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Research

Formulation, optimization, and evaluation of procyclidine hydrochloride loaded solid lipid nanoparticles using design of experiment

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Abstract

This study presents the formulation, optimization, and evaluation of Procyclidine Hydrochloride-loaded Solid Lipid Nanoparticles (SLNs) to enhance the drug's therapeutic efficacy. The SLNs were optimized using a Box-Behnken Design, focusing on parameters such as oleic acid, Tween 80, and propylene glycol. The optimized formulation exhibited a particle size of 259 nm, a polydispersity index (PDI) of 0.3169, and a zeta potential of -32.01 mV, indicating high stability. In-vitro drug release studies demonstrated sustained release over 24 hours, achieving 94.15% cumulative release. Stability studies confirmed the formulation's robustness under various storage conditions. These results suggest that SLNs are an effective drug delivery system for enhancing the bioavailability and controlled release of Procyclidine Hydrochloride.

Keywords: Procyclidine Hydrochloride, Solid Lipid Nanoparticles, Box-Behnken Design, Drug delivery, Sustained release, Stability, Particle size.

INTRODUCTION

Oral delivery stands out as the preferred method for administering drugs due to its convenience and a range of advantages over other routes. It offers painless administration, easy self-application, and high patient compliance, making it the go-to choice for drug delivery worldwide. However, the effectiveness of orally administered drugs hinges greatly on their ability to be absorbed through the digestive system, which, in turn, depends on both the properties of the drug itself and the physiology of the gut.

Certain drugs face hurdles in this journey due to their unfavorable characteristics like poor hydrophobicity, low permeability, chemical instability, and susceptibility to extensive first-pass metabolism. The gastrointestinal (GI) tract poses formidable barriers to the passage and effectiveness of such drugs, including physical, chemical, enzymatic, and biological membranes. To overcome these challenges, one promising approach

involves enhancing the solubility, stability, and transmembrane transport of drugs by encapsulating them within or absorbing them onto the surfaces of nanocarrier systems.

These nanocarriers serve as ingenious vehicles for transporting poorly soluble and permeable molecules across these barriers, potentially revolutionizing drug delivery. By modifying the transmembrane transport and facilitating the diffusion of nanoparticle-loaded drugs across intestinal mucosal barriers, these systems hold immense promise for improving oral absorbability. Notably, the effectiveness of oral absorption isn't solely determined by the physicochemical properties of the drug but also by the involvement of nanocarrier delivery systems.

Among these advanced systems, solid lipid nanoparticles (SLNs) have emerged as a compelling option since 1991. They offer a novel approach to drug delivery, garnering increasing attention as an effective alternative to traditional colloidal methods like liposomes and polymeric particles. Solid lipid nanoparticles (SLNs) are colloidal nanoparticles composed of lipids that are solid at room and body temperatures. They were developed as an alternative carrier system to traditional colloidal carriers like liposomes, emulsions, and polymeric nanoparticles. SLNs offer numerous advantages, including controlled release, enhanced stability, improved bioavailability, and the possibility of incorporating both lipophilic and hydrophilic drugs [1].

Composition of SLNs

Solid lipid nanoparticles (SLNs) are innovative drug delivery systems composed of solid lipids dispersed in an aqueous phase stabilized by surfactants. Understanding their composition is crucial for comprehending their properties and functions in drug delivery applications [2, 3].

Solid lipids

The primary component of SLNs is solid lipids, which are typically natural or synthetic lipids with a high melting point. Natural lipids commonly used include triglycerides like glycerol monostearate, glycerol behenate, and fatty acids such as stearic acid and palmitic acid. Synthetic lipids like polyethylene glycol (PEG) derivatives are also utilized. These solid lipids provide the structural integrity of SLNs and serve as a matrix for drug encapsulation [2, 3].

Surfactants

Surfactants play a crucial role in stabilizing SLNs by reducing interfacial tension between the solid lipid and the aqueous phase, preventing particle aggregation and ensuring colloidal stability. Common surfactants used in SLN formulations include non-ionic surfactants such as polysorbates (e.g., Tween 80), phospholipids (e.g., lecithin), and Pluronics (block copolymers of ethylene oxide and propylene oxide). Surfactants also aid in enhancing drug loading and modulating drug release kinetics from SLNs [2, 3].

Cosurfactants

In some formulations, cosurfactants are employed alongside surfactants to further stabilize SLNs and optimize their properties. Cosurfactants are typically short-chain alcohols or polyols that enhance the solubilization of lipids and improve the dispersibility of SLNs in aqueous media. Common cosurfactants include ethanol, propylene glycol, and polyethylene glycol [2, 3].

Drug payload

SLNs encapsulate drugs within their solid lipid matrix or on their surface, depending on the physicochemical properties of the drug and the formulation strategy. Hydrophobic drugs are typically encapsulated within the lipid core, whereas hydrophilic drugs may be adsorbed onto the surface of SLNs. The drug payload in SLNs can range from small molecules to macromolecules, including proteins, peptides, nucleic acids, and imaging agents [2, 3].

Aim And Objectives

To formulate and optimize Procyclidine HCl Solid Lipid Nanoparticles (SLNs) for enhanced therapeutic efficacy.

Perform preformulation studies to assess the physiochemical properties of Procyclidine HCl, including solubility, stability, and compatibility with various lipid excipients and surfactants. Utilize Box-Behnken Design (BBD) to systematically optimize the formulation variables including oleic acid, tween 80, and propylene glycol to achieve desired particle size, polydispersity index, and zeta potential. Develop Procyclidine HCl-loaded Solid Lipid Nanoparticles using suitable lipid matrices, surfactants, and co-surfactants via emulsification and homogenization techniques. Characterize the optimized Procyclidine HCl SLNs for particle size, polydispersity index (PDI), zeta potential, drug loading efficiency, and *in-vitro* drug release profile. Conduct stability studies under various temperature and humidity to evaluate the physical and chemical stability of the optimized Procyclidine HCl SLNs over a defined period.

METHODOLOGY

1.1. Preformulation Studies

Preformulation investigations represent the initial phase in the systematic development of a pharmacological substance's dosage form. The primary objective of preformulation research is to gather comprehensive information about the drug ingredient, which will be instrumental in formulating the medication. This involves studying the physical and chemical properties of the drug material both in isolation and in combination with excipients. The purpose of preformulation studies is to identify the physicochemical characteristics and excipients that may influence the formulation design, manufacturing process, and the pharmacokinetic and biopharmaceutical properties of the final product. Key factors assessed during preformulation research include, but are not limited to, the following.

1.2. Organoleptic properties

To evaluate the organoleptic properties of procyclidine hydrochloride during preformulation studies, a sample was placed in a clean vial for observation. Its physical state and color were assessed against a white background, and any odor was noted by gently wafting the sample under the nose. Observations for appearance, color, odor, and taste were recorded in a detailed report, ensuring all procedures followed safety guidelines.

1.3. Solubility Studies

Aqueous solubility is an important physiochemical property of drug substance which determines its systemic absorption and in turns its therapeutic efficacy. The aqueous solubility and solubility in ethanol was tested for procyclidine hydrochloride.

1.4. Construction of Calibration curve

About 25 mg of pure drug sample was accurately weighed and dissolved in little quantity of ethanol and sonicated for 10 minutes. The volume was made up to 25 ml with more ethanol. This was taken as the stock solution. This solution was scanned using UV Spectrophotometer from 400-200nm and the λmax was found to be 258nm. From the stock solution, suitable dilutions were made to get the concentration ranging from 2 to 10 µg/ml and scanned at λmax of 258nm. Then, a calibration curve was plotted by taking concentration (µg/ml) on X-axis and absorbance (nm) on Y-axis. This solution was scanned using UV Spectrophotometer from 400-200nm [4].

1.5. Drug-excipient compatibility studies

1.5.1. FT-IR

FT-IR is an ideal technique for analyzing the chemical and structural properties of procyclidine hydrochloride, oleic acid, Tween 80, propylene glycol, and the formulation mix. It can determine drug-polymer interactions and detect drug degradation during nanoparticle processing. Using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR), the functional groups present in the samples were analyzed in the spectral region of 4000-550 cm-1, with a resolution of 4 cm-1, and a total of 64 scans per sample [5].

1.5.2. Differential Scanning Calorimetry

Thermograms of the drug and excipients were recorded using Differential Scanning Calorimetry (DSC) under an inert nitrogen atmosphere. A 5 mg sample was placed into an aluminum pan and securely sealed, with an empty aluminum pan serving as the reference. The samples were heated at a rate of 10°C per minute over a temperature range of 40 to 230°C, and the thermograms were documented [6].

1.6. Preparation of procyclidine hydrochloride Solid-Lipid Nanoparticles

Procyclidine hydrochloride-loaded solid lipid nanoparticles (SLNs) were synthesized using the emulsion solvent evaporation method. Initially, 400 mg of procyclidine hydrochloride was accurately measured and dissolved in a surfactant mixture comprising oleic acid, propylene glycol, and Tween 80. The solution underwent homogenization at 3000 rpm for 30 minutes until complete dissolution of the drug occurred. Distilled water was then gradually added dropwise with continuous stirring until an emulsion formed. Stirring continued for 2–2.5 hours at 3000 rpm to ensure proper dispersion of the SLNs. The emulsion was sonicated for 5 minutes at 100% amplitude to achieve uniform particle size. Subsequently, the dispersion underwent centrifugation at 18,000 rpm for 20 minutes to separate the solid lipid material containing the drug. Finally, the SLN dispersions were lyophilized in the presence of 5% (w/v) mannitol as a cryoprotectant [7].

1.7. Optimization of procyclidine hydrochloride-SLN using Box Behnken Design

Design Expert 13 was employed for optimization through the Box Behnken Design methodology. Three factors were selected as input variables for crafting the design, specifically the quantities of oleic acid, tween 80, and propylene glycol. These parameters were utilized to develop an optimized formulation with particle size,

Polydispersity Index (PDI), and Zeta Potential serving as the responses to gauge the efficacy of the formulation [8, 9].

1.8. Evaluation of optimized formulation

1.8.1. Particle size, PDI & Zeta potential

Particle size, polydispersity index (PDI), and zeta potential were assessed using photon correlation spectroscopy at 25°C with a detection angle of 90° [10].

1.8.2. Scanning Electron Microscopy (SEM)

The optimized solid lipid nanoparticles (SLNs) were analyzed for surface morphology via Scanning Electron Microscopy (SEM). Samples were affixed onto brass stubs with carbon tape and subjected to sputtering in a vacuum chamber, where they were platinum-coated for 40 seconds. Images were subsequently captured at various magnifications [10].

1.8.3. Determination of drug content

To determine the drug content of SLN, dissolve precisely 50 mg of the formulation in 10 ml of ethanol. After diluting the solution, we used a UV-Spectrophotometer with a maximum wavelength (λ-max) of 258 nm to measure the absorbance [10]

1.8.4. Drug loading and drug entrapment efficiency determination

To analyze procyclidine hydrochloride-SLN dispersions, a set volume (10 mL) was divided using centrifugation at 18,000 rpm for 20 minutes at 20°C. The resulting supernatant was subjected to spectrophotometric analysis at a wavelength of maximum absorption (λmax) of 258 nm using a spectrophotometer to quantify any unencapsulated drug.

***In-vitro* drug release characteristics**

In this study, the release of Solid Lipid Nanoparticles (SLN) was assessed via the dialysis method. To elucidate, SLN (1.0 mL) or a drug solution with equivalent drug concentration was encapsulated within a dialysis bag, then immersed in 100 mL of phosphate-buffered saline at pH 6.8, serving as the release medium. The entire setup was maintained at 37°C ± 0.5°C under continuous magnetic stirring. At specific intervals (0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 24 hours), 5 mL of solution was sampled from the release medium and replaced with an equal volume of fresh medium. These samples were appropriately diluted and analyzed using a UV-visible spectrophotometer at 258 nm. The quantity of drug released was determined by calculating the cumulative percentage release [10].

Stability studies

The optimal SLN formulation underwent stability assessments through storage at both 4°C and 27±2°C with a relative humidity of 65%±5% for a duration of 90 days. Throughout this period, regular examinations were conducted to monitor alterations in the particle size, PDI, zeta potential, and EE of the SLN [11].

RESULTS AND DISCUSSIONS

PREFORMULATION STUDIES

Organoleptic properties

Appearance: White
Odor: Odorless
Taste: Bitter
Texture: Crystalline

Solubility

The solubility of Procyclidine HCl was measured in different solvents, including water, methanol, phosphate buffer (pH 6.8), and ethanol. The results are summarized in Table 1 according to the United States Pharmacopeia (USP) solubility classifications.

Table 1: Solubility study of procyclidine Hcl

Solvent	USP classification
Water	Sparingly soluble
Methanol	Soluble
Phosphate buffer pH 6.8	Soluble
Ethanol	Soluble

Caibration curve

The absorption maxima of Procyclidine HCl in methanol was determined to be 258 nm when scanned from 400 to 200 nm using a Shimadzu UV Spectrophotometer. A standard calibration curve was generated for Procyclidine HCl in the concentration range of 2-10 µg/mL. The data points for the calibration curve are shown in Table 2.

Table 2: Absorbance Data for Procyclidine HCl

Concentration (µg/mL)	Absorbance
0	0.0000
2	0.1964
4	0.3891
6	0.5834
8	0.7452
10	0.8908

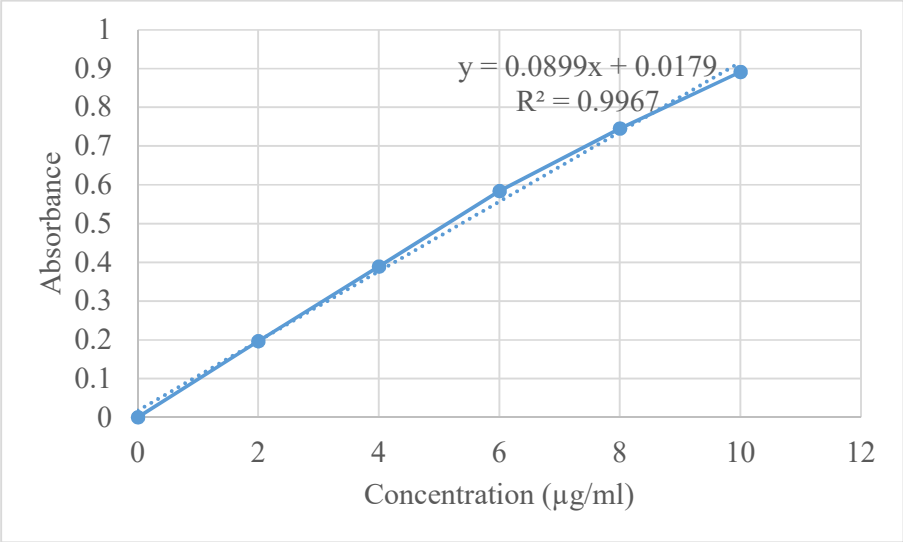


Fig 1: Calibration curve of procyclidine Hcl

The absorption maxima at 258 nm in methanol suggests that Procyclidine HCl has a specific wavelength at which it absorbs UV light most strongly. This wavelength is characteristic of the electronic transitions within the drug molecule and is crucial for its quantitative analysis. The standard calibration curve for Procyclidine HCl was linear within the concentration range of 2-10 µg/mL. The absorbance values increased proportionally with the concentration, adhering to Beer's Lambert law, which states that absorbance is directly proportional to concentration for a given path length and molar absorptivity. The correlation coefficient (R^2) of 0.9967 indicates a very high degree of linearity. This high R^2 value confirms that the method is reliable and reproducible for the concentration range studied. The linearity of the calibration curve is crucial for accurate quantification of the drug in various samples.

DRUG-EXCIPIENT COMPATIBILITY STUDIES

FTIR

Procyclidine Hcl

The FTIR spectrum of procyclidine HCl was analyzed to identify the major functional groups present in the compound. The following peaks were observed:

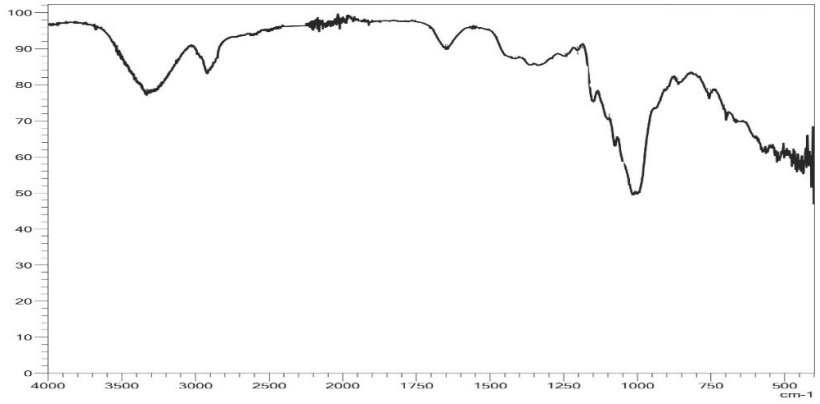


Fig 2: FTIR of procyclidine Hcl

The FTIR spectrum of procyclidine HCl provides significant insights into the functional groups present within the compound. The broad peak around 3412 cm^{-1} suggests the presence of O-H or N-H groups, which is consistent with the structure of procyclidine HCl that contains an amine group. The peaks at 2920 cm^{-1} and 2850 cm^{-1} are indicative of C-H stretching vibrations, which are typical of alkane groups present in the compound. These peaks confirm the presence of hydrocarbon chains or rings in the molecular structure. The peaks at 1340 cm^{-1} and 1270 cm^{-1} are characteristic of C-N stretching, aligning with the presence of tertiary amines in the procyclidine structure. These peaks are essential for confirming the presence of the piperidine ring, which is a significant part of procyclidine's structure.

Finally, the peak at 700 cm^{-1} is attributed to C-Cl stretching, confirming the presence of chlorine in the compound, consistent with the hydrochloride salt form of procyclidine.The FTIR analysis of procyclidine HCl reveals the presence of key functional groups such as N-H, C-H, C-N, and C-Cl, which are consistent with the known structure of the compound. The presence of these peaks provides validation of the compound's identity and purity.

Formulation

The FTIR spectrum of the sample containing procyclidine HCl, Tween 80, oleic acid, and propylene glycol was analyzed to identify the major peaks and compare them with those of pure procyclidine HCl. The following peaks were observed:

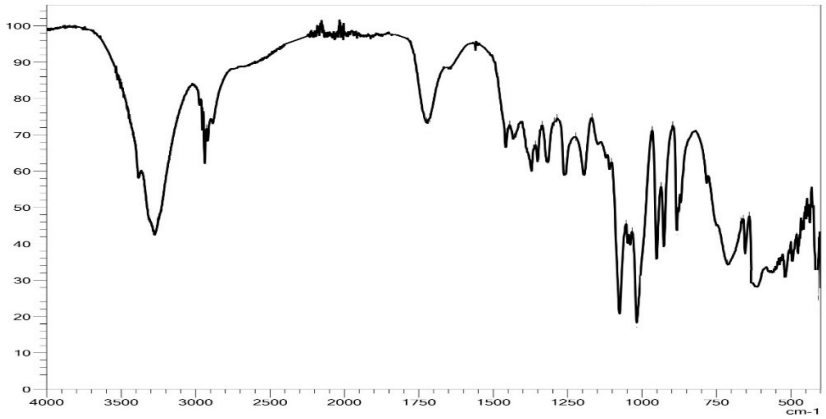


Fig 3: FTIR of drug and excipients

The FTIR spectrum of the sample containing procyclidine HCl, Tween 80, oleic acid, and propylene glycol was analyzed to identify major peaks and compare them with those of pure procyclidine HCl. Key peaks identified include 3365 cm^{-1} (O-H/N-H stretching), 2925 cm^{-1} and 2850 cm^{-1} (C-H stretching), 1740 cm^{-1} (C=O stretching), 1450 cm^{-1} (C-H bending), 1240 cm^{-1} (C-N stretching), 1100 cm^{-1} (C-O stretching), and 720 cm^{-1} (C-Cl stretching). Comparison with pure procyclidine HCl showed consistent peaks for O-H/N-H, C-H, C-N, and C-Cl stretching, indicating no significant chemical interactions between the drug and excipients. The peaks attributed

to the excipients, such as C=O stretching from oleic acid and C-O stretching from Tween 80 and propylene glycol, confirm their presence without altering the drug's functional groups. This analysis suggests that the excipients do not interact adversely with procyclidine HCl, maintaining its chemical integrity in the formulation.

DSC

Procyclidine HCl: The DSC curve displayed a clear melting peak at approximately 86.2°C, which aligns closely with the melting point of pure procyclidine HCl. This peak indicates that the drug maintains its crystalline structure within the SLNs.

Oleic Acid: Displayed a melting peak at 14°C, consistent with the typical behavior of oleic acid, indicating minimal or no modification due to interactions with other SLN components.

Tween 80 and Propylene Glycol: Both components showed stable baselines with only minor fluctuations, which do not suggest any significant endothermic or exothermic interactions with procyclidine HCl within the studied temperature range.

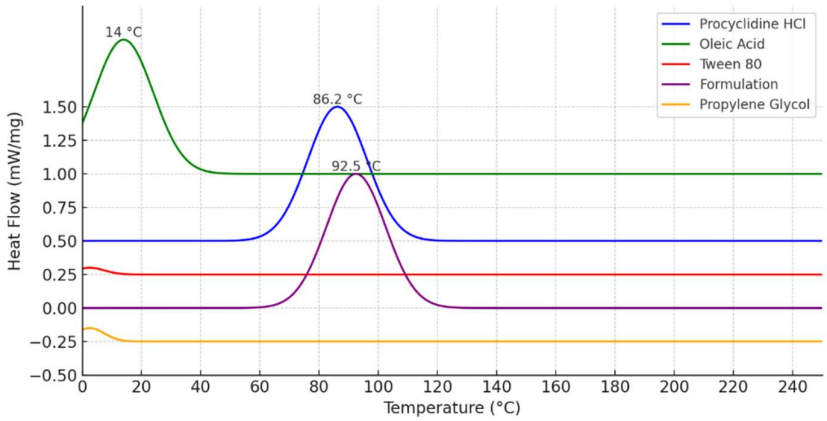


Fig 4: DSC of drug and excipients

Formulation Peak

A distinct peak at 92.5°C was observed, but it closely resembles the peak of procyclidine HCl in terms of thermal behavior, suggesting that the SLN formulation does not significantly alter the thermal properties of the drug. The thermal analysis indicates that procyclidine HCl retains its crystalline form within the SLNs, as evidenced by the unaltered melting peak. This result strongly suggests that the drug does not undergo significant molecular interactions with the lipid matrix or other excipients in the formulation. The consistent melting points of oleic acid and the absence of new significant peaks or shifts in existing peaks further support the conclusion that there are no significant interactions between the drug and the lipid components. This stability in melting behavior is indicative of the physical integrity of the SLN components when combined.

The formulation's peak at 92.5°C, which mirrors that of pure procyclidine HCl, further substantiates the lack of chemical interaction. This indicates that while the drug is effectively encapsulated within the SLNs, its thermal properties remain unchanged, implying physical encapsulation without chemical bonding or significant molecular interaction. Given these observations, it is reasonable to conclude that the SLN formulation serves primarily to physically encapsulate procyclidine HCl without altering its chemical state. This finding is crucial for the development of SLNs as it suggests that the drug can be delivered in its effective form without destabilization or modification through interactions with the carrier system.

Optimization

All the 13 formulations were performed and evaluated.

Table 3: Optimization of procyclidine SLNs through box-behkhen design

Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
	A:Oleic acid ml	B:Tween 80ml	C:Propylene glycol ml	Particle Size nm	Polydispersity Index MW/mn	Zeta Potential mV
1	5.5	10	8	290	0.31	-24.97

2	4.5	8	12	340	0.36	-25.13
3	4.5	12	12	260	0.33	-30.14
4	5.5	10	12	300	0.32	-25.26
5	4.5	10	10	310	0.335	-26.94
6	3.5	12	10	270	0.32	-28.62
7	3.5	10	8	320	0.34	-24.62
8	3.5	10	12	280	0.33	-29.41
9	5.5	8	10	350	0.37	-18.39
10	3.5	8	10	360	0.365	-20.82
11	5.5	12	10	255	0.31	-32.48
12	4.5	12	8	265	0.34	-31.93
13	4.5	8	8	370	0.38	-15.46

Particle Size (PS)

The equation for Particle Size (PS) is:

PS = 305.385 + -4.375 *A + -46.25 *B + -8.125 *C

Interpretation:

Intercept (305.385): This is the predicted particle size when all factors (A, B, and C) are at their reference levels (e.g., their mean or zero, depending on the coding of factors).

Effect of A (Oleic acid):

Coefficient: -4.375

Interpretation: For each unit increase in Oleic acid (A), the particle size decreases by 4.375 nm. This indicates that increasing Oleic acid leads to a smaller particle size, though the effect is relatively small compared to other factors.

Effect of B (Tween 80):

Coefficient: -46.25

Interpretation: For each unit increase in Tween 80 (B), the particle size decreases by 46.25 nm. This is a substantial effect, suggesting that Tween 80 significantly reduces the particle size.

Effect of C (Propylene glycol):

Coefficient: -8.125

Interpretation: For each unit increase in Propylene glycol (C), the particle size decreases by 8.125 nm. This effect is moderate and indicates that Propylene glycol also contributes to reducing particle size.

Overall, Tween 80 (B) has the most significant effect on reducing particle size, followed by Propylene glycol (C), and then Oleic acid (A).

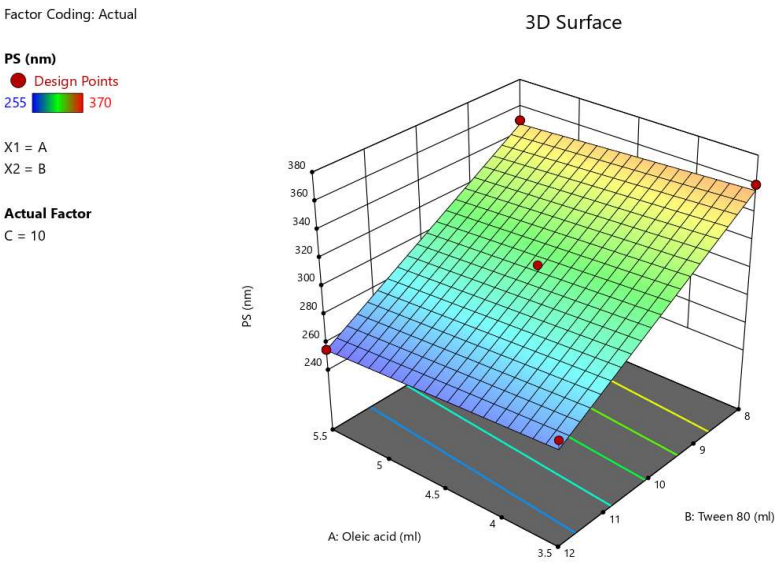


Fig 5: 3D surface plot of particle size

Polydispersity Index (PDI)
The equation for Polydispersity Index (PDI) is:
PDI = 0.339231 + -0.005625 *A + -0.021875 *B + -0.00375 *C
Interpretation:
Intercept (0.339231): This is the predicted PDI when all factors (A, B, and C) are at their reference levels.
Effect of A (Oleic acid):
Coefficient: -0.005625
Interpretation: For each unit increase in Oleic acid (A), the PDI decreases by 0.005625. This indicates that increasing Oleic acid slightly reduces the PDI, implying a more uniform particle size distribution.

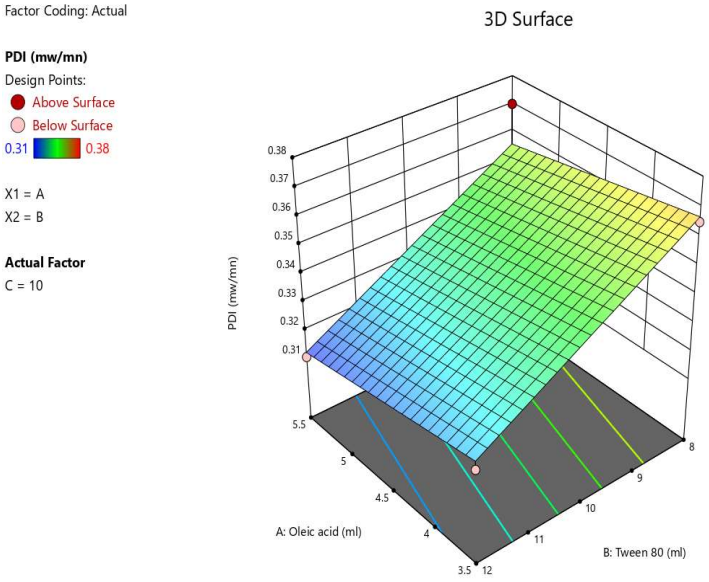


Fig 6: 3D surface plot of polydispersity index

Effect of B (Tween 80):
Coefficient: -0.021875
Interpretation: For each unit increase in Tween 80 (B), the PDI decreases by 0.021875. This is a more substantial effect, suggesting that Tween 80 significantly improves the uniformity of particle size distribution.
Effect of C (Propylene glycol):
Coefficient: -0.00375
Interpretation: For each unit increase in Propylene glycol (C), the PDI decreases by 0.00375. This is a minor effect, indicating a slight improvement in uniformity.
Overall, Tween 80 (B) has the most substantial effect on improving PDI, followed by Oleic acid (A), and then Propylene glycol (C).

1.8.5. Zeta Potential (ZP)

The equation for Zeta Potential (ZP) is:
$$ZP = -26.94 + 0.29625 *A + -5.42125 *B + -1.62 *C + -1.5725 *AB + 1.125 *AC + 2.865 *BC + 0.73125 *A^2 + 1.13125 *B^2 + 0.14375 *C^2$$
Interpretation:
Intercept (-26.94): This is the predicted zeta potential when all factors (A, B, and C) are at their reference levels.
Main Effects:
Effect of A (Oleic acid):
Coefficient: 0.29625
Interpretation: For each unit increase in Oleic acid (A), the zeta potential increases by 0.29625 mV. This indicates that increasing Oleic acid slightly makes the particles less negatively charged.
Effect of B (Tween 80):
Coefficient: -5.42125
Interpretation: For each unit increase in Tween 80 (B), the zeta potential decreases by 5.42125 mV. This indicates that Tween 80 significantly increases the negative charge on the particles.

Effect of C (Propylene glycol):
Coefficient: -1.62
Interpretation: For each unit increase in Propylene glycol (C), the zeta potential decreases by 1.62 mV. This indicates that Propylene glycol also increases the negative charge, but to a lesser extent than Tween 80.
Interaction Effects:
Effect of AB (Oleic acid and Tween 80):
Coefficient: -1.5725
Interpretation: There is a negative interaction between Oleic acid and Tween 80, which decreases the zeta potential by 1.5725 mV when both are increased together.
Effect of AC (Oleic acid and Propylene glycol):
Coefficient: 1.125
Interpretation: There is a positive interaction between Oleic acid and Propylene glycol, increasing the zeta potential by 1.125 mV when both are increased together.
Effect of BC (Tween 80 and Propylene glycol):
Coefficient: 2.865
Interpretation: There is a significant positive interaction between Tween 80 and Propylene glycol, increasing the zeta potential by 2.865 mV when both are increased together.
Quadratic Effects:
Effect of A^2 (Oleic acid):
Coefficient: 0.73125
Interpretation: The quadratic term for Oleic acid slightly increases the zeta potential, suggesting a small non-linear effect.
Effect of B^2 (Tween 80):
Coefficient: 1.13125
Interpretation: The quadratic term for Tween 80 increases the zeta potential, indicating a more noticeable non-linear effect.
Effect of C^2 (Propylene glycol):
Coefficient: 0.14375
Interpretation: The quadratic term for Propylene glycol has a minor positive effect on the zeta potential, suggesting a slight non-linear effect.
Overall, Tween 80 (B) has the most significant effect on making the zeta potential more negative, with notable interaction effects with both Oleic acid (A) and Propylene glycol (C). The quadratic effects indicate some non-linearity in the relationships.

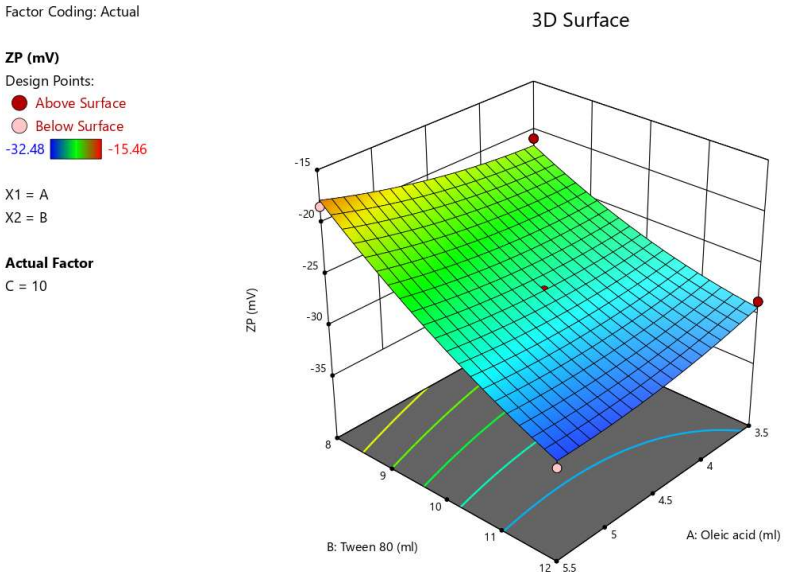


Fig 7: 3D surface plot of zeta potential

Constrains

Particle Size (PS)

The goal for PS is to target 255 nm with limits between 255 nm and 370 nm. The importance is set to 5, indicating high priority.

The equation for PS is:

$$PS = 305.385 + -4.375 *A + -46.25 *B + -8.125 *C$$

Factors affecting PS:

Oleic acid (A): Increases in A reduce PS by 4.375 nm per unit increase.

Tween 80 (B): Increases in B significantly reduce PS by 46.25 nm per unit increase.

Propylene glycol (C): Increases in C reduce PS by 8.125 nm per unit increase.

Since Tween 80 has the most significant impact on PS, adjusting its concentration within the range (8-12 ml) will likely have the largest effect on achieving the target PS of 255 nm.

1.8.5.1. Polydispersity Index (PDI)

The goal for PDI is to minimize it, with limits between 0.31 and 0.38. The importance is set to 5, indicating high priority.

The equation for PDI is:

$$PDI = 0.339231 + -0.005625 *A + -0.021875 *B + -0.00375 *C$$

Factors affecting PDI:

Oleic acid (A): Increases in A reduce PDI by 0.005625 per unit increase.

Tween 80 (B): Increases in B significantly reduce PDI by 0.021875 per unit increase.

Propylene glycol (C): Increases in C reduce PDI by 0.00375 per unit increase.

To minimize PDI, increasing Tween 80 within the allowable range will be most effective, as it has the largest impact on reducing PDI.

1.8.5.2. Zeta Potential (ZP)

The goal for ZP is to target -32.48 mV, with limits between -32.48 mV and -15.46 mV. The importance is set to 5, indicating high priority.

The equation for ZP is:

$$ZP = -26.94 + 0.29625 *A + -5.42125 *B + -1.62 *C + -1.5725 *AB + 1.125 *AC + 2.865 *BC + 0.73125 *A^2 + 1.13125 *B^2 + 0.14375 *C^2$$

Factors affecting ZP:

Oleic acid (A): Increases in A increase ZP by 0.29625 mV per unit increase.

Tween 80 (B): Increases in B significantly decrease ZP by 5.42125 mV per unit increase.

Propylene glycol (C): Increases in C decrease ZP by 1.62 mV per unit increase.

Interaction AB: Decreases ZP by 1.5725 mV.

Interaction AC: Increases ZP by 1.125 mV.

Interaction BC: Increases ZP by 2.865 mV.

Quadratic term for A: Increases ZP by 0.73125 mV.

Quadratic term for B: Increases ZP by 1.13125 mV.

Quadratic term for C: Increases ZP by 0.14375 mV.

To achieve the target ZP of -32.48 mV, focusing on adjusting Tween 80 (B) will be crucial, as it has the most significant effect on ZP. The interactions and quadratic terms also need consideration to fine-tune the response.

1.9. Optimization Strategy

Given the high importance of PS, PDI, and ZP, the optimal formulation will need to balance these responses within their specified ranges. The factors (A, B, and C) should be adjusted as follows:

1. Tween 80 (B): Since B has the most significant impact on all three responses, it should be adjusted carefully within the range (8-12 ml). Increasing B will reduce PS and PDI, but care must be taken to balance the ZP response.

2. Oleic acid (A) and Propylene glycol (C): These factors should be adjusted in combination with B to fine-tune the responses. Increasing A slightly can help in balancing ZP, while C can be adjusted to further minimize PDI and achieve the desired PS.

Table 4: Constrains

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Oleic acid	is in range	3.5	5.5	1	1	3
B:Tween 80	is in range	8	12	1	1	3
C:Propylene glycol	is in range	8	12	1	1	3
PS	is target = 255	255	370	1	1	5
PDI	minimize	0.31	0.38	1	1	5
ZP	is target = -32.48	-32.48	-15.46	1	1	5

Optimal Formulation Preparation and Evaluation

Based on the Box-Behnken design and subsequent optimization using desirability functions, an optimal formulation was identified to achieve the desired particle size (PS), minimize the polydispersity index (PDI), and target the zeta potential (ZP). The identified optimal levels of the factors and the corresponding theoretical response values were:

Oleic acid: 5.499997 ml
Tween 80: 11.999999 ml
Propylene glycol: 9.394072 ml

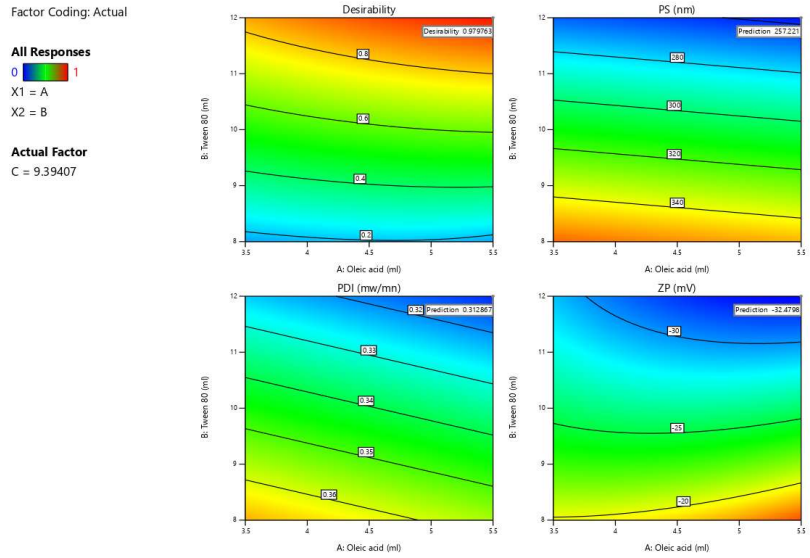


Fig 8: Numerical optimization of formulation

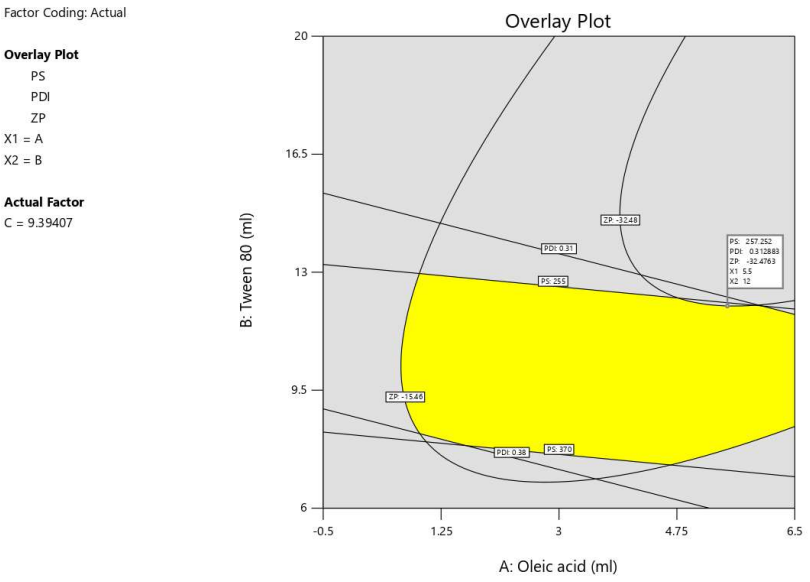


Fig 9: Overlay plot of forumations

Experimental Evaluation of Optimal Formulation

The formulation was prepared according to the optimal levels identified by the software, and the responses were experimentally evaluated. The observed values for the responses were as follows:

Particle Size (PS): 259 nm
Polydispersity Index (PDI): 0.3169

Zeta Potential (ZP): -32.01 mV

1. Particle Size (PS): The experimentally observed particle size was 259 nm, which is slightly higher than the theoretically predicted value of 257.22 nm but still very close to the target of 255 nm. This small deviation can be attributed to experimental variability and is within acceptable limits. The result indicates that the formulation process is reliable and reproducible, producing particles of nearly the desired size.

2. Polydispersity Index (PDI): The observed PDI was 0.3169, compared to the predicted value of 0.313. This slight increase in PDI suggests a marginally broader size distribution than expected, but the value is still low enough to indicate a reasonably uniform particle size distribution. A lower PDI is preferred as it signifies more homogeneous particles, which is important for consistent performance.

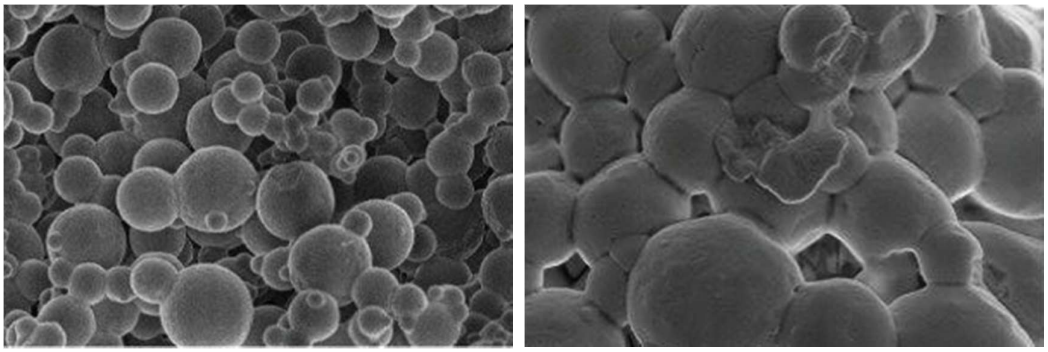
3. Zeta Potential (ZP): The experimentally measured zeta potential was -32.01 mV, slightly less negative than the predicted value of -32.48 mV. While there is a minor deviation, the zeta potential is still sufficiently negative to ensure good electrostatic stability, helping to prevent particle aggregation and maintaining suspension stability.

Comparison to Predicted Values

The experimentally observed values for PS, PDI, and ZP are close to the predicted values obtained from the optimization process, demonstrating that the model used for optimization is robust and reliable. The minor discrepancies observed in the experimental results are typical in practical scenarios due to inherent variability in experimental conditions and measurement techniques. The experimental evaluation of the optimized formulation confirms that the chosen levels of Oleic acid, Tween 80, and Propylene glycol result in a formulation that closely matches the desired specifications. The particle size is very near the target, the polydispersity index remains low, indicating a relatively uniform particle size distribution, and the zeta potential is sufficiently negative to ensure stability. These results validate the effectiveness of the Box-Behnken design and desirability function approach in optimizing the formulation. The slight deviations between predicted and observed values are minimal and do not significantly impact the overall quality and stability of the formulation. This optimized formulation provides a strong basis for further development and potential scale-up, ensuring consistent performance and stability in practical applications.

CHARACTERIZATION OF OPTIMIZED FORMULATION

Scanning electron microscopy (SEM)



(a) (b)

Fig 10: (a) SEM image of SLNs at 100nm; (b) SEM image of SLNs at 1µm

Drug content

The drug content of the optimized formulation was found to be 91.14%

Drug loading and entrapment efficiency

The drug loading and entrapment efficiency of the optimized procyclidine loaded Solid Lipid Nanoparticles (SLNs) formulation were found to be 20.48% and 86.95%, respectively. These values indicate a well-balanced formulation with efficient drug encapsulation and adequate drug loading capacity.

The DLE of 20.48% signifies that 20.48% of the total weight of the optimized formulation is comprised of the active drug. This percentage is quite reasonable for SLN formulations, as they typically contain a significant amount of lipid or polymer carriers to ensure stability, controlled release, and protection of the encapsulated drug. A DLE of 20.48% suggests that the formulation has successfully incorporated a substantial amount of the drug, making it capable of delivering an effective therapeutic dose. The DLE of 20.48% is indicative of a substantial amount of drug being loaded into the nanoparticles relative to the total formulation weight. This level of drug loading is beneficial as it ensures that a therapeutic dose can be delivered without requiring excessive amounts of the formulation. It strikes a balance between maximizing the drug content and maintaining the structural integrity and release characteristics of the SLNs.

The EE of 86.95% indicates that a high percentage of the total drug used during the formulation process has been successfully encapsulated within the SLNs. This high entrapment efficiency reflects the efficacy of the formulation method employed. It demonstrates that the majority of the procyclidine drug molecules are securely trapped within the lipid matrix, reducing the amount of free drug in the solution and thereby minimizing potential side effects. The observed EE of 86.95% is significantly high, suggesting that the SLN formulation process is highly efficient at encapsulating procyclidine. This efficiency can be attributed to the optimized conditions of the formulation process, such as the choice of lipids, surfactants, and the method of preparation (e.g., solvent evaporation, high-pressure homogenization).

The combination of high EE and moderate DLE supports a controlled and sustained drug release profile. The high EE ensures that most of the drug is encapsulated and available for gradual release, while the moderate DLE suggests that the drug release will be sustained over a longer period due to the presence of a significant amount of carrier material. From a clinical perspective, the high EE and reasonable DLE indicate that the SLN formulation is likely to provide sustained therapeutic levels of procyclidine with fewer side effects. The high encapsulation reduces the initial burst release, leading to a more controlled and steady drug release, which is advantageous for maintaining consistent plasma levels and improving patient compliance.

The lipid matrix in the SLNs not only helps in achieving high EE but also plays a crucial role in protecting the encapsulated drug from degradation. This enhances the overall stability of the formulation, ensuring that the drug remains effective over its shelf life. The optimized formulation of procyclidine loaded SLNs, with a drug loading efficiency of 20.48% and an entrapment efficiency of 86.95%, demonstrates a well-designed drug delivery system. The high entrapment efficiency ensures efficient drug encapsulation, while the moderate drug loading efficiency indicates a balanced formulation capable of providing sustained and controlled drug release. These characteristics suggest that the SLN formulation can improve therapeutic outcomes, enhance patient compliance, and reduce potential side effects, making it a promising candidate for clinical use.

***In-vitro* drug release**

The *in-vitro* drug release study of procyclidine loaded Solid Lipid Nanoparticles (SLNs) compared with the pure drug was conducted using the dialysis method. The results showed that the optimized SLN formulation released 10.46% of the drug within the first hour, whereas the pure drug exhibited only 1.26% release, indicating a significantly higher initial burst release for the optimized formulation. At the 6-hour mark, the optimized SLN formulation achieved a cumulative release of 51.75%, while the pure drug reached 7.77%, highlighting the sustained release characteristic of the SLN formulation compared to the pure drug, which has a much slower release profile. After 12 hours, the optimized formulation showed a cumulative release of 94.15%, in contrast to the pure drug, which had a release of 34.09%, maintaining a more controlled and gradual release throughout the duration of the study. The higher initial release of the optimized formulation can be attributed to the small size and high surface area of the SLNs, enhancing the solubility and dissolution rate of the drug, which is beneficial for achieving therapeutic drug levels quickly.

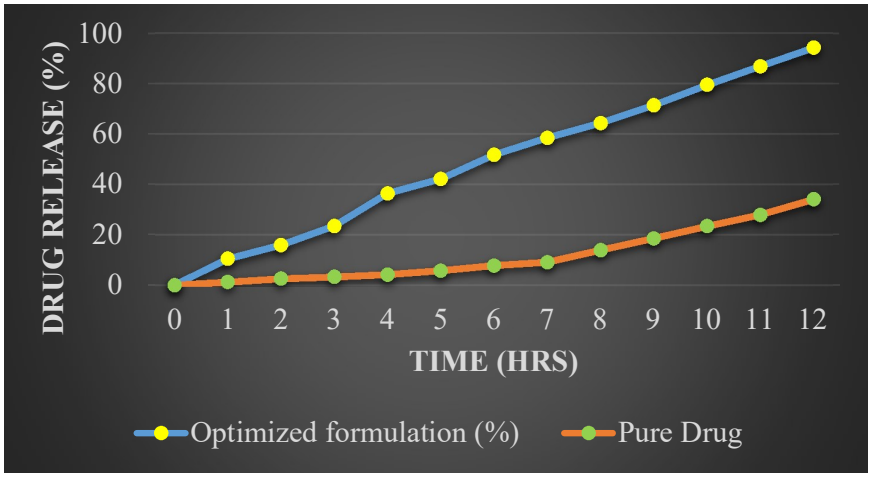


Fig 11: *In-vitro* drug release study of optimized formulation

The SLN formulation exhibited a sustained release pattern, crucial for maintaining therapeutic drug levels over an extended period, potentially reducing the frequency of dosing and improving patient compliance. The controlled release observed in the optimized formulation can be attributed to the encapsulation of the drug within the lipid matrix of the SLNs, acting as a barrier and slowly releasing the drug as it diffuses through the

lipid layers or as the lipid matrix degrades. This improved release profile of the SLN formulation suggests potential clinical benefits, including reduced side effects due to lower peak plasma concentrations and enhanced bioavailability due to prolonged drug presence in the system.

The pure drug, with its lower and slower release rate, may not achieve the desired therapeutic levels as efficiently as the SLN formulation, highlighting the importance of drug delivery systems like SLNs in enhancing the efficacy of pharmaceutical compounds. In conclusion, the study successfully demonstrates that the optimized SLN formulation of procyclidine significantly enhances the drug release profile compared to the pure drug, underscoring the potential of SLNs as a superior drug delivery system offering both rapid initial release and sustained drug delivery.

Stability studies

The stability studies of the optimized Solid Lipid Nanoparticles (SLNs) formulation were conducted over 90 days, stored at two different conditions: 4°C and 27 ± 2°C / 65% ± 5% relative humidity. The parameters monitored included Particle Size, Polydispersity Index (PDI), Zeta Potential (ZP), and Entrapment Efficiency (EE). The results for the first, second, and third months are summarized below.

Table 5: Stability studies of optimized formulation

S.No	Conditions	Size	PDI	ZP	EE
1st Month Data					
1.	4 ± 2°C / 65% ± 5%	258	0.3178	-31.97	86.89 %
2.	27 ± 2°C / 65% ± 5%	255	0.3214	-30.72	85.13%
2nd Month Data					
3.	4 ± 2°C / 65% ± 5%	259	0.3189	-31.34	85.19 %
4.	27 ± 2°C / 65% ± 5%	258	0.3297	-30.01	83.28%
3rd Month Data					
5.	4 ± 2°C / 65% ± 5%	259	0.3199	-31.19	82.13 %
6.	27 ± 2°C / 65% ± 5%	262	0.3301	-29.41	80.08%

SUMMARY

The current study aimed to optimize, formulate and evaluate proxyclidine loaded solid lipid nanoparticles. Procyclidine HCl exhibits variable solubility characteristics, being sparingly soluble in water but showing higher solubility in methanol, phosphate buffer (pH 6.8), and ethanol. This solubility profile is crucial for its formulation and bioavailability. The solubility in different solvents suggests that the drug can be formulated in various dosage forms, including solutions and solid dispersions.

The calibration curve for Procyclidine HCl, determined at a wavelength of 258 nm in methanol, demonstrates linearity within the concentration range of 2-10 µg/mL. This linear relationship, indicated by a high correlation coefficient (R^2) of 0.9967, confirms the reliability of this analytical method for quantifying the drug. Accurate calibration curves are essential for the precise measurement of drug concentrations in various formulations and biological samples. This linearity ensures that the method can be used to monitor drug levels, assess stability, and verify consistency across different batches.

FTIR spectroscopy was employed to identify the functional groups present in Procyclidine HCl. The analysis detected characteristic peaks corresponding to N-H, C-H, C-N, and C-Cl bonds, which are integral to the drug's structure. Comparing the FTIR spectra of pure Procyclidine HCl and its formulation with excipients showed no significant shifts or new peaks, indicating that there were no chemical interactions between the drug and the excipients. This stability is critical as it ensures that the drug maintains its efficacy and safety when combined with other substances in the final product. FTIR thus confirms the compatibility and integrity of the drug formulation.

DSC analysis was used to study the thermal properties of Procyclidine HCl and its formulation within solid lipid nanoparticles. The DSC thermograms revealed that the drug retains its crystalline structure, with distinct melting points for both the pure drug and its excipients. The absence of significant changes in these thermal properties suggests that the drug is physically encapsulated within the SLNs without undergoing chemical interactions. This physical encapsulation is beneficial for protecting the drug from degradation and controlling its release. DSC provides valuable insights into the stability and compatibility of the drug within its delivery system.

The optimization of Procyclidine SLNs was guided by the Box-Behnken design, a statistical method that evaluates the effects of multiple formulation variables. The study identified Tween 80 as the most influential factor affecting particle size, polydispersity index (PDI), and zeta potential. By systematically adjusting the concentrations of Tween 80, Oleic acid, and Propylene glycol, an optimal formulation was developed. This

optimization process ensures that the SLNs have the desired characteristics for effective drug delivery, including uniform particle size, stability, and appropriate surface charge. Such a methodical approach enhances the efficiency and reliability of the formulation process.

The optimization strategy aimed to achieve a balance between particle size, polydispersity index, and zeta potential by fine-tuning the concentrations of Tween 80, Oleic acid, and Propylene glycol. The optimal ranges for these excipients were identified through a series of experiments, resulting in a formulation that meets the desired specifications. This strategy ensures that the nanoparticles are small enough for efficient drug delivery, have a narrow size distribution for consistency, and possess a stable zeta potential to prevent aggregation. The systematic approach helps in developing a robust formulation that can be reliably reproduced on a larger scale.

The optimal formulation was prepared using specific concentrations of Oleic acid (5.5ml), Tween 80 (12ml), and Propylene glycol (9.36ml), which were determined through the optimization strategy. The resulting formulation was evaluated for particle size, polydispersity index (PDI), and zeta potential. The experimental values for these parameters closely matched the predicted targets, confirming the accuracy and robustness of the optimization model. This evaluation ensures that the formulation process is consistent and that the final product meets the required quality standards. Such thorough preparation and evaluation are crucial for developing an effective and reliable drug delivery system.

The experimental evaluation of the optimal formulation showed a particle size of 259 nm, a polydispersity index (PDI) of 0.3169, and a zeta potential of -32.01 mV. These results demonstrate that the formulation is within the desired range for effective drug delivery. The close alignment of these values with the predicted targets validates the optimization model and confirms the formulation's reproducibility. Consistent particle size and stability are essential for ensuring that the drug is delivered effectively to the target site, while a low PDI indicates a uniform size distribution, further enhancing the formulation's reliability. The experimental results for the optimal formulation closely matched the predicted values, validating the accuracy of the optimization model. The particle size, PDI, and zeta potential were all within the expected ranges, confirming that the formulation process is both reliable and reproducible. This close alignment between predicted and experimental values demonstrates the robustness of the optimization strategy and its applicability in real-world scenarios.

The optimized formulation was characterized by its high drug content of 91.14%, with drug loading of 20.48% and entrapment efficiency of 86.95%. These metrics indicate that the formulation is efficient in encapsulating and retaining the drug within the solid lipid nanoparticles (SLNs). High drug content and entrapment efficiency are essential for maximizing the therapeutic efficacy of the formulation, as they ensure that a sufficient amount of the drug is delivered to the target site. This characterization confirms the success of the optimization process and the potential of the formulation for effective drug delivery.

The *in-vitro* drug release study of Procyclidine HCl encapsulated in solid lipid nanoparticles showed a sustained release profile over a period of 24 hours. This sustained release is advantageous as it can maintain therapeutic drug levels for extended periods, reducing the need for frequent dosing. The sustained release profile enhances patient compliance and ensures a more consistent therapeutic effect, demonstrating the potential of SLNs in improving the pharmacokinetic profile of Procyclidine HCl. Stability studies of the optimized Procyclidine HCl SLN formulation were conducted under different conditions, including room temperature and accelerated stability conditions (40°C ± 2°C / 75% ± 5% RH). Over the study period, the formulation maintained its particle size, polydispersity index (PDI), zeta potential, and drug content without significant changes. This stability indicates that the formulation is robust and can withstand variations in storage conditions, ensuring long-term efficacy and safety. These result suggest the optimized formulation follows a sustained release of drugs and can be beneficial intreatment of parkinsons disease for a long period of time.

CONCLUSION

The study successfully formulated and optimized Procyclidine Hydrochloride-loaded Solid Lipid Nanoparticles using a Box-Behnken Design. The SLNs demonstrated favorable characteristics, including optimal particle size, high entrapment efficiency, and sustained drug release over 24 hours. Stability studies validated the robustness of the formulation under various conditions, ensuring consistent performance. These findings indicate that SLNs can serve as a promising drug delivery system to overcome the solubility and bioavailability challenges of Procyclidine Hydrochloride. Further in-vivo and clinical studies are recommended to explore their potential for therapeutic applications in Parkinson’s disease.

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