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Original Article

# Design and Optimization of Agmatine Sulphate Loaded Solid Lipid Nanoparticles for Neurological Disorders: A DOE Approach

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## ABSTRACT

**Objectives:** This study focuses on the formulation and optimization of Solid Lipid Nanoparticles (SLNs) containing agmatine sulfate, with the goal of enhancing its bioavailability and minimizing rapid metabolic degradation, thereby improving its therapeutic application in neurological conditions. **Materials and Methods:** Solid Lipid Nanoparticles (SLNs) containing agmatine sulfate were formulated using the hot melt homogenization method. Glyceryl Monostearate (GMS) served as the lipid matrix, while Tween 80 and Span 80 were employed as surfactants. A Box-Behnken Design (BBD) was utilized to systematically assess the impact of three independent variables-concentrations of GMS, Tween 80, and Span 80-on critical formulation parameters: particle size, Polydispersity Index (PDI), and entrapment efficiency. The optimized formulation was thoroughly characterized using Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), particle size analysis, and *in vitro* drug release profiling. **Results:** The optimized SLN formulation exhibited a mean particle size of 130.5 nm, a PDI of 0.189, and an entrapment efficiency of 95%. Compatibility studies confirmed the absence of significant interactions between agmatine sulfate and formulation excipients, verifying successful encapsulation within the lipid matrix. *In vitro* drug release displayed a biphasic profile characterized by an initial burst followed by sustained release over 24 hr. Kinetic modeling revealed a strong fit with the Higuchi model ($R^2$=0.9644), indicating diffusion-controlled drug release. **Conclusion:** The developed agmatine sulfate-loaded SLNs exhibit favorable properties for targeted brain delivery and prolonged release, highlighting their potential utility in the treatment of neurological disorders. This formulation strategy could substantially enhance the clinical performance of agmatine by improving both its bioavailability and therapeutic effectiveness.

**Keywords:** Agmatine Sulphate, Box-Behnken Design, Brain Targeting, Neurological Disorders, Solid Lipid Nanoparticles, Sustained Release.

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## NTRODUCTION

Agmatine is a naturally occurring biogenic amine produced through the decarboxylation of the amino acid L-arginine. It has gained considerable attention for its neuroprotective capabilities. These protective effects stem from various mechanisms, including antioxidant activity, anti-apoptotic properties, anti-inflammatory responses, maintenance of Blood-Brain Barrier (BBB) integrity, and the mitigation of cerebral edema (Xu *et al*., 2018). Evidence suggests that agmatine offers therapeutic benefits in managing several neurological conditions such as ischemic stroke, traumatic brain injury, epilepsy, Parkinson's disease, and Alzheimer's disease (Liu *et al*., 2023).

Despite its promising pharmacological profile, the clinical application of agmatine remains limited due to its rapid metabolic degradation and poor permeability across the BBB. To address these challenges, the formulation of Solid Lipid Nanoparticles (SLNs) encapsulating agmatine sulfate has been explored as a strategy to improve its pharmacokinetics and therapeutic efficacy. SLNs serve as effective carriers for hydrophilic and lipophilic drugs, providing advantages like enhanced stability, targeted delivery, and controlled release. Nanoparticles, typically sized between 10 and 1000 nm, offer favorable properties such as a high surface area-to-volume ratio, substantial drug-loading capacity, and the ability to modify drug release kinetics, all of which

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enhance pharmaceutical performance (Pawar *et al*., 2022; Patel *et al*., 2024; Lingayat *et al*., 2017; Satapathy *et al*., 2021; Naqvi *et al*., 2020; Kaur *et al*., 2008). Notably, SLNs have shown potential in the treatment of central nervous system disorders by facilitating drug transport across the BBB.

To develop SLNs with desirable attributes such as optimal particle size, drug entrapment efficiency, and sustained release, systematic formulation optimization is crucial. The Design of Experiments (DoE) methodology provides a structured framework to evaluate the influence of formulation and process variables, enabling the identification of optimal conditions. For example, DoE has been successfully employed to improve the encapsulation efficiency of SLNs from around 30% to over 60%, demonstrating its utility in optimizing drug delivery systems (Naqvi *et al*., 2020; Kaur *et al*., 2008).

Agmatine sulfate exerts its neuroprotective effects by modulating NMDA receptor activity, lowering intracellular calcium levels, and influencing neurotransmitter pathways. It also plays a role in regulating second messenger systems and alleviating inflammatory pain responses. However, limitations in bioavailability and rapid clearance have hindered its therapeutic use. Nanotechnology-based drug delivery systems offer a promising approach to overcome these drawbacks by improving drug stability, targeting specific tissues, and ensuring prolonged drug release (Kumari *et al*., 2023).

The objective of this study is to design and optimize a nanoparticulate delivery system for agmatine sulfate using a DoE approach, with the ultimate goal of enhancing its safety and therapeutic potential in the management of neurological disorders.

## MATERIALS AND METHODS

### Preformulation Studies

Preformulation is a critical phase in the rational design of pharmaceutical formulations, aimed at evaluating the physicochemical properties of the Active Pharmaceutical Ingredient (API). These investigations help determine the feasibility of formulation development and guide the selection of appropriate excipients and processing methods. The objective of the current preformulation studies was to assess the key physicochemical characteristics of agmatine sulfate and identify any potential formulation challenges.

### Organoleptic Evaluation

Organoleptic properties, including color, odor, taste, and appearance, were assessed visually and manually to obtain preliminary information about the drug's physical characteristics.

### Melting Point Determination

To evaluate the purity and crystalline nature of agmatine sulfate, its melting point was determined using the capillary tube method. A small amount of the drug was introduced into a capillary tube sealed at one end, which was then placed in a calibrated melting point apparatus. The temperatures at the onset and completion of melting were noted. This procedure was conducted in triplicate, and the mean value obtained was cross-verified with both literature values and the certificate of analysis for confirmation.

### Ultraviolet Spectroscopy of Agmatine Sulfate

#### *Preparation of Standard Stock Solution*

A precisely weighed 10 mg of agmatine sulfate was placed into a 10 mL volumetric flask. The volume was brought up to the mark using distilled water, yielding a standard stock solution with a final concentration of 1000 µg/mL.

### Construction of Calibration Curve

A working standard solution (10 µg/mL) was prepared by diluting 1 mL of the stock solution to 100 mL with distilled water. From this working solution, aliquots of 1, 2, 4, 6, 8, and 10 mL were pipetted into separate 10 mL volumetric flasks and the volume in each flask was made up with distilled water. These samples, corresponding to concentrations of 1-10 µg/mL, were scanned between 200 and 400 nm using a UV-visible spectrophotometer to determine the maximum wavelength of absorption (λ) Each measurement was performed in triplicate. The average absorbance values were plotted against concentration to obtain a calibration curve. A linear relationship was observed, confirming adherence to Beer-Lambert's law within the tested range. The regression equation was derived from the slope of the standard curve.

### Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was utilized to identify characteristic functional groups and to evaluate potential interactions between the drug and excipients. The infrared spectra of moisture-free agmatine sulfate were recorded using an FTIR spectrophotometer. The scanning range was set from 400 to 4000 $cm^{-1}$, with a resolution of 1 $cm^{-1}$. The resulting spectrum was analyzed to confirm the identity of the compound and assess any notable chemical interactions.

### Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was employed to determine the thermal behavior, including melting point and thermal transitions, of agmatine sulfate. A sample weighing approximately 3 mg was placed in a standard aluminum pan and hermetically sealed. The analysis was conducted using a TA Instruments Q10 DSC system (USA). The heating rate was maintained at 10°C/min over a temperature range of 50 to 300°C under a continuous flow of nitrogen gas. Endothermic and exothermic events were

recorded to assess purity and crystalline properties and to detect any possible interaction with excipients.

## X-ray Diffraction (XRD) Analysis

X-ray diffraction analysis was performed to investigate the crystalline nature of the pure drug. The diffraction pattern was obtained using a JEOL JDX-8030 diffractometer equipped with a copper target, operated at 40 kV and 30 mA. The scanning was carried out over a 2θ range of 10° to 80° at a step size of 2°. The resulting diffractogram was analyzed to assess the crystalline structure and phase purity of agmatine sulfate.

## Preparation of Agmatine Sulphate-Loaded Solid Lipid Nanoparticles (SLNs)

The water-soluble nature of agmatine sulphate, the drug was initially combined with the aqueous surfactant solution. Specifically, 400 mg of Tween 80 and 300 mg of Span 80 were dissolved in an appropriate volume of distilled water in one beaker. To this surfactant mixture, 10 mg of agmatine sulphate was added and stirred to ensure complete dissolution. In a separate beaker, 1 g of GMS (as shown in Table 1) was weighed and melted.

Solid Lipid Nanoparticles (SLNs) incorporating agmatine sulphate were formulated using the hot melt homogenization method, with Millipore water serving as the dispersion medium. Glyceryl Monostearate (GMS) was selected as the lipid matrix, while Tween 80 and Span 80 were used as surfactants. The proportions of the excipients were optimized using a Design of Experiments (DoE) approach.

Both the lipid and aqueous phases were uniformly heated to 65°C. The aqueous phase, containing the drug and surfactants, was stirred magnetically at 500 rpm for 10-15 min. Subsequently, it was added dropwise to the molten lipid phase while maintaining continuous stirring at 500 rpm. The mixture was then emulsified using a high-shear homogenizer operating at 13,000 rpm for 20 min. To further reduce particle size and enhance formulation stability, the emulsion was subjected to probe sonication for 5 min at 50% amplitude, employing a pulsed cycle of 5 sec on and 5 sec off.

The final SLN formulation was stored at refrigerated conditions (4-8°C) until further characterization. Representative images of the formulation process and resulting nanoparticles are presented in Figure 1.

## Optimization of Formulation Parameters Using Design of Experiments (DoE)

The formulation was optimized using the Box-Behnken Design (BBD) strategy, executed through Design-Expert® software version 13 (Stat-Ease Inc., Minneapolis, MN, USA). This response surface methodology was selected for its ability to efficiently assess the influence of multiple formulation parameters while minimizing the number of required experiments, offering a more streamlined alternative to full factorial designs. In this investigation, three key independent variables-Glyceryl Monostearate (GMS), Tween 80, and Span 80 were evaluated at three concentration levels, as shown in Table 1. Their effects on three dependent responses particle size (R1), polydispersity index (R2), and entrapment efficiency (R3) were systematically analyzed. The BBD enabled the examination of both individual and interaction effects, allowing for a comprehensive understanding of formulation behavior. Seventeen experimental runs, including five center points, were conducted to ensure statistical robustness. The collected data were modeled using a quadratic polynomial equation, and the statistical validity of the model was determined through Analysis of Variance (ANOVA), including lack-of-fit testing. This approach facilitated the identification of an optimized formulation with favorable physicochemical properties (Khishvand *et al*., 2024; Zhang *et al*., 2020; Cassayre *et al*., 2025).

## Evaluation of the Optimized SLN Formulation

### *Scanning Electron Microscopy (SEM)*

The morphological characteristics of the optimized Solid Lipid Nanoparticles (SLNs) were observed using a Scanning Electron Microscope (JEOL JSM T330A). The surface structure and shape of the nanoparticles, as well as physical mixtures of lipid excipients and pure drug, were examined under various magnifications. The images were digitally captured and analyzed to determine particle shape, surface texture, and agglomeration.

## Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was conducted to assess the thermal characteristics and physical state of agmatine sulfate in its pure form as well as within the nanoparticle formulation. Around 5 mg of each sample was placed in sealed aluminum pans and subjected to heating at a rate of 10°C per minute across a temperature range of 50-300°C. This analysis aimed to detect alterations in crystallinity and to identify any possible interactions between the drug and formulation excipients.

## Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed to identify any possible chemical interactions between agmatine sulphate and the excipients used in the formulation. The IR spectra of pure drug and optimized SLNs were recorded using a Bruker Vertex 70 FT-IR spectrometer within a scanning range of 400-4000 $cm^{-1}$, and resolution of 1 $cm^{-1}$. Characteristic peaks were compared to assess any shift or disappearance, indicating drug-excipient compatibility.

## X-ray Diffraction (XRD)

XRD analysis was conducted to determine the crystalline or amorphous nature of the nanoparticles. The diffraction patterns of the optimized SLNs were compared with those of the pure drug. Any reduction in peak intensity or disappearance of sharp

peaks was used as an indication of a transition from crystalline to amorphous form upon formulation.

## Particle Size and Polydispersity Index (PDI)

The mean particle size and Polydispersity Index (PDI) of the optimized SLNs were measured using a Zetasizer (Model ZEN 3690, Malvern Instruments, UK) based on the dynamic light scattering principle. Samples were diluted with double-distilled water and gently shaken before analysis. Measurements were performed at 25°C and 90° scattering angle to ensure accurate characterization. A narrow PDI value indicated a uniform particle distribution.

## Drug Content Estimation and *in vitro* Release Studies

### *Entrapment Efficiency (%EE)*

The Entrapment Efficiency (%EE) of the optimized solid lipid nanoparticles was determined to assess the proportion of drug successfully encapsulated within the formulation. The freshly prepared SLN dispersion was centrifuged at 4000 rpm for 10 min to separate the unentrapped drug. From the supernatant (aqueous phase), 1 mL was accurately withdrawn using a sterile hypodermic syringe and diluted appropriately with phosphate buffer (pH 6.8). The resulting solution was filtered through a 0.22 µm Millipore membrane filter and analyzed for drug content using a UV-visible spectrophotometer (Shimadzu-1800, Japan) at a wavelength of 238 nm. Each sample was analyzed in triplicate to ensure precision.

The entrapment efficiency was calculated using the formula:

$$\text{Entrapment Efficiency (\%)} = \frac{(\text{Total drug added} - \text{Free drug in supernatant})}{\text{Total drug added}} \times 100$$

## *In vitro* Drug Release and Kinetic Modeling

The *in vitro* release profile of agmatine sulfate from the optimized Solid Lipid Nanoparticles (SLNs) was evaluated using a USP

**Table 1: Composition of ingredients and independent variables used in the preparation and optimization of Agmatine Sulphate-loaded Solid Lipid Nanoparticles (SLNs).**

| Sl. No. | Component | Quantity Used | Responses | Description |
|---|---|---|---|---|
| 1. | Agmatine Sulphate | 10 mg | - | - |
| 2. | Glyceryl Monostearate | 1 g | R1 | Particle size (nm) |
| 3. | Tween 80 | 400 mg | R2 | Polydispersity Index (PDI) |
| 4. | Span 80 | 300 mg | R3 | Entrapment Efficiency (%) |
| 5. | Millipore water | 20 mL | - | - |

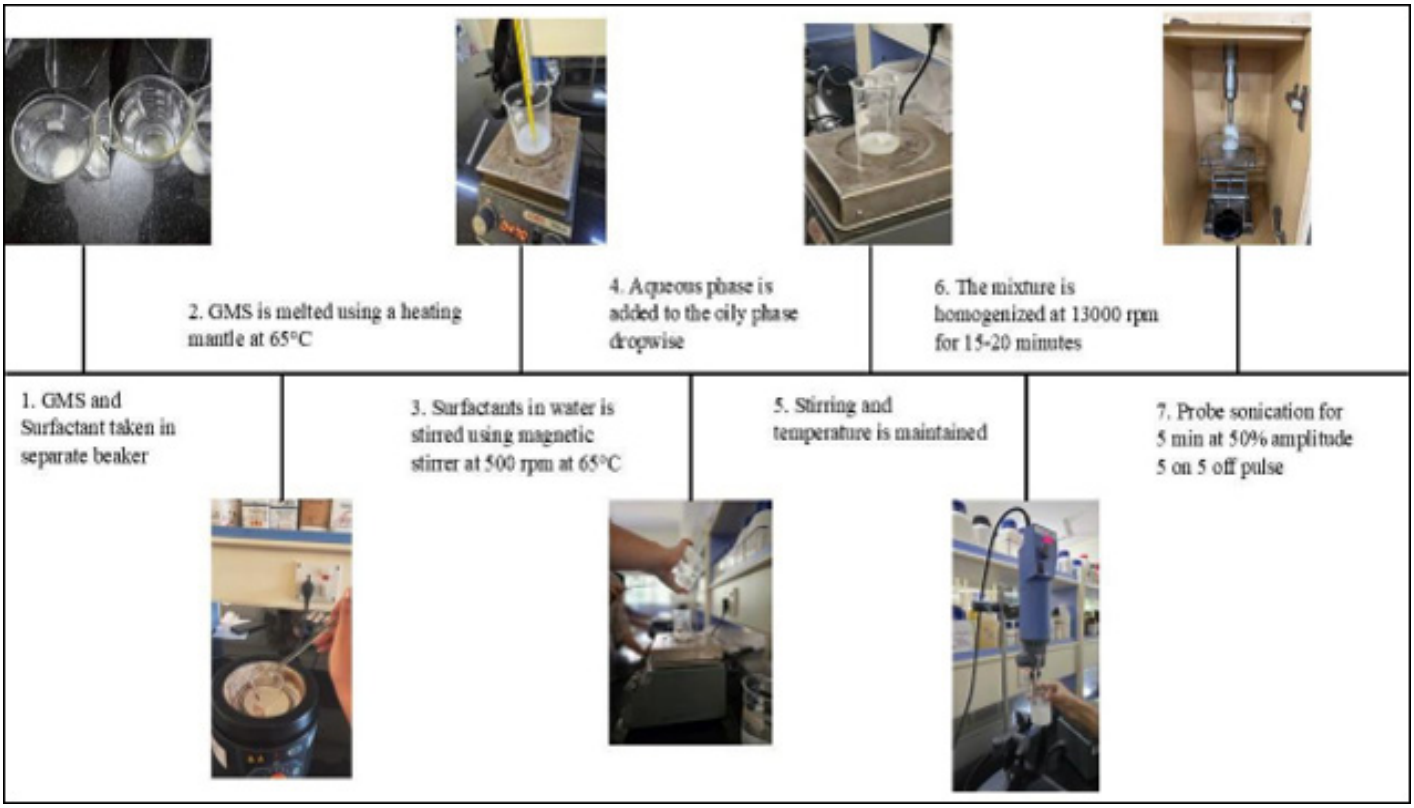


**Figure 1:** Schematic representation of the preparation process for agmatine sulphate-loaded Solid Lipid Nanoparticles (SLNs) using the hot melt homogenization method.

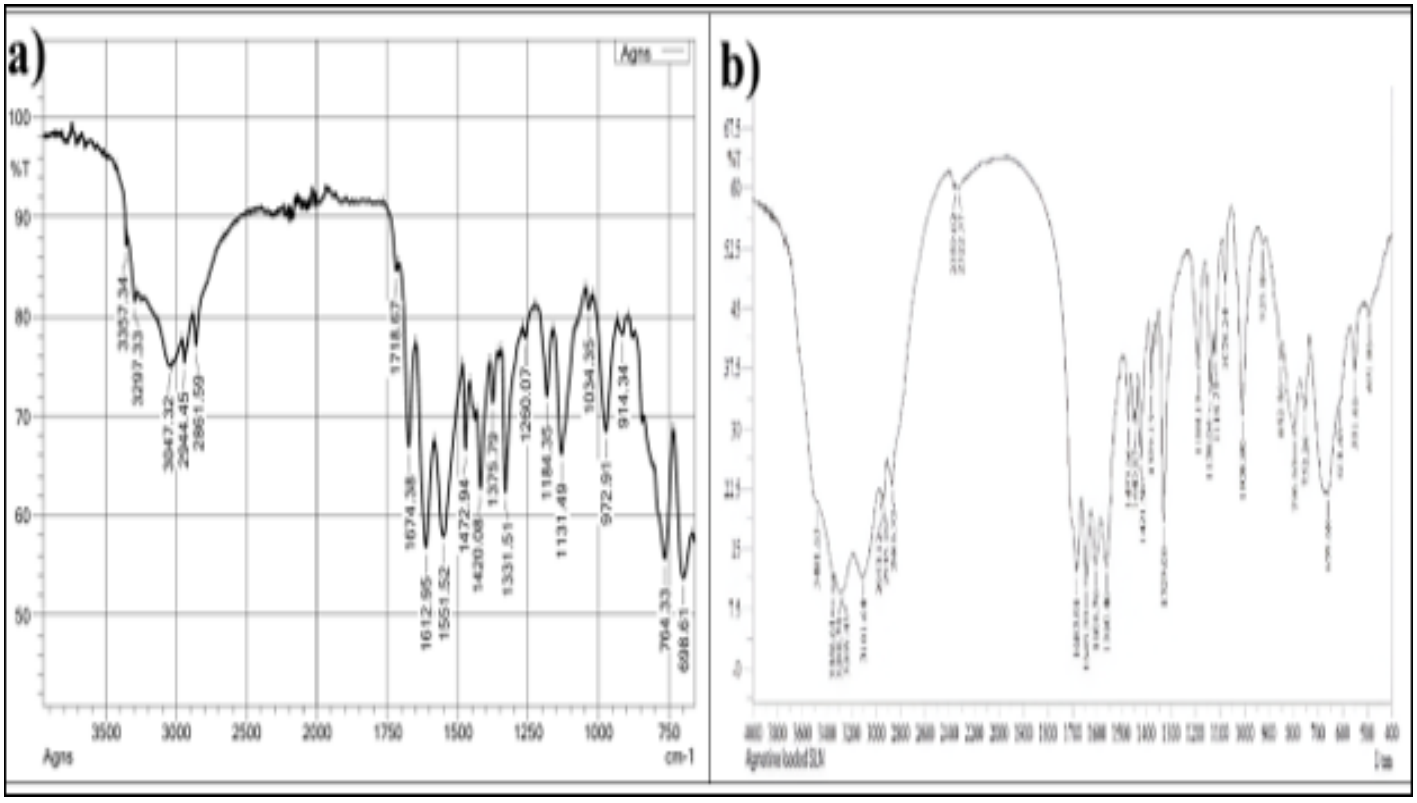


**Figure 2:** FTIR spectrum a) Agmatine sulphate b) Agmatine Sulphate-loaded SLNs.

Type II dissolution apparatus (Electrolab EDT-08L, India). The study was carried out in 900 mL of phosphate buffer (pH 6.8) maintained at 37±0.5°C, with stirring at 75 rpm. At predetermined intervals (0.5, 1, 2, 4, 6, 12, and 24 hr), 6 mL aliquots were withdrawn and replaced with an equal volume of fresh buffer to ensure sink conditions. The collected samples were filtered and analyzed at 238 nm using a UV spectrophotometer to quantify drug concentration.

The cumulative drug release was calculated and plotted as a function of time. To understand the release mechanism, the data were fitted into multiple kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Results were reported as Mean ± Standard Deviation (SD).

## RESULTS

### Preformulation Studies

The preliminary evaluation of agmatine sulphate was carried out to establish its physicochemical characteristics.

### Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR analysis was conducted to identify functional groups and confirm the chemical structure of the pure drug. The FTIR spectrum of agmatine sulphate is presented in Figure 2, and the corresponding characteristic peaks are summarized in Table 2.

FTIR spectroscopy was conducted to evaluate potential interactions between agmatine sulphate and the excipients used in the formulation of Solid Lipid Nanoparticles (SLNs). The FTIR spectrum of the pure drug is presented in Figure 2a, while the spectrum of the SLN formulation is shown in Figure 2b.

Agmatine sulphate exhibited characteristic peaks corresponding to N-H stretching, O-H/N-H stretching, C=O stretching (carboxylate), and C-N stretching vibrations. These peaks were also observed in the SLNs formulation, with slight shifts in wave numbers, indicating the presence of the same functional groups post-formulation.

### Differential Scanning Calorimetry (DSC) Analysis

DSC was used to examine the thermal behavior of Agmatine Sulphate in its pure form and within the SLN formulation (Figure 3).

### X-ray Diffraction (XRD) Analysis

X-ray Diffraction studies were carried out to investigate the solid-state characteristics of agmatine sulphate in its pure form and within the formulated SLNs. The diffractograms are shown in Figure 4a, and the overlaid patterns of the pure drug, physical mixture, and optimized nanoparticle formulation are presented in Figure 4b.

### Formulation and Optimization Using Design of Experiment

The formulation of Agmatine sulphate-loaded Solid Lipid Nanoparticles (SLNs) was optimized using a Box-Behnken Design

(BBD), executed via Design-Expert 13 software (Stat-Ease, Inc., Minneapolis, MN). This statistical approach facilitated systematic evaluation of the influence of three critical formulation variables:

**$X_1$:** Glyceryl Monostearate (GMS, 800-1200 mg).

**$X_2$:** Tween 80 (300-400 mg).

**$X_3$:** Span 80 (250-350 mg).

These independent variables were studied at three coded levels (−1, 0, +1) to observe their effect on three key dependent responses:

**$Y_1$:** Particle Size (target: 200-230 µm).

**$Y_2$:** Polydispersity Index (PDI, target: 0.05-0.30).

**$Y_3$:** Entrapment Efficiency (EE%, target: 85-90%).

The BBD model, a Response Surface Methodology (RSM), was selected due to its efficiency in modeling quadratic interactions with fewer experimental runs than a full factorial design. A total of 17 experimental runs were carried out, including five center points to evaluate reproducibility and assess pure error.

The experimental data were fitted to a second-order (quadratic) polynomial model described by the following equation:

$$Y=\beta0+\beta1X1+\beta2X2+\beta3X3+\beta12X1X2+\beta13X1X3+\beta23X2X3 +\beta11X12+\beta22X22+\beta33X32$$

Where:

Y represents the response variable ($Y_1$, $Y_2$, or $Y_3$).

β0 is the intercept.

β1, β2, β3 are the linear coefficients.

β12, β13, β23 are the interaction coefficients.

β11, β22, β33 are the quadratic coefficients.

The design matrix included randomization of run order to minimize bias. The center points (Runs 3, 7, 8, 9, and 10), set at GMS=1000 mg, Tween 80=350 mg, and Span 80=300 mg, were essential for detecting curvature and ensuring reproducibility.

Analysis of Variance (ANOVA) was conducted to evaluate the statistical significance of model terms. The design enabled identification of optimal formulation conditions that minimized particle size and PDI while maximizing entrapment efficiency. The response surface plots and numerical optimization tools guided the selection of the best formulation based on desirability criteria.

## Experimental Design Trials and Model Analysis

To optimize the formulation of Solid Lipid Nanoparticles (SLNs), 17 experimental runs were performed based on the Box-Behnken Design (BBD). The effects of GMS, Tween 80, and Span 80 on particle size, Polydispersity Index (PDI), and entrapment efficiency were systematically evaluated. The experimental matrix and corresponding results are shown in Table 3.

## Response $Y_1$: Particle Size

The quadratic model developed for particle size was found to be highly significant (ANOVA F-value=1035.47, $p<0.0001$), indicating a reliable fit and strong predictive ability. The lack-of-fit

**Table 2: Characteristic FTIR absorption bands of pure Agmatine Sulphate and SLN formulation.**

| Functional Group | Pure Drug ($cm^{-1}$) | SLNs Formulation ($cm^{-1}$) |
|---|---|---|
| N-H stretching | 3297.33 | 3268.14 |
| O-H / N-H stretching | 3460.91 | 3481.63 |
| C=O stretching (Carboxylate) | 1612.95 | 1606.70 |
| C-N stretching | 1034.35 | 1078.24 |

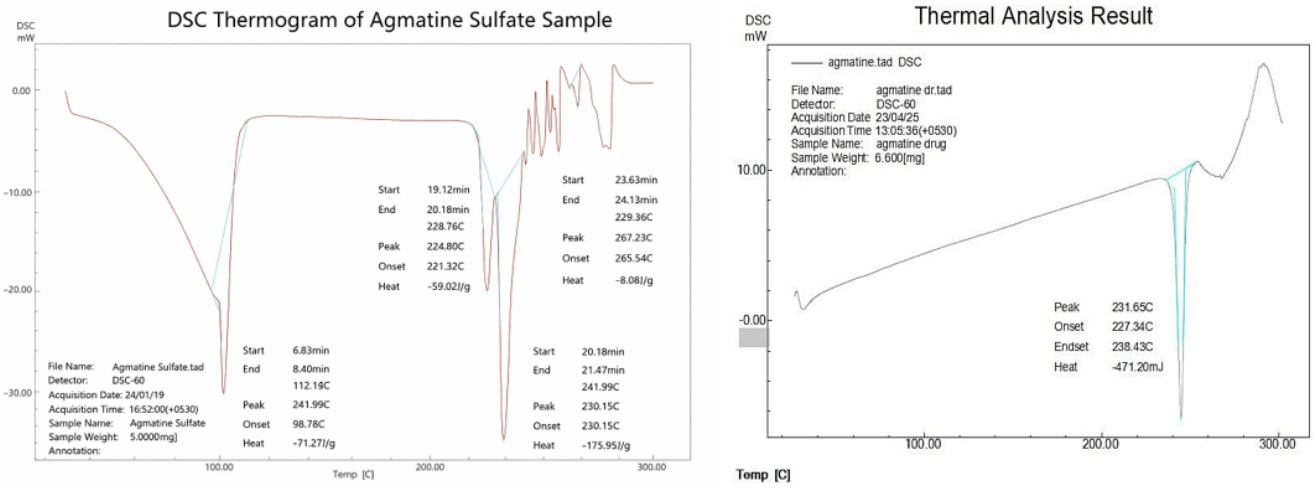


**Figure 3:** DSC thermogram of a) Agmatine Sulphate pure drug b) agmatine sulphate-loaded SLNs.

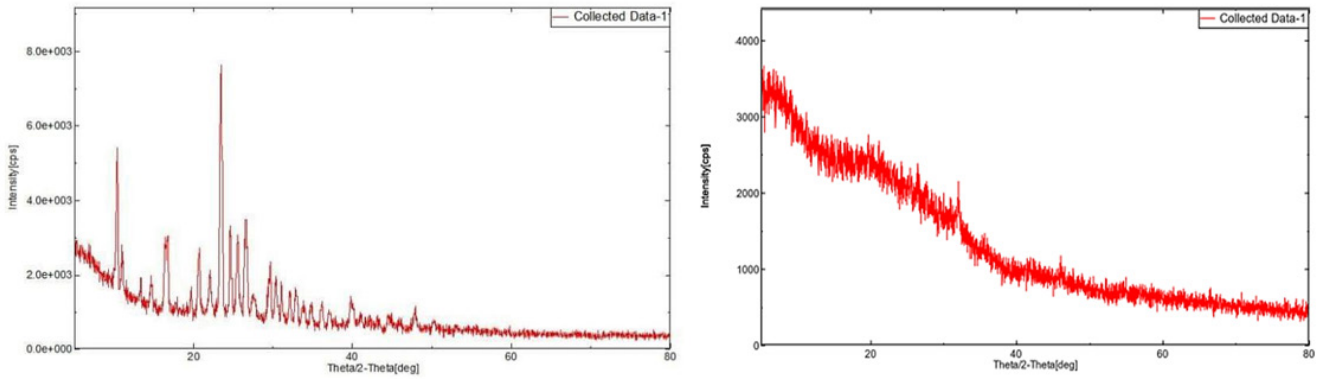


**Figure 4:** X-ray diffraction pattern a) Agmatine Sulphate pure drug b) agmatine sulphate-loaded SLNs.

was not significant (F=2.41, $p$=0.2062), confirming the model's suitability.

Significant terms affecting particle size:

- **Main effects:** A (GMS), B (Tween 80).
- **Quadratic effect:** $A^2$ ($GMS^2$).

These results demonstrate that GMS and Tween 80 significantly influence particle size, particularly in nonlinear (quadratic) relationships.

## Regression equation (coded form)

$$\text{Particle Size}=202.79-33.4X1-5.125X2+7.652X12$$

Where:

X1=GMS.

X2=Tween 80.

This equation implies that increasing GMS reduces particle size to a point, but at higher levels, the particle size increases due to the quadratic behavior. Figure 5 illustrates the predicted vs. actual plot, contour, and 3D response surface confirming these trends.

## Response $Y_2$: Polydispersity Index (PDI)

The reduced quadratic model for PDI was also found to be statistically significant (F-value=146.48, $p$<0.0001). The lack-of-fit was not significant (F=1.90, $p$=0.2799), confirming that the model fits well.

## Significant terms for PDI

**Main effects:** A (GMS, $p$<0.0001), B (Tween 80, $p$=0.0005).

**Quadratic term:** $A^2$ ($GMS^2$, $p$<0.0001).

These findings indicate that both the linear and quadratic effects of GMS significantly impact particle distribution uniformity.

## Regression equation (coded form)

$$PDI=1.21417-0.00238X1-0.00065X2+1.53819\times10-6X12$$

Where:

X1=GMS.

X2=Tween 80.

Lower levels of GMS and Tween 80 favor a more uniform size distribution (lower PDI). The quadratic term for GMS again indicates nonlinear influence. Figure 5 presents visual interpretations through response surface and contour plots.

## Response 3: Entrapment Efficiency

The ANOVA for the reduced quadratic model of Entrapment Efficiency showed the model was statistically significant (F-value=12.63, $p$=0.0003), confirming its predictive reliability. Among the variables, B-Tween 80 ($p$=0.0008), the interaction term AB ($p$ = 0.0004), and quadratic terms $A^2$ ($p$=0.0133) and $B^2$ ($p$=0.0201) had significant effects, all with $p$-values < 0.05. In contrast, A-GMS ($p$=0.1225) did not significantly impact Entrapment Efficiency, suggesting a limited role in influencing this response.

The model equation, in terms of coded factors, was derived as:

Entrapment Efficiency ($Y_3$): $Y_3=-151.33+0.0331X_1+1.208X_2-0.00046X_1X_2+6.668\times10^{-5}X_1^2-0.000983X_2^2$

The model indicates a strong positive influence of Tween 80 on entrapment efficiency, as well as a significant synergistic interaction between GMS and Tween 80 (AB term). The error analysis residual (38.05) and pure error (1.13) demonstrate the model's robustness, with only 0.03% likelihood that the observed trends are due to random noise. The coded factor model allowed precise analysis of each variable's contribution to response variability.

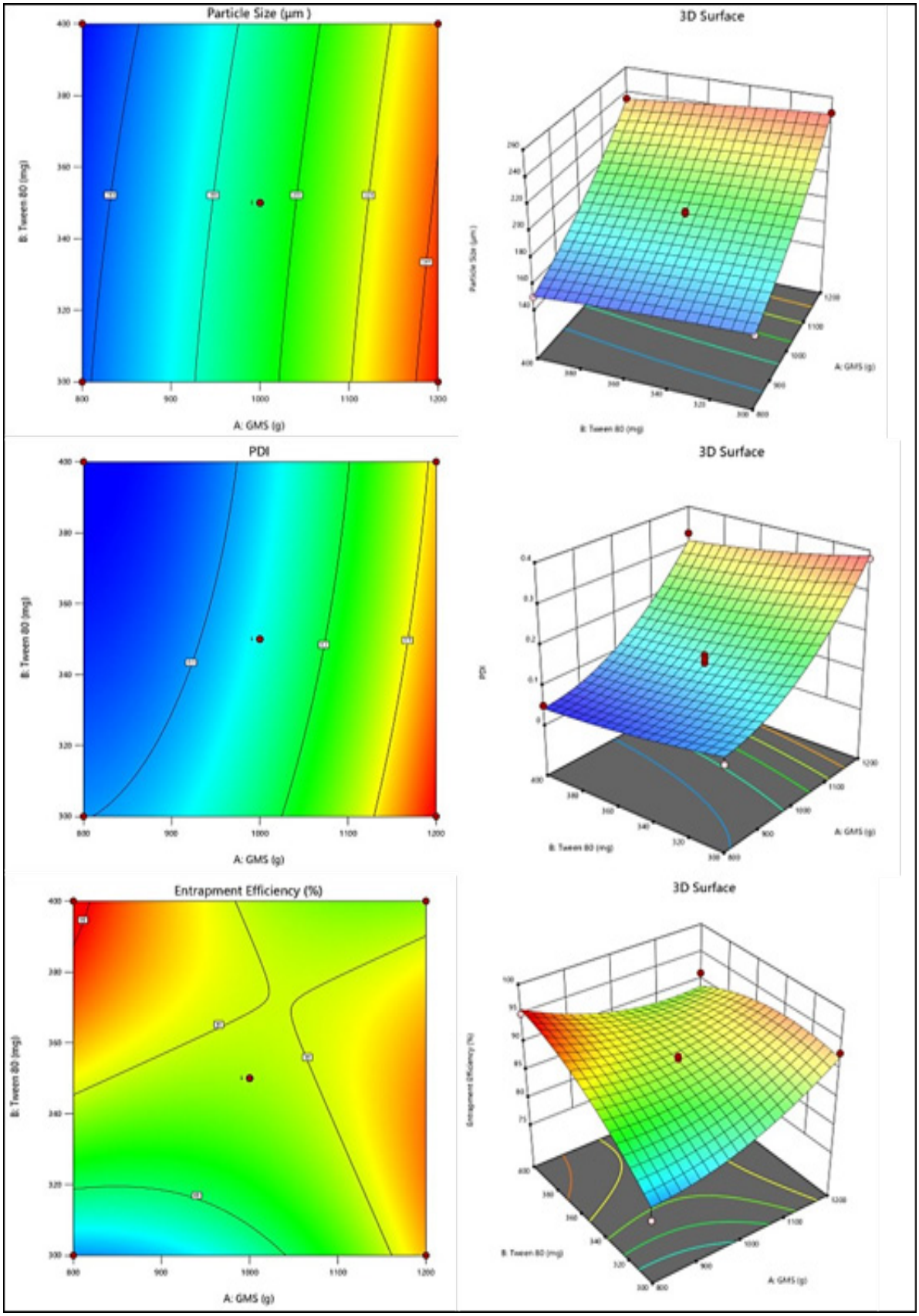


**Figure 5:** Contour and Three-Dimensional (3D) response surface plots illustrating the effect of formulation variables A: Glyceryl Monostearate (GMS) and B: Tween 80 on the responses: (a) Particle size (µm), (b) Polydispersity Index (PDI), and (c) Entrapment Efficiency (%). The contour plots show the interaction between the two variables in two-dimensional space, while the corresponding 3D surface plots depict the response variation across the studied levels of GMS and Tween 80.

**Table 3: Experimental Runs for Optimization of SLNs Using Box-Behnken Design.**

| Std | Run | GMS (mg) | Tween 80 (mg) | Span 80 (mg) | Particle Size (µm) | PDI | Entrapment Efficiency (%) |
|---|---|---|---|---|---|---|---|
| 6 | 1 | 1200 | 350 | 250 | 245 | 0.36 | 93 |
| 1 | 2 | 800 | 300 | 300 | 158 | 0.08 | 78.2 |
| 17 | 3 | 1000 | 350 | 300 | 192 | 0.15 | 88.8 |
| 12 | 4 | 1000 | 400 | 350 | 188 | 0.11 | 89.9 |
| 3 | 5 | 800 | 400 | 300 | 150 | 0.05 | 95 |
| 4 | 6 | 1200 | 400 | 300 | 235 | 0.33 | 91.2 |
| 15 | 7 | 1000 | 350 | 300 | 190 | 0.14 | 89.2 |
| 13 | 8 | 1000 | 350 | 300 | 191 | 0.13 | 89.5 |
| 16 | 9 | 1000 | 350 | 300 | 189 | 0.12 | 90 |
| 14 | 10 | 1000 | 350 | 300 | 193 | 0.16 | 88.7 |
| 8 | 11 | 1200 | 350 | 350 | 240 | 0.30 | 92.5 |
| 11 | 12 | 1000 | 300 | 350 | 195 | 0.18 | 86.7 |
| 9 | 13 | 1000 | 300 | 250 | 198 | 0.20 | 85.2 |
| 7 | 14 | 800 | 350 | 350 | 155 | 0.06 | 94.5 |
| 10 | 15 | 1000 | 400 | 250 | 185 | 0.09 | 90.7 |
| 2 | 16 | 1200 | 300 | 300 | 248 | 0.38 | 92.8 |
| 5 | 17 | 800 | 350 | 250 | 160 | 0.07 | 93 |

The overlay plot (Figure 6) further confirmed that this formulation lies within the optimal design space, meeting the desired physicochemical criteria.

This optimized solid lipid nanoparticle formulation demonstrates promising characteristics for further *in vitro* and *in vivo* evaluation, owing to its high encapsulation efficiency, nanoscale particle size, and homogeneity parameters essential for effective drug delivery systems.

## Evaluation Parameters

### *SEM analysis*

The surface morphology of the Agmatine sulphate pure drug and the Solid Lipid Nanoparticle (SLN) formulation was characterized using Scanning Electron Microscopy (SEM).

Figure 7a shows that the pure drug crystals exhibited a coarse, irregular shape with an approximate size of 200 µm. In contrast, the SLN formulation (Figure 7b) revealed a uniform, spherical morphology with significantly reduced particle size. The transformation from crystalline to spherical particles in the formulation indicates successful encapsulation and dispersion of the drug within the lipid matrix.

The disappearance of the original crystalline morphology and the formation of smaller, more compact particles in the formulation suggest improved surface characteristics. The notable reduction in particle size contributes to an increased surface area, which is likely to enhance the solubility and, consequently, the bioavailability of the drug.

## Particle Size and PDI

The particle size and Polydispersity Index (PDI) are critical parameters influencing drug release kinetics, stability, and overall performance of nanoparticulate formulations. The optimized formulation obtained through Design of Experiments (DoE) exhibited a mean particle size of 130.5 nm with a PDI of 0.189, as shown in Figure 7.

These results indicate a uniform particle distribution and nanoscale size, both of which are desirable attributes for enhancing drug solubility and bioavailability. The low PDI reflects a narrow size distribution, contributing to the physical stability of the SLN formulation.

## Estimation of drug content

The drug content of the optimized agmatine sulfate-loaded SLNs was found to be 92%, indicating efficient encapsulation and minimal drug loss during the formulation process. This high drug content supports the reliability of the preparation method and its suitability for consistent therapeutic performance.

## *In vitro* drug release

*In vitro* drug release studies are crucial for assessing the release profile, kinetics, and mechanism of drug delivery systems. For nanocarrier-based formulations such as Solid Lipid Nanoparticles (SLNs), achieving controlled and sustained drug release is essential for prolonging circulation time, enhancing bioavailability, and ensuring site-specific delivery.

The optimized formulation was subjected to dissolution testing, and the results revealed a sustained drug release of 89.9% over 24 hr, as illustrated in Figure 7. This indicates effective retention and gradual release of the drug from the lipid matrix.

To further analyze the drug release mechanism, various kinetic models were applied. The release pattern suggests a diffusion-controlled mechanism, consistent with the structural characteristics of lipid-based nanoparticles.

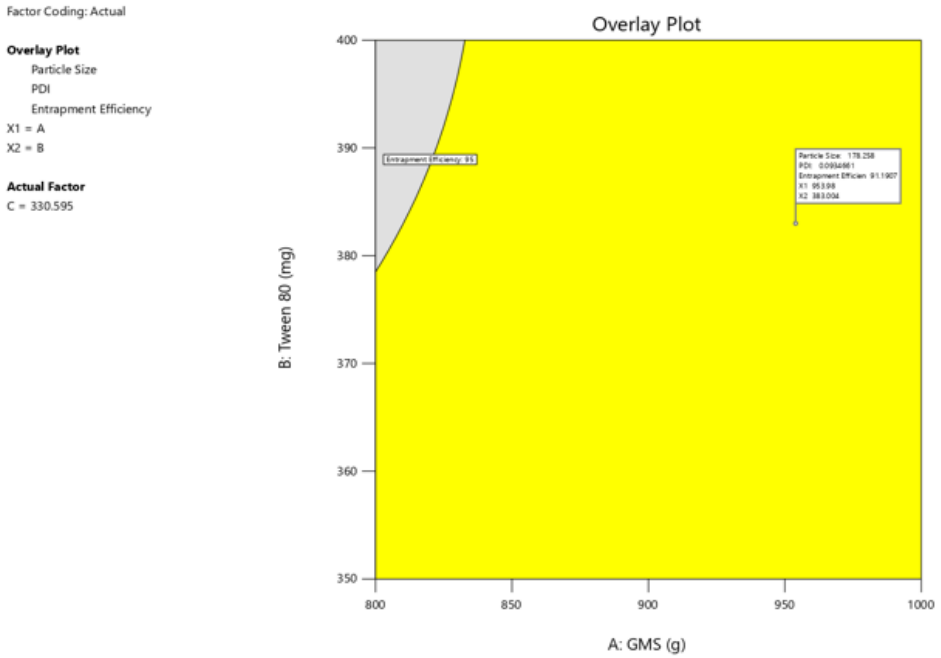


**Figure 6:** Overlay plot highlighting the design space for optimal SLN formulation based on Tween 80 and GMS.

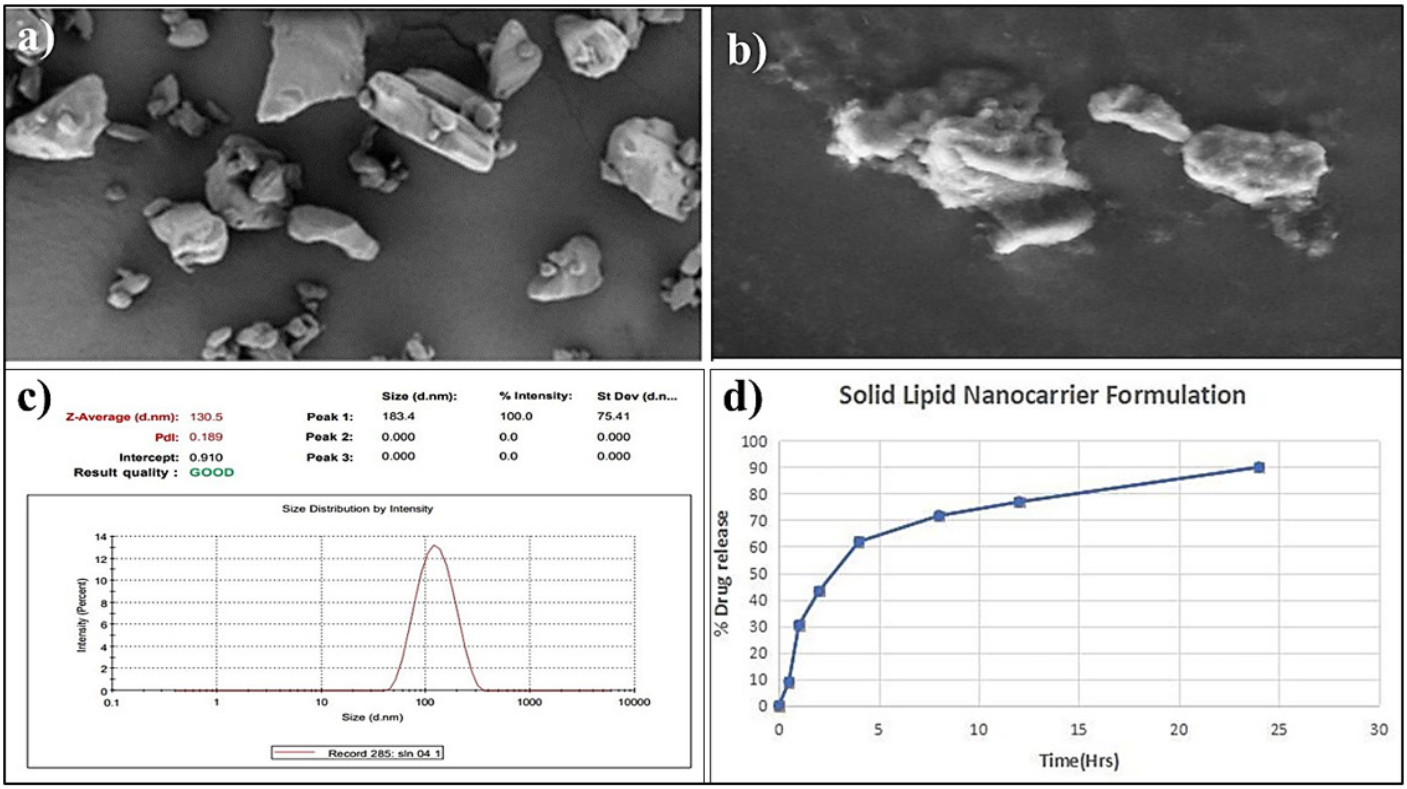


**Figure 7:** a) SEM Image of Agmatine sulphate pure drug. b) SEM image of SLNs. c) Particle size and PDI of optimized formulation. d) *In vitro* drug release of Agmatine sulphate loaded SLNs.

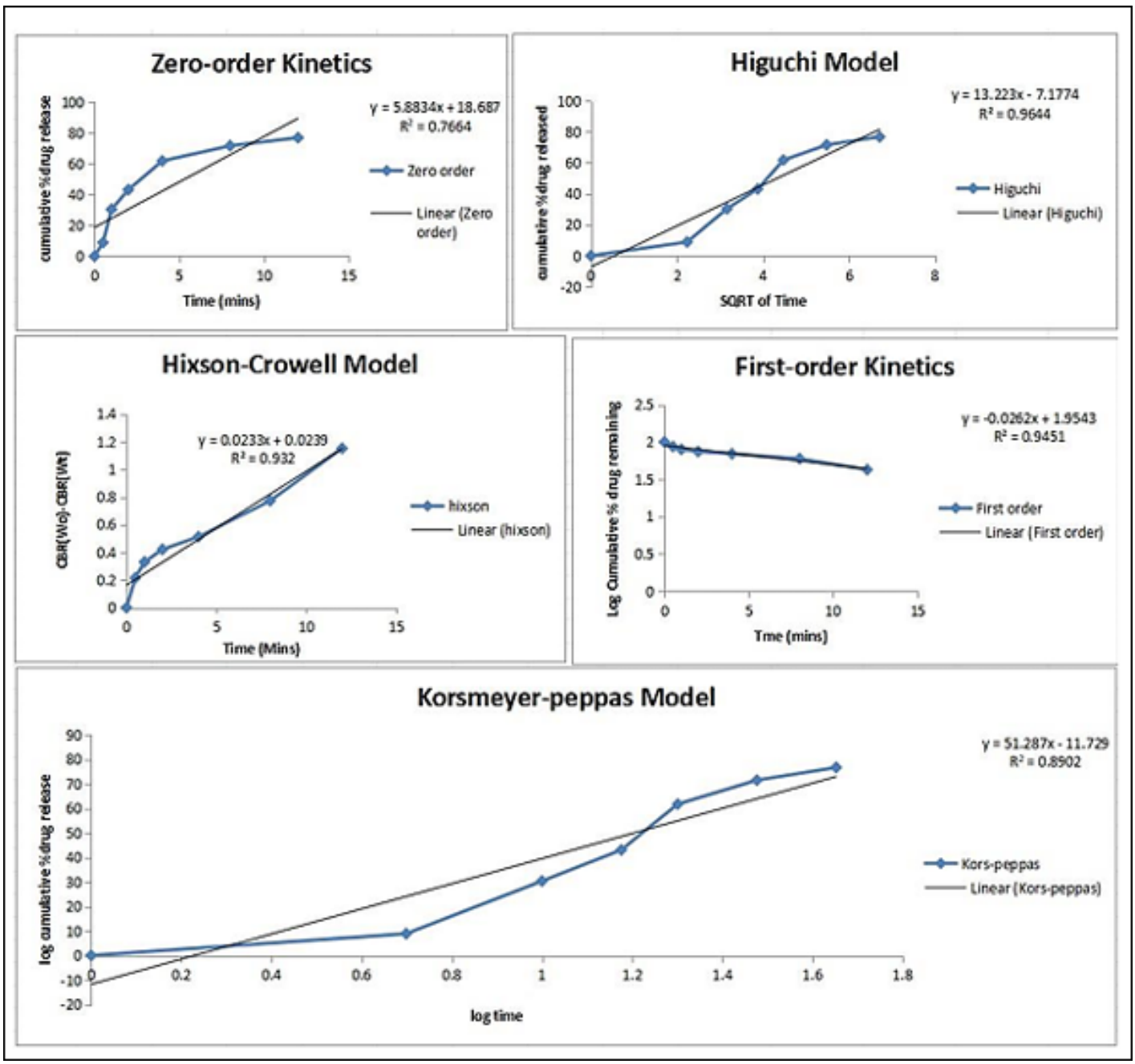


**Figure 8:** Drug release kinetics plots for various models.

## Drug release kinetics

The First-order model also showed a relatively high $R^2$ value (0.9451), suggesting a possible concentration-dependent release mechanism to a lesser extent. The Hixson-Crowell model ($R^2$=0.9320) and Korsmeyer-Peppas model ($R^2$=0.8902) further indicate that erosion and anomalous (non-Fickian) transport may contribute, albeit to a secondary degree. The Zero-order model ($R^2$=0.7664) showed the least fitting, confirming that the drug was not released at a constant rate (Figure 8).

Overall, the results confirm that diffusion is the predominant drug release mechanism from the SLN formulation, aligning with the controlled-release properties desirable for enhanced and sustained therapeutic effect.

## DISCUSSION

The drug Agmatine sulphate appeared as a solid, white crystalline powder with no discernible taste or odor. The melting point was recorded at 231°C, indicating high purity and thermal stability, which are essential parameters for subsequent formulation development. FTIR analysis revealed absorption bands correspond to specific vibrational modes of functional groups, confirming the structural integrity of the compound and absence of significant impurities.

The FTIR spectrum similarity in peak positions between the pure drug and the SLN formulation indicates the absence of any significant chemical interactions. Moreover, no new peaks emerged, nor were any characteristic peaks lost in the spectra of the physical mixture or the final formulation, suggesting that the drug retained its structural integrity throughout the formulation process. These results confirm that the excipients used were compatible with agmatine sulphate and did not chemically alter its functional groups.

The pure drug showed a sharp endothermic peak at 232.45°C, confirming its crystalline nature. In contrast, the SLN formulation displayed a broadened peak at 243.87°C, indicating drug incorporation into the lipid matrix and partial conversion to an amorphous state. This change suggests improved drug dispersion and potential for enhanced release and stability.

The XRD analysis of the pure agmatine sulphate exhibited distinct and sharp diffraction peaks, indicating its crystalline nature. In contrast, the optimized SLN formulation displayed a markedly reduced intensity and absence of sharp peaks, suggesting a transition of the drug from a crystalline to an amorphous or molecularly dispersed state upon entrapment within the lipid matrix.

This transformation is indicative of successful encapsulation and may contribute to enhanced solubility and bioavailability. The physical mixture retained the characteristic peaks of the pure drug, implying that the crystallinity was preserved prior to nanoparticle formation.

Optimization Summary and Final Formulation Selection: Based on the results obtained from the Box-Behnken Design (BBD), Run 5 was identified as the optimal formulation, achieving all three targeted outcomes: the smallest particle size (150 nm), the lowest Polydispersity Index (PDI) of 0.05, indicating excellent uniformity, and the highest entrapment efficiency of 95%. The composition of this optimized formulation included 800 mg of GMS, 400 mg of Tween 80, and 300 mg of Span 80.

The ANOVA data and response surface analysis clearly demonstrated that Tween 80 played a significant role in enhancing drug entrapment efficiency, while GMS and its interactions predominantly influenced particle size and distribution. Although the lack of fit for the entrapment efficiency model was marginally significant, the overall optimization objectives were successfully achieved with Run 5.

The agmatine sulfate-loaded SLNs showed a biphasic release profile with an initial burst followed by sustained release over 24 hr. Release data were fitted to various kinetic models. The Higuchi model showed the best fit ($R^2$=0.9644), indicating that drug release was primarily diffusion-controlled through the lipid matrix. The study conducted by Deshkar et al. (2025), optimized formulation was further characterized by FTIR, FESEM, DSC, XRD, and evaluated for *in vitro* drug release. The similar studies conducted by Mujtaba *et al.* (2024), also showed Development and Optimization of Proniosomal Formulation of Irbesartan Using a Box–Behnken Design to Enhance Oral Bioavailability.

## CONCLUSION

The present study successfully demonstrates the formulation, evaluation, and optimization of agmatine sulphate-loaded Solid Lipid Nanoparticles (SLNs) using the hot melt homogenization technique as an effective strategy to improve the delivery of poorly permeable drugs. Systematic optimization using a Box-Behnken Design enabled the development of an optimized SLN formulation exhibiting high drug entrapment efficiency (95%), small particle size (150 nm), narrow size distribution (PDI 0.05), and sustained drug release over 24 hr, predominantly governed by diffusion kinetics.

Encapsulation of agmatine sulphate within SLNs enhanced its bioavailability, potentially allowing for reduced dosing frequency and lower systemic side effects, which is particularly advantageous in the management of neuropathic pain and related conditions requiring prolonged therapeutic action.

The study highlights the critical role of formulation parameters particularly surfactant and lipid concentrations-in influencing the physicochemical and functional properties of SLNs. Moreover, the findings support the broader applicability of the hot melt homogenization technique in developing nanocarrier systems for other poorly permeable or poorly soluble drugs.

Future research may explore pharmacokinetic evaluations, alternative lipid matrices or surfactants, and *in vivo* assessments to fully realize the clinical potential of agmatine sulphate-loaded SLNs. Overall, this work contributes to the advancement of sustained-release nanocarrier-based drug delivery systems and offers a robust platform for improving therapeutic outcomes in chronic and refractory conditions.

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## ABBREVIATIONS

**SLNs:** Solid Lipid Nanoparticles; **BBB:** Blood-Brain Barrier; **DoE:** Design of Experiments; **BBD:** Box-Behnken Design; **GMS:** Glyceryl Monostearate; **PDI:** Polydispersity Index; **EE:** Entrapment Efficiency; **FTIR:** Fourier-Transform Infrared Spectroscopy; **DSC:** Differential Scanning Calorimetry; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy; **UV:** Ultraviolet; **nm:** Nanometer; **µg/mL:** Micrograms per milliliter; **mg:** Milligram; **°C:** Degree Celsius; **rpm:** Revolutions per minute; **$R^2$:** Coefficient of Determination; **USP:** United States

Pharmacopeia; **SD:** Standard Deviation; **$C_{max}$:** Maximum Plasma Concentration.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

Harish K H: Conceptualized the study, methodology, data collection, analysis and drafting of the manuscript. Agadi Hiremath Viswanatha Swamy: Supervised the overall research process and assisted in final revisions.

## SUMMARY

This research successfully utilized a Box-Behnken Design to optimize Agmatine Sulphate-loaded SLNs for neurological applications. The optimized nanoparticles achieved high entrapment efficiency (95%) and a small particle size (150 nm), ensuring a sustained release profile over 24 hr governed by diffusion. These findings suggest that the SLN platform can effectively overcome the pharmacokinetic limitations of agmatine, such as rapid degradation and poor blood-brain barrier permeability.

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