the expedited stability investigation. After 90 days, there were no significant increases in the particle size of SLN. During storage, the entrapment efficiency of SLN decreased, indicating that the medication remained in the lipid nanoparticles for 90 days. The PDI of SLN dispersion remained nearly constant during storage.

There was no significant difference in percentage drug release storage. The findings are shown in table 3.8

Table 3.8: stability study data for Rasagiline Mesylate-SLNs(M9)

Sr.no.	Parameters	0 Days	30 Days	60 Days	90 Days
1	Particle size(nm)	181.3±0.84	183.1±0.95	184.2±0.47	185.9±1.24
2	PDI	0.46±0.04	0.48±0.06	0.53±0.05	0.54±0.04
3	Zeta potential (mV)	-11.9±1.83	-12.4±1.6	-12.8±1.57	-13.6±2.01
4	Drug release	97.99±2.4%	95.45±1.9%	94.38±2.1%	92.39±1.2%

3.12 Optimization Analysis Data

Box-Behnken Design was chosen for optimization because it allows for the investigation of the effect of independent variables on the parameters of interest, creates fewer necessary runs, and is more efficient. Independent variables such as lipid concentration, surfactant concentration, and stabilizer affect drug content, release, and entrapment efficiency. The desirability function (0.836) was tested to provide an optimum formulation utilizing design expert software. The software estimated the following parameters for the ideal SLN formulation: lipid ratio (mg), surfactant concentration (mg), and stabilizer concentration (%). The table displays the ANOVA output of the Box-Behnken design for optimizing Rasagiline mesylate SLN. There appears to be no statistically significant difference between theoretical and real values for EE ($p < 0.0008$) and ($p > 0.0805$). This shows that the proposed model is valid. The drug content of the improved formulation was 99.02%, the EE was 80.12%, and the drug release was 97.99%.

3.13 Particle size and PDI

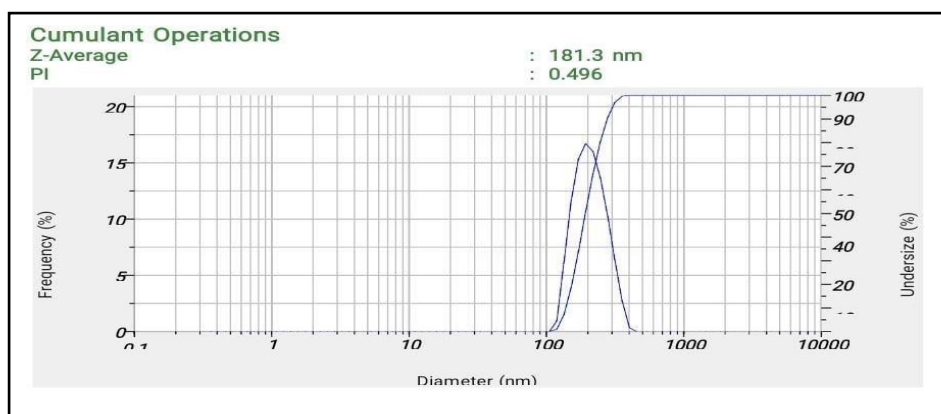
The particle size and polydispersity index (PDI) of the produced SLNs were assessed. The nanoparticle size varied amongst formulations, ranging from 323.6 ± 3.5 nm to 648.6 ± 2.6 nm. The SLN-8 formulation with a 0.98 lipid-to-drug ratio, 325 mg PEG 2000, and 2% surfactant concentration produced the lowest particle size (323.6 ± 3.5 nm). In contrast, SLN-9 with a lipid-to-drug ratio of 5, 500 mg PEG 2000 and 1% surfactant concentration had the highest particle size (648.6 ± 2.6 nm). The particle size distribution homogeneity index (PDI) ranged from 0.40 ± 0.12 to 0.52 ± 0.14 . The formulation SLN-6 had the lowest PDI (0.40), indicating more uniform particle size distribution. These findings suggest that various combinations of lipid-to-drug ratio, PEG 2000, and surfactant concentration influenced the size and dispersion of SLNs. The findings are shown in figure 3.13[36]

3.14 Zeta potential

The zeta potential of the prepared SLNs ranged from -21.45 ± 1.65 mV to -37.63 ± 2.83 mV. The formulation SLN-4 had the lowest zeta potential of -37.63 ± 2.83 mV. It was made with a lipid-to-drug ratio of 5, 150 mg PEG 2000, and 3% surfactant content. High zeta potential levels, whether positive or negative ($> \pm 30$ mV), are ideal for pharmaceutical stability. A zeta potential of -11 to -20 mV can cause agglomeration, but -41 to -50 mV is considered stable. The findings are shown in figure 3.14[37]

Practical size graph

Formulation (batchM9)

**Figure 3.16:Practical size of formulation batch M9**

**Zetapotential graph:
Formulation(batchM9)**

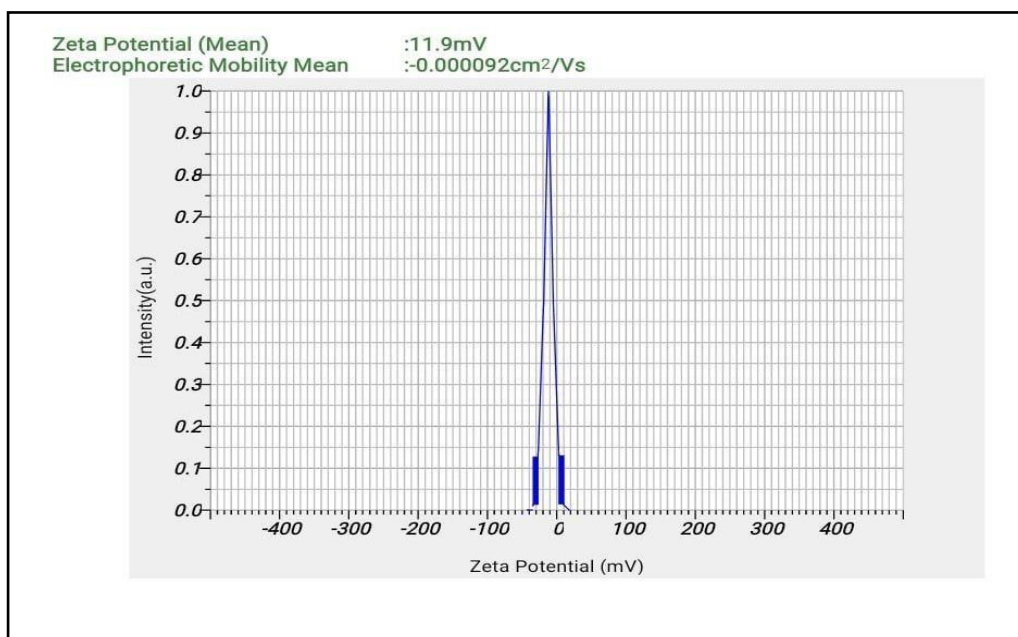


Figure 3.17: - Zeta potential of formulation batch M9

3.15 Surface morphological studies by Scanning electron microscopy(SEM)

Figure 3.15 shows a dense spherical pattern of optimized SLNs. SEM scans revealed SLNs with a spherical shape, which can easily breach the blood brain barrier in all three symmetrical dimensions.

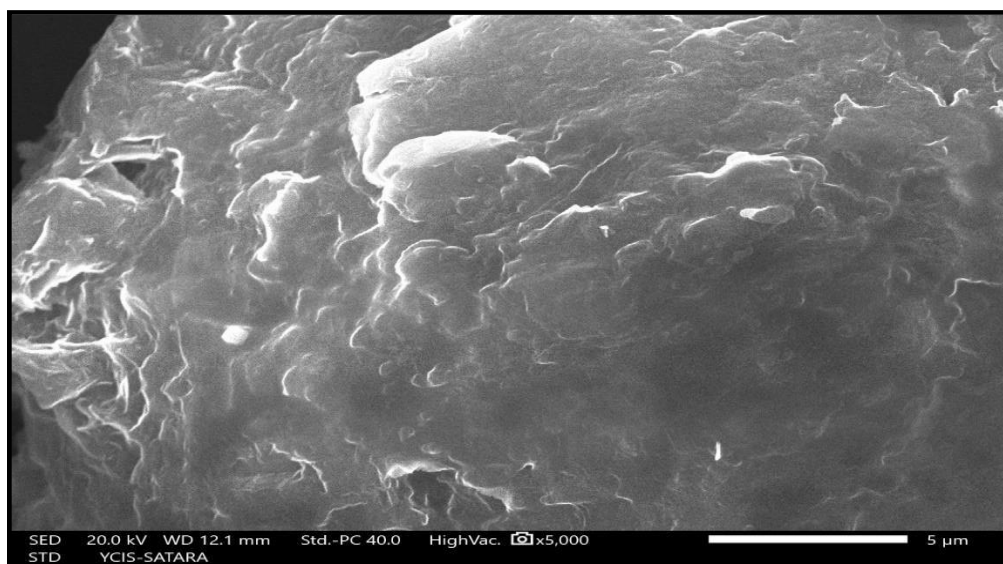


Figure 3.18: SEM of Rasagiline Mesylate loaded solid lipid nano particles depicts smooth particles of size (M 9).

3.16 Differential Scanning Calorimeter Analysis (DSC)

DSC is viewed as a method for studying the melting behavior of crystalline materials such as solid lipid nanoparticles. The melting point of the blended lipid matrix is lower than that of the solid lipid. For example, the melting point of Kolliwax S Fine was 67.81 °C, but the mixture of Kolliwax S Fine and Rasagiline Mesylate had a melting point depression of 66.78 °C. Formulation SLN-M9 was chosen as a representation of Rasagiline Mesylate SLN because it had the maximum entrapment efficiency. The DSC thermogram of Rasagiline Mesylate revealed an endothermic peak at 157.37 °C, which

corresponds to the drug's claimed melting point. Drug-loaded SLN demonstrated a significant endothermic peak at 58.71°C (Figure 3.16,3.17).[30-36]

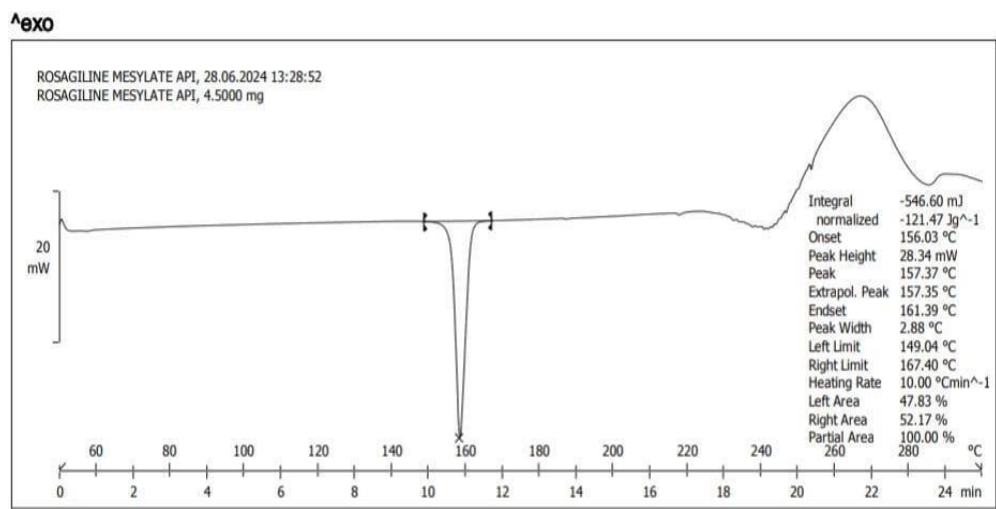


Figure 3.19:-DSC graph of Rasagiline Mesylate.

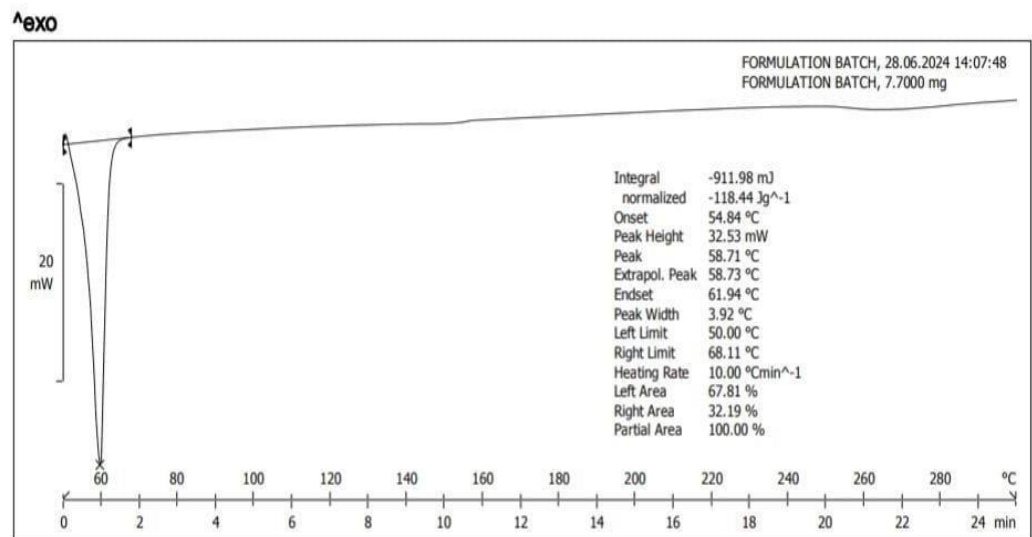


Figure 3.20: DSC graph of Drug loaded SLN(M9).

CONCLUSION

To achieve the goals of the current study endeavor, two CNS acting medicines (RM) were chosen and purchased. Based on physicochemical characterization and identification examinations, the obtained drug samples were found to be pure and authentic. This study highlights the use of Quality by Design (QbD) concepts to optimize RM-loaded Solid Lipid Nanoparticles (SLNs) for PD. The QbD approach entails defining the Quality Target Product Profile (QTPP), understanding the product and process, and using Design of Experiments (DoE) to create an optimum formulation. Identified critical material and process parameters to achieve required product quality. In the preliminary research, important process parameters were established through trial and error, whereas excipients were chosen using a literature survey and solubility experiments. the DoE was conducted using Central Composite Design (CCD) with lipid-to-drug ratio, lipid quantity, and surfactant concentrations as independent variables to assess their effect on drug content, entrapment efficiency, and release. Design Expert software was used to examine the data, which included perturbation, contour, and 3D surface plots. The formulation with the highest attractiveness value was cross-validated, resulting in good agreement between anticipated and actual production values. "The optimized SLNs have favourable features, including high drug content, high entrapment, and optimal drug release. Lyophilization with mannitol as a cryoprotectant was used to increase physical stability and shelf life. SEM showed tiny vesicles with smooth exterior surfaces. The optimized SLNs showed high drug content (99.02%), entrapment efficiency (72.84%), drug release

(97.99%), indicating their potential as effective drug delivery systems. The study effectively met the goal QTPP for SLN formulation.

CONFLICT OF INTEREST

Authors declares there is no any conflict of interest.

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