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Research Article

Fabrication of Mebendazole-loaded Solid Lipid Nanoparticles: Formulation, Optimization, Characterization, Stabilization, and *In-vitro* Evaluation

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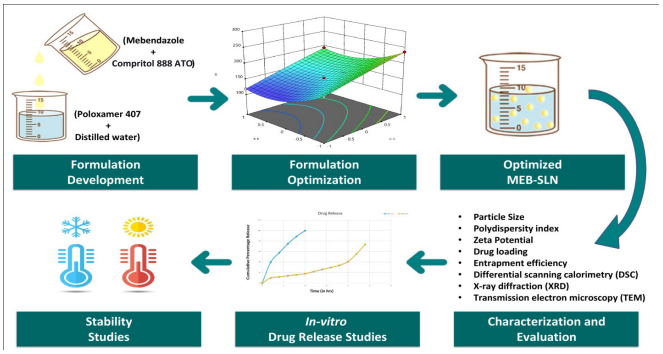
ABSTRACT

The research focused on the formulation, optimization, characterization stabilization and *in vitro* evaluation of Mebendazole loaded solid lipid nanoparticles (MEB-SLN) using response surface methodology. MEB-SLN was developed using the melt-emulsification ultrasonication method. The optimized formulation contains Compritol 888 ATO as a solid lipid and Poloxamer 407 as a surfactant. The optimized MEB-SLN was spherical, and had an average size of 230.4nm, and zeta potential of -21.9mV. The physiochemical characterization included the evaluation of surface morphology, drug entrapment, x-ray diffraction studies, and differential scanning calorimetry. *In vitro* drug release studies revealed that MEB-SLN would release drugs for up to 24 hrs. The formulation was found to be significantly quite stable in the refrigerator than at room temperature, as per stability tests. As a result of these findings, MEB-SLN was identified as a possible drug carrier. This provides a framework for further exploration of the developed formulation.

Introduction

In the field of drug development and technology, the formulation of lipid-based nanoparticles has gained prominence nowadays. It is one of the most advanced systems for embedding lipophilic and hydrophilic substances into lipid matrices. Lipid nanoparticles have been reported substantial attention in recent years as they are made of natural or synthetic lipids, have strong biocompatibility, and can deliver drugs in a controlled manner.[1] Lipid nanoparticles can also boost drug absorption by improving dissolution and solubilization inside the intestinal surroundings, delaying gastric emptying, and increasing mucosal permeability. Lipids have been seen to enhance lymph production while also increasing lymph flow volume. As a result, lipid nanoparticles can improve both the total degree of absorption and the amount of what is absorbed and transported.[2]

Graphical Abstract



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Solid lipid nanoparticles (SLN) are colloidal particles with diameters ranging from 50 to 1000 nm. It is composed of lipids that remain solid at room and body temperatures and stabilized in an aqueous solution by one or more surfactants. [3-4] SLN also have a fantastic opportunity for the advancement of new approaches to cure diseases due to their size-based properties.[2,5] Hydrophilic bioactive molecules can be inserted outside of the lipid matrix, while lipophilic bioactive compounds are typically scattered in the lipid matrix. The use of solid lipid nanoparticles to improve the biopharmaceutical properties and therapeutic effect of plant-derived molecules and herbal extracts has been recorded in the scientific literature.[4,6]

The leading nanocarrier seems to be SLN, which is an alternate carrier mechanism to conventional colloidal carriers such as emulsions, liposomes, microemulsions, self-emulsifying drug delivery systems, and polymeric nanoparticles.[2] SLN is used to encapsulate hydrophilic drugs to achieve improved sustained release and entrapment efficiency. [6] They have numerous advantages over traditional colloidal systems, including improved physical stability, protection against drug degradation in the body, low or no toxicity, and a variety of administration routes. They also have high biocompatibility, drug targeting, and the control drug release profile.[1] Liposomes and traditional emulsions have issues with membrane stabilization and drug leaching, which SLN could be able to solve. SLN surpasses emulsions and polymeric nanoparticles in terms of biocompatibility, oral bioavailability, and targeted drug delivery. As compared to other polymeric nanoformulations, lipid molecules have physiological and biodegradable properties that can minimize harmful side effects and chronic toxicity. [3]

Mebendazole (MEB) is a benzimidazole-class broad-spectrum anthelmintic. Methyl-5-benzoyl benzimidazole-2-carbamate is the chemical formula for MEB. For the prevention of filariasis and other helminthic infections, it is available as tablets or a suspension dosage type. It has low oral bioavailability and aqueous solubility. [7-8] In high doses, it produces plenty of side effects, including anemia and liver injury. It is a well-known anthelmintic drug, but it has not attained therapeutic efficacy due to its limited aqueous solubility, reduced bioavailability, and fast first-pass metabolism.[9,10]

The MEB formulations such as floating microsphere,[11] solid dispersion,[12] self micro emulsifying drug delivery system,[13] self nano emulsifying drug delivery system,[14] nanofibers,[15] nanoparticles,[16] nanostructured lipid carriers,[17] and silver nanoparticles[18] have all been proposed by the researchers for various biological activities.

Various experimental designs are now helpful in designing a formulation that requires less experimentation while also offering assessments of the relative importance of various variables. In recent years, it has been shown that applying a predictive experimental design to pharmaceutical formulation is effective in obtaining the knowledge needed to explain the interaction between independent and dependent variables in a formulation. [17,19] On a specified number of experiments or runs, the experimental design software tool is being used to examine the impact of the independent variable on the dependent variables and their relationship. The Box-Behnken design (BBD) was used to develop and optimize certain formulations as well as to investigate the effect of independent and dependent variables systemically. [20-21]

In light of the above, the current study aimed to avoid the problems associated with MEB and reformulate drugs by using the benefits of nanotechnology across a wide range of nanoformulations, to improve drug entrapment efficiency and further minimize particle size. As a result, the MEB-SLN was developed, optimized, and evaluated for physicochemical characteristics like surface morphology, particle size, zeta potential, entrapment efficiency, and *in vitro* drug release behavior.

Materials And Methods

Chemicals and Reagents

The MEB was obtained as a gift sample from Medopharm, Chennai, Tamil Nadu, India. The surfactant Poloxamer 407 was provided from Sigma-Aldrich (St Louis, USA). All the other chemicals and reagents used in the study were of analytical grade and were procured from Sigma–Aldrich (St. Louis, USA).

MEB Solubility in Lipids and Surfactants

Accurately weighed 1-gm of the solid lipids (stearic acid, cetyl palmitate, cholesterol, and compritol 888 ATO) and heated to 5°C above their melting point with continuous stirring using a magnetic stirrer with a hot plate (Remi Instrument Ltd., Mumbai, India) at 200 rpm. MEB was applied in 1-mg amounts until fully dissolved in the molten solid lipids, and the amount of solid lipids required to solubilize MEB was estimated.[22] The analysis was carried out three times; with the outcomes expressed as mean value (mg/g) ± standard deviation (SD). Surfactants were chosen based on aqueous titration observations. Melting and agitating the lipid with a magnetic stirrer, various surfactants were applied, accompanied by drop-wise addition of double-distilled water, which resulted in primary emulsions. The collected preparations were examined for clarification and homogeneity.[23]

Formulation and Optimization of Mebendazole loaded Solid Lipid Nanoparticles (MEB-SLN)

The MEB-SLN was prepared using the melt-emulsification ultrasonication process, with minor modifications, as previously stated.[17] In a nutshell, appropriate amounts of solid lipid (Compritol 888 ATO) were blended and melted at 5°C above their melting point. MEB was applied and dissolved to create a consistent and transparent oil phase. In the meanwhile, the aqueous phase was made

by dissolving a surfactant (Poloxamer 407) in Milli-Q water and heating it to the same temperature as the lipid phase. The lipid phase was then poured into a hot aqueous phase and homogenized for 5 mins with a probe sonicator mounted to a 75% amplitude frequency. The resulting dispersion was left to cool to form MEB-SLN, which was then preserved at 4°C.[6, 23]

The Box–Behnken statistical factorial design was used for optimization. Design-Expert is one of the best experimental design tools for investigating quadratic response surfaces and creating second-order polynomial models.[19,20] BBD was used in this analysis to examine the impact of independent variables on specific dependent variables as well as to refine the formulation parameters of MEB-SLN. [17, 24] The independent variables chosen were the drug: solid lipid (X_1), surfactant concentration (X_2), and sonication time (X_3), as well as their low (1), medium (0), and large (1) levels calculated in previous trials. With constraints applied to SLN formulations, particle size (Y_1) and entrapment efficiency (Y_2) were chosen as dependent variables. The software [Stat Ease Design expert software version 13.0.5.0] produced the design matrix, which included 17 experimental runs. Table 1 shows the variables and their levels used in the factorial design.[25,26]

Characterization, Stabilization, and *In-vitro* Evaluation of Optimized MEB-SLN

Particle Size, Polydispersity Index, and Zeta Potential

The particle size (PS), polydispersity index (PDI), and zeta potential (ZP) of optimized MEB-SLN were determined using the differential light scattering (DLS) technique on a particle size analyzer (Beckman Coulter, DelsaNano C, CA) at 25±1°C. Optimized MEB-SLN was diluted with purified water to achieve a suitable concentration for this analysis. [17, 27] All measurements were taken in triplicate, and the results were expressed as mean ± SD.

Entrapment Efficiency (EE)

By studying the drug content in the nanoformulation, the entrapment efficiency of MEB in prepared nanoformulation could be predicted. The SLN (5 ml) sample was centrifuged for 15 mins at 12,500 rpm. The drug content was determined by UV spectroscopy at 286 nm after the supernatant was isolated, purified, and correctly diluted with acetonitrile.[23,25]

Differential Scanning Calorimetry (DSC)

It is the most widely used method of thermal analysis. DSC measures variations in enthalpy in samples as a result of temperature or time due to changes in their physical and chemical properties. DSC has proven to be a very valuable tool in the pharmaceutical industry for characterizing drug delivery mechanisms and investigating drug-nanocarrier interactions. Under nitrogen gas, 10 mg each of MEB, Compritol ATO 888, and lyophilized MEB-SLN were mounted on an aluminum pan and scanned between 50° and 350°C at a rate of 10°C/min.[17,22]

X-ray Diffraction (XRD) Studies

The XRD technique is used to determine whether a substance is crystalline or amorphous.[5] Inorganic materials are evaluated using XRD to determine phase purity and crystallinity. An X-ray diffractometer (X'Pert Powder PAN analytical device, Netherlands) was used to capture XRD patterns of the optimized SLNs using Cu Ka radiation at 40 mA, 35 kV. The MEB, Compritol ATO 888, and lyophilized MEB-SLN were scanned in the 5° to 50° range.[22]

Transmission Electron Microscopy (TEM)

The TEM is a method for imaging nanoparticle samples that uses an electron beam to achieve much higher precision than light-based imaging techniques. The TEM is an excellent tool for analyzing structural and chemical properties at the nanoscale. A drop of SLN dispersion was applied to a 200-mesh copper grid that had been coated with carbon film and stained with phosphotungstic acid at 2% w/v. The grid was dried at room temperature and the TEM was used to inspect it (TEM Jeol JEM-14000).[25,28]

In-vitro Drug Release Studies

The *in vitro* drug release study of MEB-SLN formulation was carried using USP-II dissolution apparatus (paddle

Table 1: Variables and their levels used in Box–Behnken statistical factorial design for the development of MEB-SLN

	Levels		
Variables	*Low (-1)*	*Medium (0)*	*High (+1)*
Independent variables			
X_1= Drug: solid lipid (MEB : Compritol ATO 888) (% w/w)	1:50	1:100	1:200
X_2= Surfactant concentration (Poloxamer 407) (% w/w)	0.5	1	1.5
X_3= Sonication time (min)	5	10	15
Dependent Variables	*Constraints*		
Y_1= Particle size (nm)	Minimum		
Y_2= Entrapment efficiency (%)	Maximum		

apparatus).[17,22] A dissolution medium of 900 mL phosphate buffer pH6.8 was used in this analysis. The samples (2 mL) were taken out at daily intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 hours) and substituted with the same volume of dissolution medium to keep the sink state. A UV spectrophotometer set to 286 nm was used to determine the amount of dissolved drug.[27,29]

Stability Studies

The stability of nanoparticles is important in several ways. Stability is one of the most important factors in maintaining the quality and effectiveness of drug products, and as a result, its evaluation has become vital in the pharmaceutical industry. The stability study was conducted as per International Conference on Harmonisation (ICH) Q1A (R2) guideline. For six months, the formulation was refrigerated (4 ± 1°C) and placed in a stabilization chamber (25 ± 1°C) in a sealed container. The stability study was checked by observing the influence of particle size, entrapment efficiency, phase separation, and redispersibility in the formulation over a period of six months.[25,30]

Statistical Analysis

The values are analyzed as a mean and standard deviation (SD). Student's t-test and ANOVA were used to compare the experimental findings statistically. p values less than 0.05 were considered relevant in all cases.

Table 2: Independent variables, along with their coded and actual values, along with their respective responses for different batches of MEB-SLN

	Coded Value factor			*Responses value*	
Run	X_1	X_2	X_3	Y_1: *Particle Size (nm)*	Y_2: *Entrapment Efficiency (%)*
1	-1	0	1	166.1 ± 0.51	70.4 ± 0.25
2	0	0	0	154.2± 0.53	74.8 ± 0.51
3	0	-1	1	250.4± 0.32	77.4 ± 0.72
4	0	0	0	154.3 ± 0.25	75.2 ± 0.39
5	1	0	1	230.4 ± 0.56	81.6 ± 0.53
6	1	0	-1	255.3 ± 0.87	85.4 ± 0.74
7	-1	-1	0	171.4 ± 0.94	70.8 ± 0.52
8	0	0	0	154.3 ± 0.26	74.6 **± 0.94**
9	1	1	0	193.5 ± 0.72	77.8 ± 0.51
10	0	-1	-1	274.5 ± 0.33	80.1 ± 0.58
11	-1	0	-1	183.5 ± 0.82	72.3 ± 0.86
12	1	-1	0	236.9 ± 0.27	83.7 ± 0.21
13	0	0	0	154.1 ± 0.75	74.9 ± 0.39
14	-1	1	0	118.6 ± 0.25	71.8 ± 0.66
15	0	1	-1	223.6 ± 0.65	78.3 ± 0.65
16	0	1	1	204.4 **± 0.61**	76.2 ± 0.71
17	0	0	0	154.3 ± 0.52	74.7 ± 0.52

Results and Discussion

MEB Solubility in Lipids and Surfactants

The preliminary formulation trials were carried out to screen surfactants (Tween-80, Poloxamer 407, SLS) and solid lipids (stearic acid, cetyl palmitate, cholesterol, and compritol 888 ATO), as these variables would affect particle size, zeta potential, entrapment efficiency, and *in vitro* release profile. Solubility of a drug in lipid is one of the most important factors for determining the drug loading capacity of the SLN. It was observed that the MEB showed maximum solubility in compritol 888 ATO as compared to other lipids. The emulsion was found stable with poloxamer 407 and no phase separation was observed. Thus, the compritol 888 ATO and poloxamer 407 were selected as a solid lipid and surfactant respectively for the study.

Formulation and Optimization of MEB-SLN

The melt-emulsification ultrasonication process was used to generate 17 MEB-SLN formulations, which were then optimized using a three-level BBD. The drug-to-solid-lipid ratio (X_1), surfactant concentration (X_2), and sonication time (X_3) were chosen as independent variables for the optimization studies. The optimization tools were used to investigate the interactions of these independent variables on particle size (Y_1) and entrapment efficiency (Y_2). The Design-Expert software evaluated the results of all experimental design formulations, and the responses generated from this run are shown in Table 2.

Effect of Independent Variables on Particle Size (Y_1): The particle size of all 17 MEB-SLN formulations ranges from 118.6 nm to 274.5 nm. The 3D response surface plot of the effects of independent variables on particle size (Y_1) is shown in Figs. 1(a)–(c). The particle size of formulations decreases as the drug: lipid ratio decreases, surfactant concentration increases, and sonication time increase. The particle size increases as the drug: lipid ratio increases, and *vice versa*. The particle size decreases with increasing surfactant concentration. This may be attributed to a reduction in interfacial stress between the aqueous and organic phases.[22] The particle size reduces as the sonication time increases, and *vice versa*.

Effect of Independent Variables on Entrapment Efficiency (Y_2): The entrapment efficiency of all 17 MEB-SLN formulations ranges from 70.4 to 85.4%. The 3D response surface plot of the effects of independent variables on entrapment efficiency (Y_2) is shown in Fig. 1(d)–(f). Entrapment efficiency increases as the drug: lipid ratio increases, and *vice versa*. Entrapment efficiency decreases with increasing surfactant concentration. Entrapment efficiency increases as sonication time increases, and *vice versa*.

The quadratic equation generated for optimized MEB-SLN formulation is listed below:

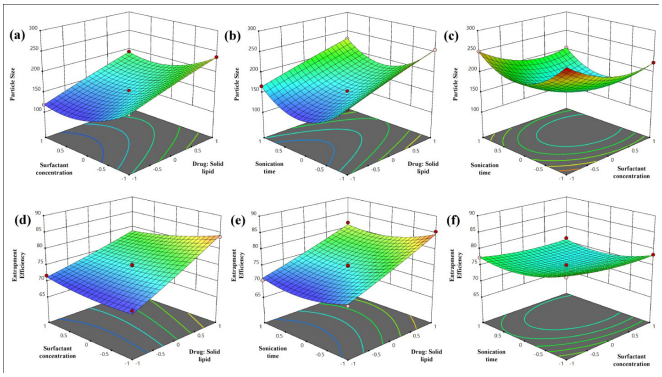

Fig 1: Optimization of MEB-SLN formulation parameters. The effect of independent variables on particle size represent in (a), (b), and (c). The effect of independent variables on entrapment efficiency represent in (d), (e), and (f).

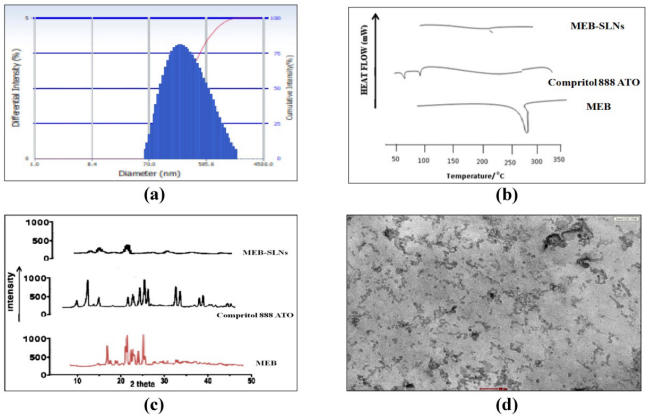

Fig 2: Characterization of optimized MEB-SLN formulation: (a) Particle size: 230.4 nm, (b) DSC, (c) XRD, (d) TEM

$$Y_1 = +154.24 + 34.56X_1 - 24.14X_2 - 10.70X_3 + 2.35X_1X_2 - 1.88X_1X_3 + 1.23X_2X_3 - 1.77X_1^2 + 27.63X_2^2 + 56.36X_3^2$$

$$Y_2 = +74.84 + 5.40X_1 - 0.9875X_2 - 1.31X_3 - 1.73\,X_1X_2 - 0.4750\,X_1X_3 + 0.1500\,X_2X_3 + 0.3050\,X_1^2 + 0.8800\,X_2^2 + 2.28\,X_3^2$$

The optimized MEB-SLN formulation was chosen based on the criterion of maximizing entrapment efficiency and minimizing particle size using the Design Expert software's point prediction process. The formulation composition with the drug-to-solid-lipid ratio (1:200), surfactant concentration (1%), and sonication time (15 mins) was found to meet the requirements of an optimal formulation, i.e. SLN5. The particle size of the optimized MEB-SLN formulation (SLN5) is 230.4 ± 0.56 nm, and the entrapment efficiency is 81.6 ± 0.53%.

Characterization, stabilization, and *In-vitro* Evaluation of Optimized MEB-SLN

Particle Size, Polydispersity Index, and Zeta Potential Measurement

The particle size of nanoformulations is crucial in determining colloid properties such as rheology, film gloss, surface area, and packing density. With a PDI of 0.231, the optimized MEB-SLN particle size was found to be 230.4 ± 0.56 nm (Fig. 2(a)). The low PDI value indicates that the particles are homogeneous. [26] The SLN has a zeta potential of -21.9 mV, which is sufficient to form a stable colloidal nanosuspension.

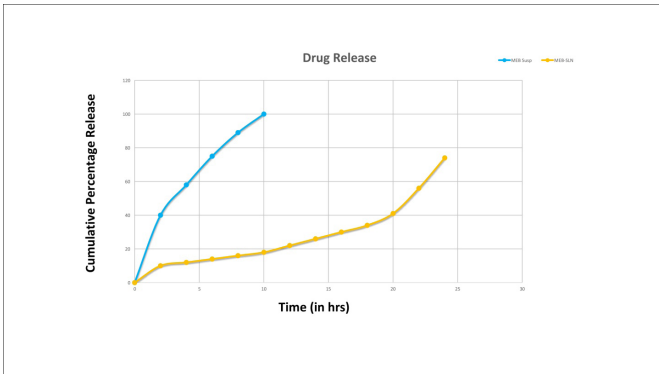

Fig 3: *In vitro* drug release studies of optimized MEB-SLN formulation

Entrapment Efficiency

Entrapment efficiency is one of the critical characteristics to assess the efficiency of carriers. The optimized MEB-SLN formulation had an entrapment efficiency of 81.6 %.[25]

Differential Scanning Calorimetry (DSC)

Fig. 2 (b) illustrates the DSC thermograms of MEB, Compritol ATO 888, and lyophilized MEB-SLN. The crystalline nature of MEB was indicated by a sharp peak that corresponded to melting at 288°C and solid lipid showed an endothermic peak at 82.5°C. The absence of an MEB melting peak in SLN suggests either amorphous dispersion of MEB in the lipid matrix or MEB solubilization in the lipid matrix upon heating. [17, 22]

X-ray Diffraction (XRD) Studies

Fig. 2 (c) illustrates the x-ray diffraction patterns of compritol ATO 888, pure drug MEB, and MEB-SLN. At an angle of 2h, the XRD spectra of pure MEB showed high-intensity characteristic sharp peaks at 16.1, 21.8, and 28.0. Peaks of Compritol ATO 888 were found at 3.9, 20.7, and 23.1. The characteristic peak of MEB diminished from the SLN curve, showing that the compound seems to be in a disrupted crystalline state in SLN.[5,22]

Transmission Electron Microscopy (TEM)

An exact morphology (size, shape, and internal structure) of developed nanoparticles could be analyzed by transmission electron microscopy. The optimized MEB-SLN formulation had an almost spherical surface with a uniform size distribution, as seen in TEM images. Fig. 2 (d) shows a TEM photomicrograph of the optimized MEB-SLN formulation.[25,28]

In-vitro Drug Release Studies

The drug was released in a biphasic pattern, with a quick initial release accompanied by a sustained release

Table 3: Stability studies of optimized MEB-SLN formulation

	Test Period					
	4 ± 2°C, 60 ± 5 % RH			*25 ± 2°C, 60 ± 5 % RH*		
Stability Parameters	*0 month*	*3 months*	*6 months*	*0 month*	*3 months*	*6 months*
Particle Size (nm)	230.4 ± 0.56	233.9 ± 0.47	236.2 ± 0.76	230.8 ± 0.56	234.9 ± 0.12	238.4 ± 0.37
Entrapment Efficiency (%)	81.6 ± 0.53	80.2 ± 0.26	79.8 ± 0.75	82.1 ± 0.53	80.4 ± 0.94	78.6 ± 0.46
Phase separation	No	No	No	No	No	No
Redispersibility	Yes	Yes	Yes	Yes	Yes	Yes

extending up to 24 hours. SLN's burst release mechanism delivers a high quantity of drugs for rapid therapeutic action and increases drug penetration while allowing for long-term release.[27,29] Around 30% of the drug was released in the first 15 mins, followed by a gradual and steady release pattern that reached 70% after 12 hours. Entrapment of the drug in the lipid matrix can be the cause of its long-term release. In comparison to a pure drug, SLN proved to be effective carriers for the continuous delivery of MEB as shown in Fig. 3.[22,27]

Furthermore, the obtained data were fitted to several mathematical models such as Korsmeyer–Peppas, Higuchi Matrix, and Hixson–Crowell to evaluate the release mechanism. The coefficient of correlation (r) was determined to be 0.994. For the *in-vitro* release analysis, the model with the highest correlation coefficient was regarded as the most acceptable.[17]

Stability Studies

At 4 and 25°C, the physical stability of MEB-SLN was evaluated using a stability test. After 180 days at 25°C, the optimized MEB-SLN formulation had particle sizes of 196.46 ± 8.64 nm and entrapment efficiency of 76.47 ± 3.58 %, respectively. After 180 days at 25°C, the optimized MEB-SLN formulation shows no phase separation or precipitate formation. Furthermore, the developed SLN formulation showed significant redispersibility after 180 days of testing. Table 3 summarizes the results of the stability studies.[30]

Conclusion

MEB-SLN was successfully formulated using melt emulsification ultrasonication methods, and the formulation parameters were tailored using Box Behnken modeling experiment tools to achieve the desired target characteristics. The optimized MEB-SLN formulation demonstrated strong particle homogeneity, robust colloidal stability, and high drug entrapment. The DSC and XRD affirm the purity, crystallinity, and interaction of drugs and excipients. TEM establishes the structural confirmation of SLN nanoparticles. *In vitro* drug release tests showed that the optimized MEB-SLN formulation released the drug more slowly than the drug solution. A stability studies revealed that the optimized formulation was stable and there was no phase separation. As a finding of this study, it was obvious that optimized MEB-SLN could be treated as a possible carrier for sustained drug delivery.

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