RESEARCH PAPER

From Molecule to Matrix: Preformulation of SLN Systems for Dual Anti-Inflammatory/Antimicrobial Therapy in Wound Healing

Aditi Singh[1, 2], Brijesh Singh[2], Shikha Srivastava[1*]

[1] Institute of Pharmacy, Shri Ramswaroop Memorial University, Barabanki, Lucknow-Deva Road, 225003,Uttar Pradesh, India.

[2] Ashoka Institute of Technology and Management, Paharia, Sarnath Road, Varanasi, 221007, Uttar Pradesh, India.

Corresponding Author

Dr. Shikha Srivastava

Professor

Institute of Pharmacy

Shri Ramswaroop Memorial University

Uttar Pradesh, India. 225003.

Email: shikha_ceutics@yahoo.co.in

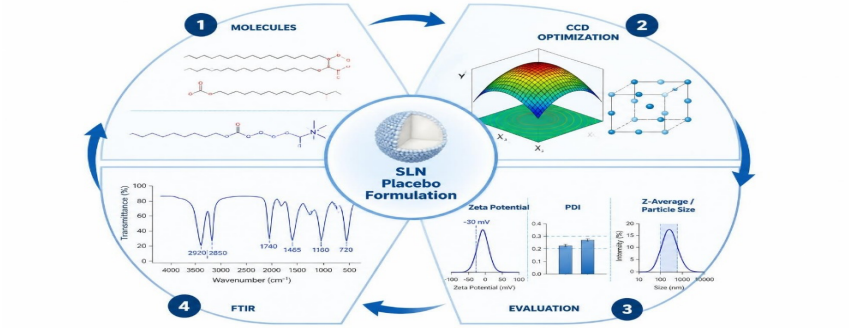
shikhasri.pharma@srmu.ac.in

Abstract

Objective: This study aimed to formulate and optimize a placebo SLN formulation as a first step to develop a suitable SLN-based topical delivery system. Preparations of SLNs were performed using Gelucire® 50/13 as a solid lipid and vitamin E was incorporated as the lipidic component. Preparation: The systematic optimization of placebo SLN formulation employed an 18 run Central Composite Design (CCD). The optimized formulation was evaluated for particle size, polydispersity index (PDI), zeta potential, viscosity, morphology, and compatibility study by FTIR. Antibacterial activity was tested on a preliminary basis. Result: The findings indicated that the particle size of the optimized formulation of placebo SLNs (F-Opt-P) was particle size of 152.3 ± 6.4 nm, PDI 0.162 ± 0.018 and ZP = −33.8 ± 1.6 mV, which indicates narrow particle-size distribution and satisfactory stability in the colloidal system. The formulation exhibited a viscosity and flow characteristics acceptable for further development of topical formulation. TEM analysis indicated separate spherical nanoparticles without aggregation. The characterstic peaks of formulation component were observed in the FTIR spectra which did not show any major incompatibility. The lack of antibacterial activity was observed in placebo SLN formulation against the microorganisms.

Conclusion: To conclude, the optimized placebo SLN exhibited satisfactory physiochemical, morphological, and compatibility properties; it offers a suitable basis for further formulation development. In the future studies conducted in this project, optimized SLN will be entered into a suitable topical gel system for its easy application and investigations of being a unique topical drug-delivery system.

Graphical Abstract



Description: Figure shows the detail illustration of Placebo SLN formation.

Keywords: Optimization, Formulation, SLN

How to cite this article: Aditi Singh1 2 Brijesh Singh2 Shikha Srivastava1*. From Molecule to Matrix: Preformulation of SLN Systems for Dual Anti-Inflammatory/Antimicrobial Therapy in Wound Healing. Int J Drug Deliv Technol. 2026;16(76s):2076-2086. DOI: 10.25258/ijddt.16.76s.201.

INTRODUCTION

Chronic wounds require longer times to heal, have a complex pathophysiology, are repetitive, and have a higher risk for microbial colonization and infection. Chronic wounds enter into a prolonged inflammatory state as compared to acute wounds that would proceed in a well-coordinated manner through hemostasis, inflammation, proliferation, and tissue remodeling phases. [1] Wounds do not close due to persistent inflammation. Furthermore, chronic

wounds are characterized by excessive oxidative stress, dysregulated inflammatory mediators, impaired angiogenesis, defective extracellular matrix deposition, and reduced proliferation and migration of reparative cells. Ultimately, all this results in delayed tissue regeneration and wound closure. Under such circumstances, chronic wound management requires therapeutic approaches, which can maintain an adequate concentration of the therapeutic agent at the wound site for a sufficient period while minimizing unnecessary systemic exposure.[2] Topical drug delivery provides several benefits for the treatment of wounds as it allows applying therapeutic agents directly to the site. The conventional topical formulations available today, such as creams, ointments, conventional gels, etc. may have low retention at the wound surface, be removed quickly by the wound exudate, have poor penetration, instability of incorporated drugs, and uncontrolled release of drug.[3] These shortcomings have stimulated the development of advanced systems to facilitate topical delivery that can enhance drug localization, protect therapeutic agents and provide controlled or sustained drug release. Solid lipid nanoparticles (SLNs) have gained much interest among the different investigated topical nanocarrier systems due to their advantageous physicochemical and biopharmaceutical properties. SLNs are systems of solid lipid matrix stabilized by suitable surfactants.[4] The solid lipid matrix creates an environment that allows incorporation of those therapeutic agents, which are poorly water-soluble or lipophilic. The agents may as well be protected from premature degradation. Additionally, SLNs' nanometric size yields a high surface area that promotes intimate contact at the site of application, making them attractive carriers for localized topical delivery. [5] The lipid matrix may also affect the release characteristics of the therapeutic agent incorporated into it, thus offering the possibility of controlled or prolonged release. Among the various nanocarrier systems investigated for topical drug delivery, solid lipid nanoparticles (SLNs) have attracted considerable attention due to their respective physicochemical and biopharmaceutical characteristics. SLNs consist of surfactants and lipid and are formulated into solid lipid nanoparticles. [6] Compounds that are poorly soluble in water can be incorporated into a liquid lipid matrix. This may help them to remain stable during degradation. Moreover, the small size of SLNs imparts a large surface area which helps to provide close contact with the site of application making it an attractive carrier for localized topical delivery. [7] The lipid matrix can also influence the release characterstics of the therapeutic agent incorporated into it. Thus, it gives the possiblity of controllable, prolonged drug release. SLNs, the colloidal carriers composed of lipids, have several advantages over conventional delivery systems. They might improve the dispersion of poorly water-soluble drugs, which have a "poor drug-receptor" interaction. Further, SLNs might protect the incorporated actives and enhance the surface contact. As a result, this can modulate the drug release. The composition of lipids can also interact with the biological surface and contribute to better localization of the formulation after topical administration. These properties categorize SLN as a promising platform for localized drug-delivery systems for wound healing.[8] However, the performance of SLN is strongly dependent on the composition of the formulation and the processing conditions used during preparation. Nanoparticle formation was influenced by type and amount of lipids, surfactant concentration, lipid surfactant ratio, process temperature, homogenization conditions, and temperatures of cooling etc. Reproducibility of SLN system with desired physicochemical properties requires systematic optimization of formulation and process variables. Variations in these parameters can influence particle size, particle-size distribution, surface charge, physical stability, drug incorporation and release behaviour. The particle size, polydispersity index (PDI), zeta potential, and among the critical quality attributes.[9] The surface area and dispersion properties of the nanoparticles are influenced by size. Likewise, the PDI provides information about the uniformity of the particles size. The charge of Zeta potential provides information on the surface charge and helps to assess the colloidal stability of the dispersion of nanoparticles. Choosing suitable lipid and surfactant components is therefore an important step in developing SLNs. In this study, the lipid components selected were Gelucire and Gelot, while oil vitamin E as lipidic component for the development of lipid matrix. [10] PF68 was chosen to serve as stabilizing surfactant. The lipid materials selected will make the solid lipid matrix while PF68 will stabilize the nanoparticles and may provide some steric protection against particle aggregation. The aim of using these components altogether was to develop a lipid–surfactant system able to produce a homogeneous and physically stable nanoparticulate dispersion. The development of a placebo SLN formulation before incorporation of the active pharmaceutical ingredient is an important preliminary step in rational formulation development. [11] The preparation of placebo SLNs without the active drug allows for the independent evaluation of the suitability of the selected lipid and surfactant system away from drug-related effects. This method will help identify the optimal concentrations of

formulation ingredients and processing conditions required to achieve suitable nanoparticles characteristics. It also serves as a reference system to evaluate changes produced by the incorporation of another drug. It is especially important as incorporation of drug into lipid matrix may change particle size, PDI, surface charge, lipid organization, and physical stability. A systematic Design of Experiments (DoE) approach provides an efficient means of optimizing pharmaceutical formulation by studying the individual and interaction effects of formulation variables.[12] One-factor-at-a-time conventional approaches may require too many experimental trials and provide little information about formulation component interactions. On the other hand, response-surface methodology can access more than one factor, as well as one Central Composite Design (CCD) is particularly useful for process optimization since it makes possible the investigation of linear, interaction and quadratic effects of chosen independent variable and the pinpointing of suitable optimization conditions within the design space.[13] Accordingly, a Central Composite Design (CCD) with 18 runs was utilized for systematic optimization of the placebo SLN formulation in the present work. According to the experimental design, the selected formulation variables were varied and the resulting placebo SLN dispersions were evaluated for particle size, PDI and zeta potential. The statistical analysis of the experimental responses was carried out to ascertain the effect of the formulation variables and their interactions and to examine the optimized formulation with desired nanoscale features and colloidal stability. The improved placebo formulation of solid lipid nanoparticles (SLN) was subsequently developed as the carrier platform for development of drug-loaded SLN formulation for potential application in chronic wound care. Establishing a placebo SLN system before drug incorporation provides a rational and reproducible basis for subsequent formulation development by enabling the effects of the drug to be distinguished from those associated with the lipid and surfactant composition. Thus, the present work aimed to develop and optimize a placebo SLN system using Gelucire, Gelot, vitamin E, and Pluronic F68. The final output of the work must be stable, homogenous, reproducible SLN system or nanoparticulate carrier. This would be further loaded with the active pharmaceutical ingredient and develop a wound-healing delivery system.

2. Materials and Methods. The principal solid lipid matrix chosen for producing a placebo solid lipid nanoparticle was Gelucire® 50/13, while vitamin E (α-tocopherol) was added as a lipidic component. During preliminary lipid-screening studies, GMS (glycerol monostearate) and Gelot® 64 were evaluated for a suitable lipid composition for subsequent SLN development. A non-ionic surfactant and stabilizer was Pluronic® F68 (PF68) which was used to promote nanoparticle formation, consequently enhancing the physical stability of SLN dispersion. For FTIR sample preparation, spectroscopic grade Potassium bromide, KBr was used. During SLN preparation, the aqueous phase consisted of double distilled water. Soaking 5g of wet SLN in 0.5% w/v liquid detergent solution takes place in a beaker. This combination gets agitated using a magnetic stirrer for 30-60 minutes at room temperature. The respective manufacturers' recommendations were followed for the storage of all the materials. Tools and Materials. The placebo SLNs were prepared and characterized using suitable laboratory and analytical instruments. A lipid phase was melted using a hot water bath maintained at about 80–85°C. A tissue homogenizer was used for high-speed homogenization of the lipid dispersion; a probe sonicator was used for the reduction of particle size wherever applicable. A magnetic stirrer was employed for monitoring the mixing and cooling of the formulation.[14] The analysis of the mean hydrodynamic particle size and the polydispersity index (PDI) will be performed using a Dynamic Light Scattering (DLS) instrument (Malvern Zetasizer Nano ZS or equivalent). The folds capillary zeta cell (DTS1070) was used to measure zeta potential.[15] The chosen formulation components' characteristic functional groups and preliminary compatibility were examined through FTIR analysis performed using an FTIR spectrophotometer (Bruker or equivalent). Morphological investigation of the optimized placebo SLNs was carried out by transmission electron microscopy (JEOL JEM or similar). [16] A UV–visible spectrophotometer was used wherever appropriate for spectral characterization. Formulation and characterization studies were conducted using calibrated digital pH meter, micropipettes, borosilicate glass containers, Eppendorf tubes, and other standard laboratory accessories. The Chemoface software generated and statistically optimized the Central Composite Design (CCD) utilized for the development of placebo SLN formulation [17].

2.1 Preliminary Lipid Screening

A lipid-screening test was carried out to select the most suitable lipid composition for developing placebo SLNs. The suitability of Gelucire® 50/13, GMS and Gelot® 64 for formation of a stable lipid dispersion and compatibility with selected formulation components was evaluated. Scientists examined vitamin E to alter lipid composition and

help in the formation of desired Nanoparticulate system. [18] The lipid components that were selected were assessed for their capacity to yield a uniform dispersion after the formulation process. From the initial observations, Gelucire® 50/13 was selected as the solid lipid matrix and a lipidic component of the placebo SLN optimization was vitamin E.

2.2 Development of Placebo Solid Lipid Nanoparticles The placebo solid lipid nanoparticle (SLN) formulation was optimized using a Central Composite Design (CCD) to determine the appropriate levels of the selected formulation variables and obtain a homogeneous and physically stable nanoparticulate dispersion. The experimental design comprising 18 runs was generated using Chemoface software. Since the objective of this stage was to optimize the drug-free SLN system, no active pharmaceutical ingredient was incorporated. The formulation variables primarily included the concentration of the lipid phase and the composition of the lipid mixture.

2.2.1. Preparation of Vitamin E–Gelucire Lipid Mixture The selected lipid mixture was prepared using Vitamin E and Gelucire® 50/13 at the predetermined ratio. The required quantities of Vitamin E and Gelucire® 50/13 were accurately weighed and transferred into a suitable glass container. For preparation of the selected 25% Vitamin E–Gelucire lipid mixture, 4 g of Vitamin E and 8 g of Gelucire® 50/13 were used. The lipid components were heated on a water bath at approximately 80°C with gentle mixing until a uniform and completely molten lipid phase was obtained. The resulting homogeneous lipid phase was maintained in the molten state until further use.

2.2.2. Preparation of Placebo SLN Dispersion Placebo SLN dispersions were prepared according to the compositions specified in the experimental design. The final volume of each formulation was maintained at 5 mL. The quantity of lipid phase was varied according to the respective experimental run, while the remaining volume was made up with 1% w/v Pluronic® F68 (PF68) aqueous solution. For the formulation containing 10% lipid, 0.5 mL of the molten Vitamin E–Gelucire lipid mixture was accurately withdrawn using a micropipette and transferred into a suitable glass container, followed by the addition of 4.5 mL of 1% w/v PF68 aqueous solution. Similarly, the required quantities of the molten lipid mixture and PF68 solution were calculated and incorporated to obtain formulations containing 15% and 20% lipid, respectively, while maintaining the final volume at 5 ml. The resulting lipid–aqueous mixtures were subjected to high-speed homogenization at 10,000 rpm for 5 min using a tissue homogenizer while maintaining the formulation under controlled heating conditions. Homogenization facilitated the dispersion of the molten lipid phase within the aqueous surfactant phase and promoted the formation of the nanoparticulate dispersion. [19] The resulting dispersion was subsequently subjected to probe sonication for 5 min to reduce particle aggregation and improve the uniformity of the dispersion. After sonication, the prepared placebo SLN dispersions were transferred into clean glass containers and allowed to equilibrate before further characterization. The prepared formulations were subsequently evaluated for their relevant physicochemical characteristics, and the experimental responses obtained from the 18 formulation runs were used for statistical analysis and selection of the optimized placebo SLN formulation.[20] The detailed central composite design (CCD)-based optimization of the SLN formulation, generated using Chemoface software, is presented in Table.1 and Figure.1 show procedure of making placebo SLN.

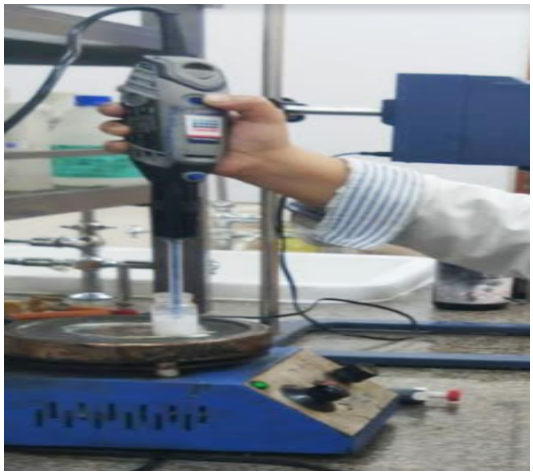


Figure.1 Schematic representation of the detailed preparation procedure of placebo solid lipid nanoparticles (SLN).

Table.1 *Central Composite Design Runs for SLN Optimization.*

S. No.	Run	Lipid Concentration	Pluronic F68 (% w/v)	Vitamin E : Gelucire 50/13 (% w/w)
1	1	10	1	25

2	2	10	1	75
3	3	10	3	25
4	4	10	3	75
5	5	20	1	25
6	6	20	1	75
7	7	20	3	25
8	8	20	2	50
9	9	6.591	0.3182	50
10	10	23.408	3.681	50
11	11	15	2	7.9551
12	12	15	2	92.044
13	13	15	2	50
14	14	15	2	50
15	15	15	2	50
16	16	15	2	50
17	17	15	2	50
18	18	15	2	50

2.3. CHARACTERIZATION AND EVALUATION OF PLACEBO SLN

2.3.1 Visual Examination of Placebo SLNs

The prepared placebo SLN formulations were subjected to preliminary visual examination to assess their macroscopic appearance and detect any visible turbidity, aggregation, or phase separation. Approximately 0.5 mL of each formulation was transferred into individually labelled Eppendorf tubes using a calibrated micropipette. The samples were allowed to stand undisturbed for approximately 5 min and were examined visually against a light background for appearance and turbidity.

2.3.2 Measure of PDI and Particle Size The Malvern Zetasizer Nano Series instrument (Malvern Instruments, Malvern, UK) was employed to determine the particle size and polydispersity index (PDI) of the developed placebo solid lipid nanoparticles (SLNs) using dynamic light scattering (DLS) technique.[21] A 5 µL aliquot of the SLN dispersion was properly diluted with distilled water to 3 mL in a disposable cuvette to get an ideal concentration for measurement and lower multiple scattering effect.[16] The diluted sample was gently swirled and transferred to the measuring cell without any bubbles forming. The measurement was done at 25°C with a detection/scattering angle of 90°. Using the two-parameter non-linear fitting analysis, the scattered light intensity measured by the instrument determined the Z-average hydrodynamic particle diameter and PDI. The PDI was used to measure the uniformity of the particle-size distribution. [22] The measurements done in triplicate and expressed as mean particle size ± standard deviation (SD) and mean PDI ± SD.

2.3.3 Zeta potential determination The ELS test of Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) was used to characterize the zeta potential of the placebo SLNs. Before making the measurement, the SLN dispersion was diluted by approximately 100 times with filtered distilled water, which was established using a 0.45 µm membrane filter.[23] This was done in order to reduce particle concentration thus minimizing multiple scattering at the time of measurement. The diluted sample was placed in a folded capillary zeta cell (DTS1070) without introducing any air bubbles into the measurement cell. The sample was equilibrated at 25 ± 1 °C before the measurement. The instrument measured the resulting electrophoretic mobility and translated that into zeta potential via suitable electro kinetic scaling analysis when the applied electric field induced the electrophoretic movement of the charged nanoparticles. The surface charge and electrostatic stability of the nanoparticle dispersion can be indicated by the zeta potential. In general, a suitably high positive or negative zeta potential promotes electrostatic repulsion between the particles and suppresses their tendency to aggregate. A zeta potential value of about ±30 mV or more is generally considered to indicate good electrostatic colloidal stability. [24] The stability of colloidal dispersions however is also determined by steric stabilization, surfactant composition, ionic strength and the dispersion medium. Every single measurement was carried out in triplets and results given in mean zeta potential ± SD. [25]

2.3.4 Morphological Examination by Transmission Electron Microscopy Transmission electron microscopy (TEM) The morphology and internal structure of the optimized solid lipid nanoparticles (placebo) as well as the blended SLN-hydrogel formulation, were examined using Transmission Electron Microscopy (TEM).[26] A small drop (approximately 5–10 µL) of the appropriately diluted sample (diluted with distilled water in a ratio of 1:100 v/v) was placed onto a carbon-coated copper grid (200 mesh) and allowed to adsorb for 2–3 minutes. Excess liquid was gently blotted off using filter paper. Negative staining was performed by placing a drop of 2% w/v uranyl acetate solution onto the grid for 1–2 minutes. The excess stain was removed by blotting, and the grid was air-dried at room temperature in a dust-free environment.[27] The dried grid was loaded into the TEM instrument and observed at an accelerating

voltage of 80–200 kV. Micrographs were captured at various magnifications to evaluate the morphology (spherical, ellipsoidal, or irregular), surface characteristics, uniformity, and particle size range of the SLNs. In the case of the blended SLN-hydrogel formulation, the images were further analyzed to visualize the SLNs embedded within or distributed throughout the hydrogel matrix, the uniformity of distribution, the mesh size and porosity of the hydrogel network, and the structural integrity of the hybrid system. The observed particle dimensions were correlated with the hydrodynamic diameter obtained from DLS analysis.

2.3.5 FTIR Compatibility Studies

FTIR spectroscopy is determined by their functional groups and structure of the molecules. It is the interaction of the matter with infrared radiation that gives the information about the substance. Infrared light is passed through or reflected from a sample in FTIR. [28] The sample absorbs frequencies that correspond to the vibrational modes of chemical bonds. The absorption "fingerprint" generates a spectrum that gives a unique fingerprint of a compound. The spectrum can be used for qualitative identification of compounds. Similarly, it can be used for monitoring changes in sample matrix composition.[29] The modern FTIR device is easily able to take readings on a solid, paste or liquid just with minimal preparations which has certainly made it popular for application in pharmaceutical material and natural product analyses. The FTIR spectra of the pure drugs, individual excipients, placebo formulations, and drug-loaded formulations were recorded in the range of 4000–400 cm^{-1}, and the characteristic absorption peaks are summarized in **Tables**. Representative overlaid spectra are depicted in **Figure**

2.3.5.1 Characterization of Excipients

Gelucire 50/13 displayed prominent peaks at 2920 and 2851 cm^{-1} (C–H stretching, aliphatic), 1735 cm^{-1} (C=O stretching, ester carbonyl), 1467 cm^{-1} (C–H bending), and 1177 cm^{-1} (C–O stretching, ester). **Vitamin E (α-tocopherol)** showed bands at 3400 cm^{-1} (O–H stretching), 2924 and 2854 cm^{-1} (C–H stretching), 1460 cm^{-1} (C–H bending), and 1080 cm^{-1} (C–O–C stretching). **Pluronic F-68** exhibited peaks at 3440 cm^{-1} (O–H stretching), 2880 cm^{-1} (C–H stretching), 1467 cm^{-1} (C–H bending), 1344 cm^{-1} (C–H wagging), and 1110 cm^{-1} (C–O–C stretching, ether).

Guar gum displayed bands at 3417 cm^{-1} (O–H stretching, broad), 2925 cm^{-1} (C–H stretching), 1647 cm^{-1} (O–H bending, adsorbed water), 1420 cm^{-1} (C–H bending), and 1023 cm^{-1} (C–O–C stretching, glycosidic linkage). **Polyvinyl alcohol** showed characteristic bands at 3200–3550 cm^{-1} (O–H stretching, broad), 2920 cm^{-1} (C–H stretching), 1710 cm^{-1} (C=O stretching, residual acetate group), 1430 cm^{-1} (C–H bending), and 1093 cm^{-1} (C–O stretching). **Boric acid** exhibited peaks at 3230 cm^{-1} (O–H stretching), 1450 and 1190 cm^{-1} (B–O stretching), and 880 cm^{-1} (B–O–H bending). The detail is shown in Table:2 display the various FTIR peaks of excipients.

Table: 2 *display the various FTIR peaks of excipients.*

Gelucire 50/13	2920, 2851, 1735, 1467, 1177	C–H, C=O (ester), C–O
Vitamin E	3400, 2924, 2854, 1460, 1080	O–H, C–H, C–O–C
Pluronic F-68	3440, 2880, 1467, 1344, 1110	O–H, C–H, C–O–C
Guar gum	3417, 2925, 1647, 1420, 1023	O–H, C–H, C–O–C (glycosidic)
PVA	3200–3550, 2920, 1710, 1430, 1093	O–H, C–H, C=O (acetate), C–O
Boric acid	3230, 1450, 1190, 880	O–H, B–O, B–O–H

3. Results and Discussion

3.1. Findings of Placebo SLN Optimization

3.1.1. PDI, Particle size and Zeta Potential

The placebo SLN formulations (F1–F18) were prepared as per the experimental design. These formulations were analyzed for appropriate particle size, polydispersity index (PDI) and zeta potential using dynamic light scattering (DLS). The Z-average particle size was between 152.3 ± 6.4 and 218.7 ± 10.3 nm while the PDI was between 0.162 ± 0.018 and 0.314 ± 0.029. The details description of Z-diameter is given in Figure.2.

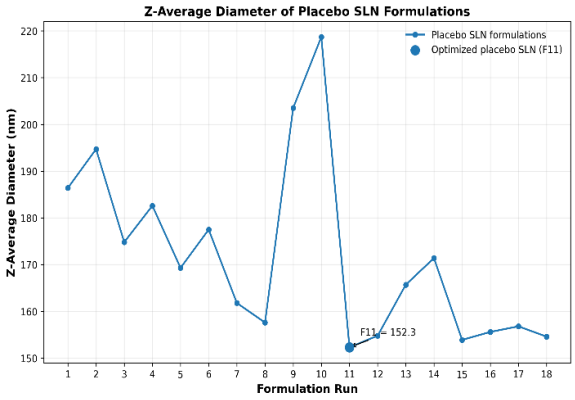


Figure.2 Schematic representation of the detailed preparation Z-average diameter of placebo solid lipid nanoparticles (SLN).

The PDI values of the formulations indicated that they had a comparably narrow particle-size distribution. Out of the 18 formulations, F11 was identified as the most suitable formulation on the basis of lowest Z-average particle size (152.3 ± 6.4 nm) and lowest PDI (0.162 ± 0.018). The formulation also exhibited a zeta potential of −33.8 ± 1.6 mV, which indicates adequate surface charge and good electrostatic stabilization of the dispersion of nanoparticles. Thus, considering minimum particle size, low PDI and suitable zeta potential, optimized placebo SLN formulation (F-Opt-P) was selected as F11 formulation. The detail result is described in Table.3.

Table.3 *Depicts the Z-Average diameter (nm), PDI and Zeta Potential (mV)*

S.no.	Run	Formulation Code	Z-Average Diameter(nm)	PDI	Zeta Potential(mV)
1.	1.	F1	186.4 ± 7.2	0.241 ± 0.021	−22.6 ± 1.8
2.	2.	F2	194.7 ± 8.1	0.268 ± 0.024	−21.4 ± 1.7
3.	3.	F3	174.8 ± 6.9	0.218 ± 0.01	−25.8 ± 1.9
4.	4.	F4	182.6 ± 7.5	0.232 ± 0.022	−24.1 ± 1.8
5.	5.	F5	169.3 ± 6.4	0.201 ± 0.018	−27.3 ± 1.6
6.	6.	F6	177.5 ± 7.1	0.214 ± 0.020	−26.2 ± 1.7
7.	7.	F7	161.8 ± 5.8	0.181 ± 0.017	−30.1 ± 1.5
8.	8.	F8	157.6 ± 6.1	0.173 ± 0.016	−31.4 ± 1.6
9.	9.	F9	203.5 ± 9.2	0.286 ± 0.026	−20.8 ± 1.9
10.	10.	F10	218.7 ± 10.3	0.314 ± 0.029	−19.6 ± 2.0
11.	11.	F11	152.3 ± 6.4	0.162 ± 0.018	−33.8 ± 1.6
12.	12.	F12	154.8 ± 6.7	0.168 ± 0.017	−32.6 ± 1.5

13.	13.	F13	165.7 ± 7.0	0.193 ± 0.019	−29.4 ± 1.7
14.	14.	F14	171.4 ± 7.3	0.207 ± 0.020	−28.2 ± 1.6
15.	15.	F15	153.9 ± 6.2	0.165 ± 0.016	−33.1 ± 1.5
16.	16.	F16	155.6 ± 6.5	0.169 ± 0.017	−32.8 ± 1.6
17.	17.	F17	156.8 ± 6.3	0.171 ± 0.018	−32.4 ± 1.7
18.	18.	F18	154.6 ± 6.6	0.166 ± 0.017	−33.0 ± 1.5

3.2 Turbidity Study of Solid Lipid Nanoparticles

The first assessment of the physical characteristics of the 18 placebo SLN formulations was based on their visual appearance. Each formulation was taken into separately labelled eppendorf tubes of 0.5 mL and allowed to stand for 5 min. The samples were subsequently examined visually using light to detect the presence of turbidity. Table X indicates that formulas have clear, not turbid appearance or turbid appearance. The 18 formulations were tested for the appearance, with 8 formulations (Runs 1, 3, 5, 7, 10, 12, 14 and 15) being translucent and non-turbid. The 10 formulations (Runs 2, 4, 6, 8, 9, 11, 13, 16, 17 and 18) were found to be visibly turbid. When formulations appear clear, it means there is no dispersion state incompatibility between the lipid and surfactant components; however, when the formulation appears turbid, this suggests that there is a dispersion state incompatibility. The experimental formulation Run 10 comprising a concentration of 23.40% lipid, a concentration of 3.68% w/v Pluronic F68 and a combination of Vitamin E: Gelucire® 50/13 in the ratio of 50:50 was found to be translucent. Nonetheless, only visual appearance was selected as a preliminary qualitative screening parameter, while selection of the optimized placebo SLN was based on quantitative characterization including particle size, PDI and zeta potential. The result of turbidity study is given in Table.4.

Table 4 *– Represents the turbidity study for Solid lipid nanoparticles*

S.N.	Run	Lipid concentration	Pluronic acid 68 (%w/v)	Vitamin E: Gelucire 50/13 (%w/w)	Turbid
1.	1	10	1	25	Not turbid (translucent)
2.	2	10	1	75	Turbid
3.	3	10	3	25	Not turbid (translucent)
4.	4	10	3	75	Turbid
5.	5	20	1	25	Not turbid (translucent)
6.	6	20	1	75	Turbid
7.	7	20	3	25	Not turbid (translucent)
8.	8	20	2	50	Turbid
9.	9	6.59	0.31	50	Turbid
10.	10	23.40	3.68	50	Not turbid (translucent)

11.	11	15	2	50	Turbid
12.	12	15	2	50	Not turbid (transluce nt)
13.	13	15	2	7.95	Turbid
14.	14	15	2	92.04	Not turbid (transluce nt)
15.	15	15	2	50	Not turbid (transluce nt)
16.	16	15	2	50	Turbid
17.	17	15	2	50	Turbid
18.	18	15	2	50	Turbid

3.3. Morphological Analysis by Transmission Electron Microscopy (TEM)

TEM analysis of the blended SLN-hydrogel formulation revealed the SLNs embedded within and distributed throughout the three-dimensional hydrogel network. The hydrogel matrix appeared as an electron-lucent, porous meshwork with interconnected channels, consistent with the cross-linked guar gum–PVA architecture formed through boric acid–mediated boronate ester linkages. The SLNs were visualized as discrete, electron-dense spherical particles (approximately 200–270 nm) distributed uniformly within the gel pores and along the polymer fibrils. The particle size of the SLNs within the hydrogel matrix was marginally larger than that observed in the free SLN dispersion, which corroborates the DLS data (234.6 ± 12.4 nm) and is consistent with swelling of the lipid core during hydrogel preparation.

The hydrogel mesh size appeared to range from approximately 50 nm to 500 nm in cross-sectional view, providing sufficient porosity for drug diffusion from the embedded SLNs through the gel matrix to the external environment. No evidence of SLN aggregation, coalescence, or disruption was observed within the hydrogel, confirming the structural integrity of the SLNs following their incorporation into the gel network. The uniform distribution of SLNs within the hydrogel matrix is a critical quality attribute for ensuring homogeneous drug delivery across the wound surface upon application. Detailed illustration Transmission Electron Microscopy of nanoformulation is given in Figure.2.

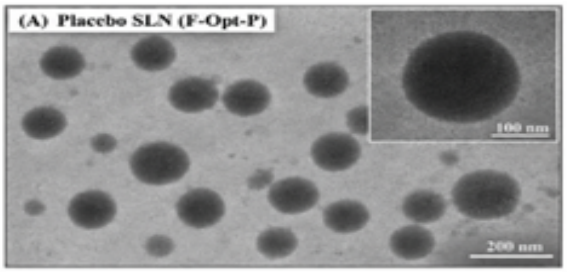


Figure.3 Schematic representation of Transmission Electron Microscopy of nanoformulation.

3.4. Compatibility Assessment of Formulated Systems

The FTIR spectra of the drug-loaded formulations were identify any evidence of excipient-excipient interactions.

Placebo SLN (F-Opt-P**):** The spectrum displayed the characteristic C=O ester stretch of Gelucire 50/13 at 1734 cm^{-1}, C–H aliphatic stretches at 2918 and 2850 cm^{-1}, and the C–O–C ether stretch of Pluronic F-68 at 1108 cm^{-1}. No anomalous peaks were observed, confirming the absence of chemical interactions among the lipid matrix components and surfactant in the absence of drug. The peaks of FTIR spectra is given in Figure.4.

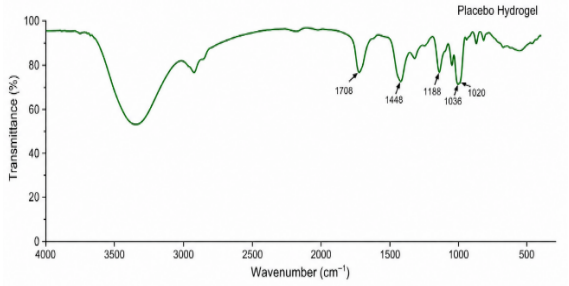


Figure.4 FTIR Absorption Peaks of Placebo-Loaded Formulations Compared with Pure Drugs.

4. Conclusion

In this study, placebo solid lipid nanoparticle (SLN) formulation was successfully established and optimized as a preliminary nanocarrier platform for preparing drug-loaded SLN intended for local topical delivery for chronic wound management. The formulation of SLN was done by using Gelucire® 50/13, Gelot®, vitamin E and Pluronic® F68 as the main formulation components. The optimized

placebo SLN demonstrated suitable nanoscale properties, somewhat narrow particle-size distribution and an appropriate negative zeta potential, which showed that it had good dispersion properties and colloidal stability. The Central Composite Design was systematically applied for evaluating and optimizing the chosen formulation variables which allowed the researcher to rationally select suitable formulation conditions. Also, the preliminary visual examination showed the selected formulation to be homogeneous, translucent, and free from any evident macroscopic aggregation. FTIR analysis of the SLN chosen components and their physical mixture shown no significant chemical incompatibility indicating that the selected excipients are suitable for further formulation development. In general, the optimized placebo SLN exhibited suitable attributes in terms of physicochemical, colloidal and compatibility aspects, conveying a reliable and reproducible nanocarrier platform. According to the results, a rational basis is established for the future addition of the therapeutic agent and preparation of the related drug-loaded SLN formulation for localized and sustained topical delivery in chronic wound treatment.

5. References

1. Falanga V, Isseroff RR, Soulika AM, Romanelli M, Margolis D, Kapp S, Granick M, Harding K. Chronic wounds. Nature reviews Disease primers. 2022 Jul 21;8(1):50.

2. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Advances in wound care. 2015 Sep;4(9):560-82.

3. Bowers S, Franco E. Chronic wounds: evaluation and management. American family physician. 2020 Feb 1;101(3):159-66.

4. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. International journal of molecular sciences. 2016 Dec 11;17(12):2085.

5. Garud A, Singh D, Garud N. Solid lipid nanoparticles (SLN): method, characterization and applications. International Current Pharmaceutical Journal. 2012 Oct 3;1(11):384-93.

6. Üner M, Yener G. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. International journal of nanomedicine. 2007 Dec 1;2(3):289-300.

7. Severino P, Andreani T, Macedo AS, Fangueiro JF, Santana MH, Silva AM, Souto EB. Current state-of-art and new trends on lipid nanoparticles (SLN and NLC) for oral drug delivery. Journal of drug delivery. 2012;2012(1):750891.

8. Dingler A, Gohla S. Production of solid lipid nanoparticles (SLN): scaling up feasibilities. Journal of microencapsulation. 2002 Jan 1;19(1):11-6.

9. Tantra R, Schulze P, Quincey P. Effect of nanoparticle concentration on zeta-potential measurement results and reproducibility. Particuology. 2010 Jun 1;8(3):279-85.

10 Karmakar SA. Particle size distribution and zeta potential based on dy

namic light scattering: Techniques to characterize stability and surface charge distribution of charged colloids. Recent Trends Mater. Phys. Chem. 2019;28:117-59.

11. Bhattacharjee S. DLS and zeta potential–what they are and what they are not?. Journal of controlled release. 2016 Aug 10;235:337-51.

12. Ghelich R, Jahannama MR, Abdizadeh H, Torknik FS, Vaezi MR. Central composite design (CCD)-Response surface methodology (RSM) of effective electrospinning parameters on PVP-B-Hf hybrid nanofibrous composites for synthesis of HfB2-based composite nanofibers. Composites Part B: Engineering. 2019 Jun 1;166:527-41.

13. Saeed MO, Azizli K, Isa MH, Bashir MJ. Application of CCD in RSM to obtain optimize treatment of POME using Fenton oxidation process. Journal of water process engineering. 2015 Dec 1;8:e7-16.

14. Jose S, Anju SS, Cinu TA, Aleykutty NA, Thomas S, Souto EB. In vivo pharmacokinetics and biodistribution of resveratrol-loaded solid lipid nanoparticles for brain delivery. International journal of pharmaceutics. 2014 Oct 20;474 (1-2):6-13.

15. Akash MS, Rehman K. Ultraviolet-visible (UV-VIS) spectroscopy. InEssentials of pharmaceutical analysis 2019 Dec 18 (pp. 29-56). Singapore: Springer Nature Singapore.

16. Lunardi CN, Gomes AJ, Rocha FS, De Tommaso J, Patience GS. Experimental methods in chemical engineering: Zeta potential. The Canadian Journal of Chemical Engineering. 2021 Mar;99(3):627-39.

17. Nunes CA, Freitas MP, Pinheiro AC, Bastos SC. Chemoface: a novel free user-friendly interface for chemometrics. Journal of the Brazilian Chemical Society. 2012;23:2003-10.

18. Anwar W, Dawaba HM, Afouna MI, Samy AM. Screening study for formulation variables in preparation and characterization of candesartan cilexetil loaded nanostructured lipid carriers. Universal Journal of Pharmaceutical Research. 2019.

19. Gao Y, Chen L, Li L, Chi H, Teng F. High-pressure homogenization assisted pH-shifting modified soybean lipophilic protein interacting with chitosan hydrochloride: Double emulsion construction, physicochemical properties, stability, and in vitro digestion analysis. Food Hydrocolloids. 2025 Mar 1;160:110834.

20. Nunes CA, Freitas MP, Pinheiro AC, Bastos SC. Chemoface: a novel free user-friendly interface for

chemometrics. Journal of the Brazilian Chemical Society. 2012;23:2003-10.
21. Nobbmann U, Connah M, Fish B, Varley P, Gee C, Mulot S, Chen J, Zhou L, Lu Y, Sheng F, Yi J. Dynamic light scattering as a relative tool for assessing the molecular integrity and stability of monoclonal antibodies. Biotechnology and genetic engineering reviews. 2007 Jan 1;24(1):117-28.
22. Peters ER, Joseph SA, Garety PA. Measurement of delusional ideation in the normal population: introducing the PDI (Peters et al. Delusions Inventory). Schizophrenia bulletin. 1999;25(3):553.
23. Németh Z, Csóka I, Semnani Jazani R, Sipos B, Haspel H, Kozma G, Kónya Z, Dobó DG. Quality by design-driven zeta potential optimisation study of liposomes with charge imparting membrane additives. Pharmaceutics. 2022 Aug 26 ;14(9):1798.
24. Clogston JD, Patri AK. Zeta potential measurement. InCharacterization of nanoparticles intended for drug delivery 2010 Oct 19 (pp. 63-70). Totowa, NJ: Humana press.
25. Barbosa JA, Abdelsadig MS, Conway BR, Merchant HA. Using zeta potential to study the ionisation behaviour of polymers employed in modified-release dosage forms and estimating their pKa. International journal of pharmaceutics: X. 2019 Dec 1;1:100024.
26. Alhelal HM, Mehta S, Kadian V, Kakkar V, Tanwar H, Rao R, Aldhubiab B, Sreeharsha N, Shinu P, Nair AB. Solid lipid nanoparticles embedded hydrogels as a promising carrier for retarding irritation of leflunomide. Gels. 2023 Jul 14;9(7):576.
27. Rao DS, Muraleedharan K, Humphreys CJ. TEM specimen preparation techniques. Microscopy: science, technology, applications and education. 2010;2:1232.
28 Magalhaes S, Goodfellow BJ, Nunes A. FTIR spectroscopy in biomedical research: How to get the most out of its potential. Applied Spectroscopy Reviews. 2021 Nov 26;56(8-10):869-907.
29**.** Balan V, Mihai CT, Cojocaru FD, Uritu CM, Dodi G, Botezat D, Gardikiotis I. Vibrational spectroscopy fingerprinting in medicine: from molecular to clinical practice. Materials. 2019 Sep 6;12(18):2884.