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Original Article

Development and Characterisation of Salicylic Acid and Benzoyl Peroxide Dual Drug-Loaded SLN for Acne: QbD-FMEA Optimisation Studies

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ABSTRACT

Background: The present study primarily aims to explore the synergistic potential of Salicylic Acid (SA) and Benzoyl Peroxide (BP) loaded Solid Lipid Nanoparticles (SLN) for treating acne. **Materials and Methods:** SA-BP SLNs were prepared using high-shear homogenisation method. Risk analysis was performed to identify the critical parameters that may impact the results and the SLNs were optimised through the Box-Behnken design. The lipid concentration, surfactant concentration, and homogenisation speed were considered as independent variables and particle size, zeta potential, Polydispersity Index (PDI) and % Entrapment Efficiency (EE) of SA and BP were considered response variables. The SA-BP SLNs were characterised and evaluated for drug content, release kinetics and stability. **Results**: The optimised SLN showed a particle size of 133 nm with a very narrow PDI of 0.17 and a zeta potential of -23.3 mV. The SA-BP SLN showed a high entrapment efficiency (>85%), sustained release and spherical surface particles. **Conclusion:** This study reported a dual drug-loaded SLN containing SA and BP as a promising candidate for acne treatment.

Keywords: Acne**,** Benzoyl peroxide, Box-Behnken design, Design of experiments, Salicylic acid, Solid lipid nanoparticles.

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INTRODUCTION

Acne vulgaris is a major problem that every teenager faces during their adolescence. It can occur in people of any age but is most common between 18 and 24 years old with an occurrence rate of 80%.[1] The oil glands and follicular hair present on the skin (pilosebaceous unit) cause scars and lesions on the face.[2] These consequences cause decreased self-confidence leading to substantial stress and psychological disturbances.[3] The guidelines on acne vulgaris by the American Academy of Dermatology state that acne vulgaris can be classified as severe, moderate or mild based on the complications.[4,5] To overcome these problems, teenagers usually get their treatment started from first-line medications, usually topical antibacterials/antimicrobials, owing to their local effects.[6] Benzoyl peroxide, retinoids, salicylic acid, azelaic acid and antibiotics such as clindamycin, tetracycline, minocycline and doxycycline were used either individually or in combinations.[7]

Benzoyl Peroxide (BP) is an antibacterial agent with comedolytic effects against *Propionibacterium acnes* and acts as a mild anti-inflammatory agent.[8] Usually, Benzoyl peroxide is the first choice for treating acne in the concentration range of 2.5-10% in combination with other agents like adapalene (0.1-0.3%), azelaic acid (1%) and clindamycin (1%).[9] It exerts its action by entering the skin and breaking down into active metabolites and the major advantage of BP is its high efficiency in reducing acne lesions.[10] Salicylic Acid (SA) is a hydroxy derivative of benzoic acid. The keratolytic and comedolytic actions of SA make it suitable for treating acne.[11] However, the irritation on the skin and lack of immediate effect make topical treatments cause very low adherence.[12] In addition, the possibility of resistance formation by the pathogen is high when BP and SA are administered individually.[13] Encapsulating both drugs into lipid-based drug delivery methods using nanocarriers can overcome skin irritation and resistance to pathogens.[3] The synergistic activity of benzoyl peroxide's antibacterial action complements salicylic acid's keratolytic and anti-inflammatory properties.[14] Balanced treatment can be ensured at lower concentrations of each, potentially reducing the risk of side effects while maintaining efficacy.[15]

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Solid Lipid Nanoparticles (SLN) possess various advantages such as biodegradability, stability, biocompatibility and enhanced skin permeability, making them a good choice of nanocarrier for topical drug delivery systems.[6] The reduced particle size in SLN enhances the onset of action rapidly, compared to drugs incorporated in free form.[16] The incorporation of SLN into gel-based formulations provides prolonged drug release and retention with minimal application.[17] In general, SLN's are the most desirable drug carriers for drugs classified under BCS classification III with low permeability and solubility.[18] Critical parameters that may affect the product are optimised using the Quality by Design (QbD) framework, which facilitates achieving a high-quality product.[19] QbD mainly focuses on Designing the Experiments (DoE) and analysing the risks that may occur during the experiments. The International Conference on Harmonization (ICH) has laid down quality guidelines that mainly discuss pharmaceutical development through scientific and risk assessments (ICH Q8), risk identification and prevention through quality risk management (ICH Q9) and ensuring the product quality through stringent pharmaceutical quality systems (ICH Q10).[20]

The One-Factor-At-a-Time (OFAT) approach is traditionally used for drug development and optimisation, which may be a major drawback that hinders the potential of exploring other factors.[21] DoE is a principal factor in pharmaceutical development and helps in overcoming the limitations of OFAT through screening and design optimisations. The initial risk assessment outlines the critical quality attributes that affect the Target Product Profile (TPP). QbD defines the final product expectations through the Quality Target Product Profile (QTPP), which plays a central role in the development of a drug. It is postulated based on factors such as dosage form, delivery system, route of administration, strength of dosage, safety and ADME studies. Final product quality is ensured by establishing the Critical Quality Attributes (CQA) through monitoring of Critical Method Parameters (CMP) and Critical Process Parameters (CPP).[22]

An extensive literature survey revealed that no studies have reported the use of salicylic acid and benzoyl peroxide together in nanoparticles. The current study focuses on developing a dual-drug-loaded SLN of Benzoyl peroxide and salicylic acid by applying risk assessment and DoE for screening and optimisation of experiments and evaluation of SLN.

MATERIALS AND METHODS

Materials

Salicylic Acid (SA) was purchased from Shoolin Pharma Chem (Gujarat, India) and Benzoyl Peroxide (BP) was purchased from Anmol Chemicals (Mumbai, India). The solid lipid used was Glyceryl Monostearate (GMS), and the surfactants used were Tween-80, Tween-20 which were received as gift samples from Esdab Medicare Pvt. Ltd., (Dindigul, India). The water used for preparation and instrumental analysis was sourced from Sisco, Chennai, India.

Methods

Quality by Design

Since the formulation is based on dual-drug-loaded SLN, applying the QbD approach will establish a superior understanding of the formulation.[23] QbD approach helps in the implementation of quality, safety and efficacious products for stakeholders.[24] It identifies the quality Target Product Profile (QTTP) as an initial step, followed by factors that may critically affect QTTP, such as Critical Quality Attributes (CQA), Critical Process Parameters (CPP) and critical material attributes (CMA).[25] A high-quality product may result from a QbD approach, which is cost-effective and time-efficient.[26]

Defining QTTP and CQA

The preliminary pathway for QbD starts by defining the Quality Target Product Profile (QTPP) to make sure that the medication reaches its objective to which it was developed. ICH Q8 guidelines lay down the criteria for identifying the QTTP based on physical characteristics and qualitative and quantitative parameters such as stability, release kinetics, particle size and Polydispersity Index (PI) for a high QSE of SA-BP SLN.[27,28]

Initial Quality Risk Assessment

An initial risk assessment was performed to evaluate actions of the variables like materials and processes involved in the formation of lipid nanoparticles. Based on the knowledge gained from previous literature and theoretical knowledge the initial risk assessment was performed by plotting the Ishikawa graph. Risk assessment was performed using Failure Mode and Effect Analysis (FMEA) in accordance with ICH Q9 quality risk management principles. The cause-and-effect relationship between the process and formulation variables is likely to influence the CQAs of drug products. The therapeutic efficiency of the drug product in nanoparticles is influenced by particle size, entrapment efficiency, zeta potential, drug release and polydispersity index.[29] The effects of CMPs such as Lipid concentration, surfactant concentration, solvent volume, drug concentration, and other excipients were studied. Furthermore, Critical Process Parameters (CPPs), such as the effect of homogenisation speed, homogenisation time,

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temperature, and solubility, were assessed. The Risk Priority Number (RPN) for each CPP regarding a specific CQA was calculated using the equation RPN=Severity x Occurrence x Detection. A standardised 3-point scale (1=Low, 2=Medium, 3=High) was utilised to assign scores for severity, occurrence and detection. These factors had a significant impact on the 8 distinct Critical Quality Attributes (CQAs), and the inter-relationship of CMAs and CPPs for SLN preparation was finally represented at three levels of Risk Assessment Matrix (RAM), that is high, medium, and low. The Risk Assessment Matrix (RAM) and final risk assessment by summing all RPNs were interpreted using Pareto charts.[30]

Design of Experiments

Practical design of experiments was performed using the statistical tool of response surface modelling called Box-Behnken Design (BBD) using JMP® Student Edition 19.0.1 software. For optimising Solid Lipid Nanoparticle (SLN) development, BBD is preferred because of its efficiency in reducing the number of experiments required to assess potential interactions.[31] Based on the results of the risk assessment matrix, concentration of lipids, surfactant concentration, and solubility were identified as Critical Method Attributes (CMA), and the homogenisation time and temperature were confirmed as Critical Process Parameters (CPP).[20] The response of the CMA and CPPs was evaluated with respect to a dependent variable known as Critical Quality Attributes (CQA), which were identified as particle size, Polydispersity Index (PDI), entrapment efficiency, and zeta potential for SA-BP SLN. A total of 15 experimental runs were designed and conducted at three levels: -1, 0, and +1 as shown in Table 4.

Preparation of SA-BP SLN

Solid lipid nanoparticles of dual-drug-loaded benzoyl peroxide and salicylic acid were prepared using high shear homogenisation in succession with the sonication method. Salicylic acid (0.5% w/v) and benzoyl peroxide (2.5% w/v) were added to Glyceryl Monostearate as a solid lipid. The above mixture was melted at a controlled temperature of 85°C and completely mixed at 500 rpm until the drugs completely dissolved. An aqueous phase containing 0.5-1.5% w/v of surfactant Tween 80 was prepared and kept in a temperature-controlled water bath at 85°C. Furthermore, while maintaining a constant temperature, the aqueous surfactant solution was introduced into the drug-lipid mixture. The mixture was then introduced into a high-shear homogeniser (BI-30K homogeniser, BR Biochem Lifesciences Pvt. Ltd., New Delhi, India) for 15 min at 10000-12000 rpm. Subsequently, the homogenised mixture was sonicated using a probe sonicator (KLPRO-750, King Lab, India) at 15 kHz frequency and 500 W power for 10 min at a controlled temperature of 30°C. The resultant emulsion was freeze-dried using a lyophiliser (Delvac Lyodel-LYO0555) by adding 5%w/v mannitol as a cryoprotectant for 6 h to obtain a free-flowing powder and stored at room temperature in an airtight container until further use.

Characterisation of SA&BP SLN

Particle size, zeta potential and polydispersity index

A smaller particle size is required for better penetration of SLN across the skin barrier, whereas a narrow size distribution (minimal PDI) is needed for more constant skin permeation.[32] Using a particle size analyser, the dynamic light scattering technique was utilised to measure the particle size and polydispersity index of SA-BP loaded SLNs (Nano ZS, Malvern Instruments Ltd.,). Optimal light scattering can be achieved by diluting the samples with double-distilled water before analysis. The measurements were performed in triplicate at 25°C at an angle of 90°. The zeta potential of the optimised SLN was measured using the same instrument to determine the charges present on the surface of the sample. The measurements were performed in triplicate and are represented as mean±Standard Deviation (SD).

%Entrapment Efficiency (%EE)

%EE was calculated by centrifuging the optimised SA-BP SLNs was determined by direct method using a cooling centrifuge for 15 min at 10000 RPM to separate entrapped and unentrapped drugs. The supernatant was discarded, and the isolated SLN pellet was completely dissolved in a solvent mixture of methanol and chloroform to lyse the lipid matrix and extract the entrapped drugs. The resulting solution was then analysed using a double-beam UV-visible spectrophotometer (Shimadzu® 1700) at 238 nm. The %EE was calculated using the following equation:

$$\% \text{ EE} = \frac{\text{Amount of drug in pellet}}{\text{Total Amount of drug}} \times 100$$

SEM Analysis

The shape and surface morphology of the optimised dual drug-loaded SLN were analysed using a Scanning Electron Microscope (SEM) (Thermo scientific Apreo S). Sample preparation was carried out by placing the samples onto adhesive carbon tape (Nisshin Em. Co. Ltd., Tokyo, Japan) and then coated with gold. The detector used in this study was InLens. The system was operated with a probe current of 200 nA and Electron High Tension (EHT) of 5 kV.

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Table 1: Quality Target Product Profile (QTPP) of SA-BP SLN.

Quality Target Product Profile (QTPP) Element	Target	Justification
Dosage Form	Solid Lipid Nanoparticle (SLN)	As the salicylic acid and Benzoyl peroxide are poorly water soluble and cause irritation to the skin. So, SLNs provide a biocompatible and biodegradable carrier system that enhances drug stability, enables controlled and sustained drug release, improves drug bioavailability, and facilitates targeted delivery, especially for topical applications. Their solid lipid matrix protects labile drugs from degradation and enhances skin penetration.[6]
Dosage Strength	Salicylic Acid 0.5%, Benzoyl Peroxide 2.5%	These concentrations are widely accepted and clinically effective doses for topical acne treatment, balancing efficacy with minimal irritation risk. The SLN formulation supports stable incorporation and controlled release of these doses, optimising therapeutic outcomes.
Therapeutic Effect	Antimicrobial, anti-inflammatory, and keratolytic for acne treatment	Combining Salicylic Acid and Benzoyl Peroxide is expected to target multiple acne pathophysiology aspects: exfoliation of dead skin cells, reduction of inflammation, and eradication of acne-causing bacteria. This multifaceted approach improves treatment efficacy and reduces resistance development.[35]
Drug Content (Salicylic Acid)	High, stable drug loading	High drug loading ensures sufficient therapeutic dose within the carrier, maximising efficacy. Stability within the lipid matrix prevents drug crystallisation or degradation, maintaining consistent release and bioavailability. This is critical to achieving predictable therapeutic outcomes.
Drug Content (Benzoyl Peroxide)	High, stable drug loading	Adequate loading ensures sustained antimicrobial activity and keratolytic effect. Stability within SLN prevents premature degradation of this peroxide-based drug, which is prone to instability, thus maintaining potency during storage and use.[56]
Drug Release	Controlled and sustained release profile	Prolonged therapeutic drug levels are maintained through controlled release, minimising dosing frequency and related side effects. Sustained release also improves patient compliance and enhances treatment outcomes by ensuring continuous drug availability at the target site.
Route of Administration	Topical	Topical delivery provides direct application to affected skin areas, maximising local drug concentration while minimising systemic absorption and related adverse effects. This route is preferred for dermatological conditions like acne.
Stability (Physical and Chemical)	High physical stability and chemical stability	Physical stability ensures maintenance of nanoparticle size, shape, and dispersity, preventing aggregation or sedimentation. Chemical stability preserves drug integrity against degradation pathways such as oxidation or hydrolysis, ensuring consistent efficacy and safety throughout shelf life.[6]
Storage	Store in a cool, dry place, protected from light and moisture	Proper storage conditions prevent degradation of both drugs and maintain SLN integrity. Benzoyl Peroxide is photosensitive and prone to oxidation; thus, protection from light and moisture is critical. Cool storage slows chemical degradation and preserves formulation stability over shelf life.[35]

Differential Scanning Calorimetry

The crystallinity of the pure drugs freeze-dried blank and freeze-dried SA-BP SLN was analysed using a differential scanning calorimeter (HO-TH-OSC, Holmarc Opto-Mechanics Pvt. Ltd., India). For reference, a blank aluminium pan was used, whereas an aluminium pan loaded with 5 mg of drug was tightly sealed using a crimping machine. At 25-350°C with a change in heat range of 10°C was used to record a thermogram. During the analysis a constant inert atmosphere was maintained using nitrogen at a flow rate of 40 mL/min.

Fourier Transform Infrared Spectroscopy (FT-IR)

The functional groups present in the pure drugs, lyophilised dual drug-loaded SLN, and lyophilised unloaded SLN were identified using a Perkin Elmer Spectrum Two FT-IR spectrometer, covering the spectral range of 400-4000 cm^{-1}. The KBr hydraulic pellet technique was applied to each sample using a force range of 4-6 tons, and subsequent spectra were recorded.

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Table 2: CQAs of SA-BP SLN with justifications.

Critical Quality Attribute (CQA)	Target	Justification	Reference
Zeta Potential	±30 mV or higher in magnitude	Ensures colloidal stability by providing sufficient electrostatic repulsion to prevent nanoparticle aggregation, enhancing shelf life and uniformity.	[57]
Polydispersity Index (PDI)	≤0.3	Indicates a narrow particle size distribution, essential for reproducible drug release and consistent therapeutic performance.	[58]
Encapsulation Efficiency (EE%) of Salicylic Acid	≥80%	High EE ensures adequate drug loading, maximising therapeutic efficacy and minimising drug loss during formulation and storage.	[59]
Encapsulation Efficiency (EE%) of Benzoyl Peroxide	≥80%	High EE is critical due to BP instability; encapsulation protects it from degradation and maintains potency.	[60]
pH	4.5 - 6.5	Maintains skin compatibility and formulation stability; avoids irritation and preserves nanoparticle integrity.	[61]
Particle Size	100 - 300 nm	Optimal size range for enhanced skin penetration and controlled drug release, while avoiding rapid clearance or irritation.	[62]
Aggregation	No visible aggregation or sedimentation	Prevents loss of efficacy and ensures uniform dosing; aggregation can alter release profiles and reduce stability.	[63]
Physical Attributes (Shape, Morphology)	Spherical, uniform nanoparticles	Consistent morphology correlates with predictable drug release and stability; irregular shapes may affect performance.	[64]

Table 3: FMEA 3-Point Scoring Scale for Risk Assessment.

Score	Severity (S) Impact on CQA	Occurrence (O) Probability	Detectability (D) Uncertainty
1 (Low)	Minor impact; formulation remains within specifications.	Failure is unlikely to occur.	High detectability; failure is easily identified.
2 (Medium)	Moderate impact; potential to compromise formulation quality.	Failure may occur occasionally.	Moderate detectability; failure might go unnoticed initially.
3 (High)	Critical impact; failure guarantees batch rejection or unsafe product.	Failure is highly likely to occur.	Low detectability; high uncertainty in identifying the failure.

Table 4: Coded and actual levels of independent variables in the BBD for SA-BP-loaded SLN.

Independent Variables (Factors)	Units	Low (-1)	Medium (0)	High (+1)
A: Lipid Concentration	mg	100	150	200
B: Surfactant Concentration	% w/v	0.5	1.0	1.5
C: Homogenisation Speed	rpm	8000	10000	12000

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Table 5: Formulations and responses of SA-BP-loaded SLN suggested by BBD.

Levels	Lipid Conc. (mg)	Surfactant Conc. (%)	Homogenisation speed (rpm)	PDI	Particle size (nm)	Zeta Potential (mV)	EE % of SA (%)	EE % of BP (%)
0+-	150	1.5	8000	0.29	204	-35.6	88.11	84.26
+0-	200	1	8000	0.38	191	-14.3	76.34	73.99
++0	200	1.5	10000	0.24	140	-38.6	85.66	80.43
-0+	100	1	12000	0.19	189	-18.5	84.19	86.57
–0	100	0.5	10000	0.82	181	-18.9	85.91	81.55
-0-	100	1	8000	0.67	217	-20.7	87.2	83.55
-+0	100	1.5	10000	0.21	196	-32.7	75.15	76.23
000	150	1	10000	0.18	152	-21.8	75.43	69.24
000	150	1	10000	0.2	160	-21.4	75.24	71.29
000	150	1	10000	0.27	154	-22.7	73.67	70.38
0-+	150	0.5	12000	0.31	149	-16.5	85.72	88.31
+-0	200	0.5	10000	0.34	143	-15.4	78.27	80.96
0++	150	1.5	12000	0.21	139	-38.2	81.53	82.48
+0+	200	1	12000	0.17	133	-23.3	90.84	88.24
0–	150	0.5	8000	0.6	186	-12.4	83.19	81.91

BP: Benzoyl Peroxide; EE: Entrapment Efficiency; PDI: Polydispersity Index; rpm: rotation per minute;
SA: Salicylic Acid

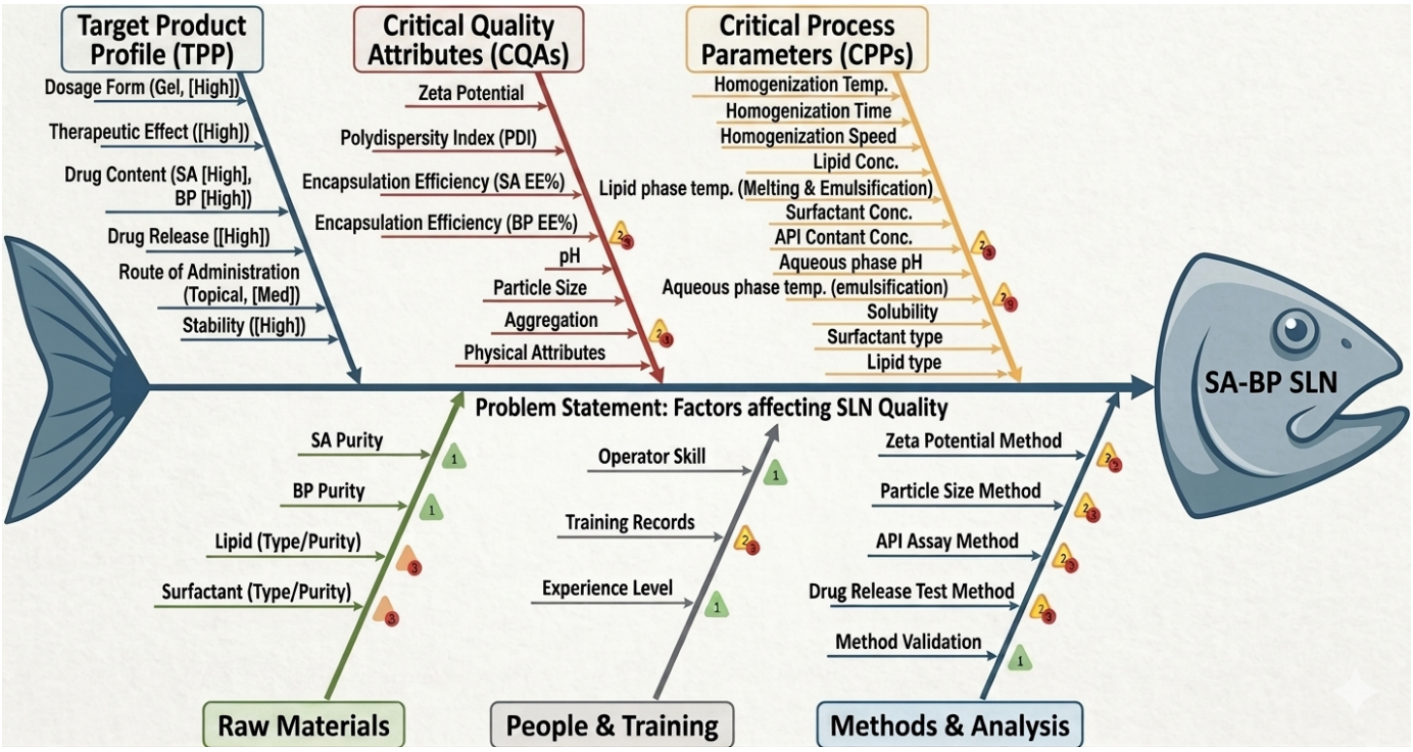


Figure 1: Ishikawa Analysis for cause-and-effect relationship between the CQA and CPP variables of SA-BP SLN.

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X-ray Powder Diffraction Analysis

X-ray diffraction analysis to assess the crystalline properties of individual drugs, lyophilised blank, and lyophilised SA-BP loaded SLN was conducted using an X-ray diffractometer (Rigaku, Japan). The monochromatic X-ray source used was Cu Kα1 (atomic number: 1.5406 ˚A), operating at a voltage of 40 keV and a current of 40 mA. The data collection specifications included an angular range from 3° to 80°, with a step size of 0.02° and a step period of 0.3 s.

In vitro Drug Release Studies

The SA-BP SLN and individual drugs were assessed for *in vitro* drug release using the dialysis bag method (mol. wt. cutoff 12-14 kDa). The dialysis bag was soaked overnight in the release media containing phosphate buffer (pH 7.4). A volume of approximately 30 mL of dissolution medium was introduced into a beaker, containing 2.0 mL of the SLNs enclosed within a dialysis bag. This assembly was subsequently sealed at both ends and maintained at a temperature of 37ºC with a stirring rate of 100 rpm. Fractions of 1 mL were collected from the above at defined time intervals (0.5, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h) and replaced with 1 mL of the same to maintain sink conditions. A 0.22 µm syringe was used to filter the sample, and the filtered sample was analysed using a UV spectrophotometer at 234 nm. The resultant data points were fitted to the zero-order, first-order, Higuchi and Korsmeyer-Peppas models to understand the release kinetics of SA and BP in the SLN. All experiments were performed in triplicate (n=3), and the results were expressed as mean±SD.

Ex vivo Skin Permeation Studies

Ex vivo skin permeation studies were performed using a vertical Franz diffusion cell with an effective diffusion area of 1.76 cm^2 and a receptor compartment volume of 12.0 mL. Fresh porcine ear skin was carefully cleaned, and the underlying fat was removed. The skin was trimmed to a uniform thickness of 1.0 ± 0.1 mm using a surgical scalpel and cleaned with a digital micrometre. Integrity of the skin was assessed via careful visual inspection against light to ensure the absence of pinholes or physical damage prior to mounting. The skin was then mounted between the donor and receptor compartments with the stratum corneum facing the donor compartment. The receptor chamber was filled with phosphate-buffered saline (PBS, pH 7.4) containing 20% (v/v) ethanol to maintain sink conditions and maintained at 37 ± 0.5ºC under continuous magnetic stirring at 600 rpm. The donor compartment received 1 mL of the SA-BP SLN formulation containing 0.5% (w/v) salicylic acid and 2.5% (w/v) benzoyl peroxide. Equivalent concentrations of pristine salicylic acid and benzoyl peroxide suspensions were used as controls. At predetermined intervals (0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 h), 1 mL samples were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh prewarmed receptor medium. A 0.22 µm membrane filter was used to filter the sample and analysed at 238 nm using a UV-Visible spectrophotometer. The cumulative amount of drug permeated per unit area (µg/cm^2) was calculated. All experiments were performed in triplicate (n=3) using three independent Franz diffusion cells and skin samples, and the results were expressed as mean±SD. The graphical plotting and data visualisations were done using OriginPro software (Version 2026, Origin Lab Corporation, Northampton, MA, USA).

Stability Studies

The optimised SA-BP SLN formulation was kept in a 10 mL sealed glass vial and stored it under distinct environmental conditions using a refrigerator at 4ºC, at room temperature (25±2ºC) and in a hot air oven (37±2ºC) for 2 months to assess its stability. The stability was assessed over a period of 2 months at predetermined intervals of 0, 1 and 2 months for colour, physical state, pH, PDI, particle size and zeta potential. All measurements were performed in triplicate (n=3) and reported as mean±SD.

Statistical Analysis

Statistical data analysis of the obtained results was performed using JMP® Student Edition 19.0.1 (JMP Statistical Discovery LLC, New Carolina, USA) and Microsoft Excel® (Microsoft Office Professional Plus 2010, Microsoft Excel 15.0.5023.100, Microsoft Corporation, Washington, USA).

RESULTS AND DISCUSSION

Describing QTTP and CQAs

The primary step for a QbD based formulation is the description of the QTTP and CQA. Since our study deals with a dual-drug-loaded SLN formulation (SA-BP SLN), QTTP and CQA were implemented through a rigorous paradigm for effective, stable and patient-friendly topical acne therapy. The poor water solubility and skin irritation of both SA and BP were addressed using QTTP, supporting the use of SLNs as the dosage form. Drug stability is enhanced by enabling controlled and sustained release through a biocompatible and biodegradable lipid matrix of SLNs.[33] Clinically validated doses (0.5% SA, 2.5%BP) minimised the irritation for stable drug incorporation and release within the SLN system. Patient adherence is improved by controlled release to maintain therapeutic levels and reduce dosage frequency and side effects. The combination of both drugs offers the potential for synergistic therapeutic action, which can improve efficacy and reduce the risk of resistance.[34] The particle size, shape and dispersion of particles are considered the main characteristics of SLN's and to maintain this, the stability of the formulation should be carefully observed. The stability of BP should be overseen to prevent its degradation because of its photosensitive nature and the QTTP suggested for cool, dry, light-protected conditions

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Table 6: ANOVA prediction of CQAs depicted in Box-Behnken design.

CQA	Source	Sum of square	Degrees of freedom	Mean square	F-Value	*p* Value
PDI	Model	0.5620	9	0.0624	11.48	0.0076 (Significant)
	Error	0.0271	5	0.0054		
	Total corrected	0.5892	14			
	Lack of fit	0.0227	3	0.0075	3.39	0.2360 (Not Significant)
	Pure error	0.0044	2	0.0022		
Particle Size	Model	10694.067	9	1188.23	110.70	<0.0001 (Significant)
	Error	53.667	5	10.73		
	Total corrected	10747.733	14			
	Lack of fit	19.00	3	6.33	0.3654	0.7893 (Not Significant)
	Pure error	34.66	2	17.33		
Zeta Potential	Model	1048.83	9	116.537	55.00	0.0002 (Significant)
	Error	10.5942	5	2.119		
	Total corrected	1059.4293	14			
	Lack of fit	9.7075	3	3.2358	7.29	0.1229 (Not Significant)
	Pure error	0.8866	2	0.4433		
% EE of Salicylic Acid	Model	401.3354	9	44.5928	5.5162	0.0373 (Significant)
	Error	40.4197	5	8.0839		
	Total corrected	441.7551	14			
	Lack of fit	38.5535	3	12.85	13.7726	0.0685 (Not Significant)
	Pure error	1.8662	2	0.9331		
% EE of Benzoyl Peroxide	Model	519.3242	9	57.7027	7.3327	0.0205 (Significant)
	Error	39.3462	5	7.8692		
	Total corrected	558.6704	14			
	Lack of fit	37.2316	3	12.4121	11.7646	0.0794 (Not Significant)
	Pure error	2.1100	2	1.0550		

Note: $p<0.05$ was considered a minimal level of significance.
CQAs: critical quality attributes; PDI: poly dispersity index; %EE: percentage entrapment efficiency

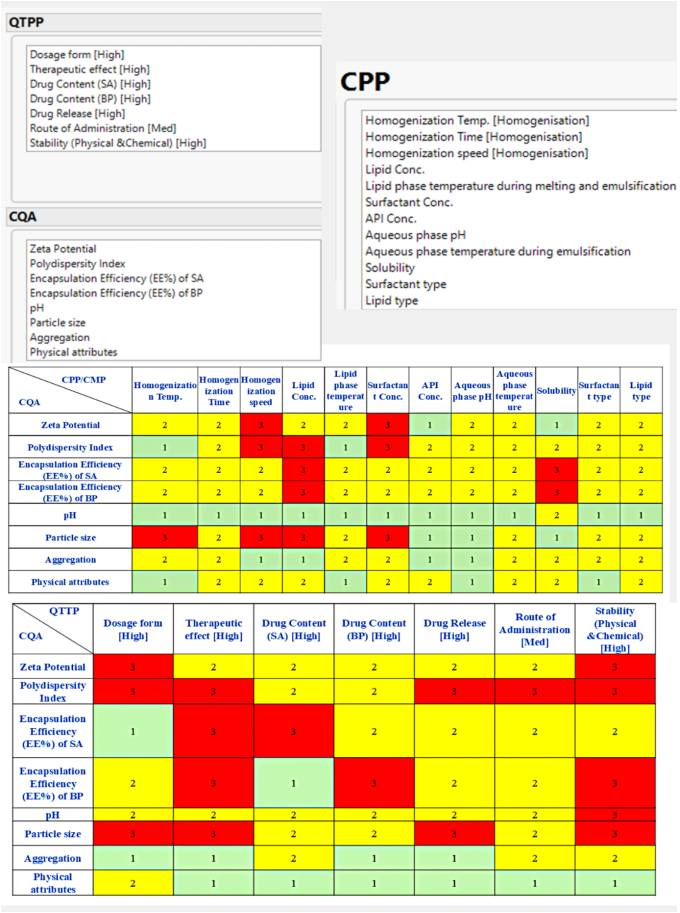
for long-term stability.[35] The QTTPs are listed in Table 1 with its targets and justifications.

CQAs are quantifiable parameters that must be determined based on the QTTP for consistent and quality SA-BP SLN. The identified CQAs affecting the SLNs are Zeta Potential (ZP), polydispersity index (PDI), Encapsulation Efficiency (%EE), pH, particle size, aggregation and physical attributes.[29] All CQAs are presented in Table 2 with targets and justifications. Furthermore, the CMP/CPPs that directly affect CQAs are identified and subjected to risk analysis.

Results of Initial Quality Risk Assessments

An initial risk assessment was conducted based on a literature survey and conceptualization by Ishikawa analysis (fishbone diagram Figure 1) to identify the probable interdependence of methods and processes on the quality of the SLN containing salicylic acid and benzoyl peroxide. Based on this further risk estimation was performed using JMP® Student Edition 19.0.1. Failure Mode and Effects Analysis (FMEA) assessed the overall risks associated with the material and process parameters based on the scores of the Risk Priority Number (RPN), as shown in Figure 2. The 3-point FMEA scoring scale is shown in Table 3. A score of 1 (low) is assigned when the impact on the

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CPP/CMP / CQA	Homogenization Temp.	Homogenization Time	Homogenization speed	Lipid Conc.	Lipid phase temperature	Surfactant Conc.	API Conc.	Aqueous phase pH	Aqueous phase temperature	Solubility	Surfactant type	Lipid type
Zeta Potential	2	2	3	2	2	3	1	2	2	1	2	2
Polydispersity Index	1	2	3	3	1	3	2	2	2	2	2	2
Encapsulation Efficiency (EE%) of SA	2	2	2	3	2	2	2	2	2	3	2	2
Encapsulation Efficiency (EE%) of BP	2	2	2	3	2	2	2	2	2	3	2	2
pH	1	1	1	1	1	1	1	1	1	2	1	1
Particle size	3	2	3	3	2	3	1	1	2	1	2	2
Aggregation	2	2	1	1	2	2	1	1	2	2	2	2
Physical attributes	1	2	2	2	1	2	2	1	2	2	1	2

QTTP / CQA	Dosage form [High]	Therapeutic effect [High]	Drug Content (SA) [High]	Drug Content (BP) [High]	Drug Release [High]	Route of Administration [Med]	Stability (Physical &Chemical) [High]
Zeta Potential	3	2	2	2	2	2	3
Polydispersity Index	3	3	2	2	3	3	3
Encapsulation Efficiency (EE%) of SA	1	3	3	2	2	2	2
Encapsulation Efficiency (EE%) of BP	2	3	1	3	2	2	3
pH	2	2	2	2	2	2	3
Particle size	3	3	2	2	3	2	3
Aggregation	1	1	2	1	1	2	2
Physical attributes	2	1	1	1	1	1	1

Figure 2: Risk assessment of quality attributes (JMP Software): 1=low risk parameter; 2=medium risk parameter; 3= high risk parameter.

CQA is minor and the formulation is likely to remain within the specifications and the failure is unlikely to happen and highly detectable. On the other extreme, a score of 3 (high) is designated for critical impacts on CQAs leading to an unsafe product with a high failure rate. Also, the prediction and detection of risk is very less at those conditions. The parameters that have a moderate impact on CQAs, with the potential to compromise formulation quality and the probability of its occurrence is occasional and failure might go unnoticed initially, are given a risk score of 2 (medium). The risks were assigned as low (1), medium (2) and high (3) to each process and materials based on their causable effects on CQA. The complete dataset containing individual S, O, D and RPN scores for every CPP-CQA combination alongside the cumulative RPN values is provided in Supplementary Table S1. RPN scores above 100 are considered major risk factors, such as surfactant concentration (RPN =162), lipid concentration (RPN=162) and homogenisation speed (RPN =102), based on the pareto chart for CPP and CMAs (Figure 3a). These parameters influence the uniformity and stability of the formulation, affecting critical quality attributes such as particle size distribution and drug encapsulation efficiency. Moderate risk factors included solubility (RPN=96), aqueous phase temperature during emulsification and homogenisation time (both RPN=60), and lipid phase temperature during melting and emulsification (RPN =52).[36] These thermal parameters can affect the physicochemical properties of the lipid matrix and emulsification efficiency, thus influencing product consistency. Lower risk factors, such as API concentration (RPN=48), lipid type (RPN=30), surfactant type, homogenisation temperature (both RPN = 28), and aqueous phase pH (RPN=24), were less critical.[37]

Another Pareto chart (Figure 3b) was plotted based on the RPN scores of the CQAs of SA-BP SLN, which identified polydispersity index, encapsulation efficiency of both SA and BP, particle size, and zeta potential as the most affected quality attributes, with RPN values ranging from 113 to 126. These CQAs are directly influenced by the identified CPPs, reinforcing the link between process parameters and product performance and are characterised as high-risk factors. Physical attributes, aggregation and pH were considered to be less risky, with an RPN of less than 100. Based on all these factors, high-risk factors were applied to BBD for systematic formulation and optimisation studies.

Experimental Design

Factors that significantly affect product quality were identified using FMEA risk analysis and preliminary screenings. Based on the RPN scores, lipid concentration (X1), surfactant concentration (X2), and homogenisation speed (X3) were identified as independent variables. The critical variables influenced by the independent variables were the polydispersity index (Y1), particle size (Y2), zeta potential (Y3), and entrapment efficiency of SA and BP (Y4 and Y5, respectively). The responses of various formulations of SA-BP-loaded SLN suggested by BBD are shown in Table 5.

Effects on PDI

The polydispersity Index is a critical factor in the assessment of an SLN for its size distribution and checks for any aggregation of particles in the formulations. A uniform particle size distribution and minimal aggregation resulted in a lower PDI value, which is considered good. A higher PDI value results in a variable-size distribution with maximal aggregation. The impact of all three independent variables on the PDI was evaluated to assess the CQAs as shown in Table 6. A quadratic and linear model were observed, and it was found to be significant with a *p*-value of 0.0076 ($p<0.05$) and a F-value of 11.483. The overall model showed an R^2 of 0.9500 with an adjusted R^2 of 0.8707. Over the 15 trials, the PDI ranged from 0.11 to 0.82. The ultimate goal is to attain a minimised PDI for maximised stability. Further, the correlation of PDI was evaluated by considering the effect of two independent factors, one of which was kept constant. The polynomial equation of PDI is as follows:

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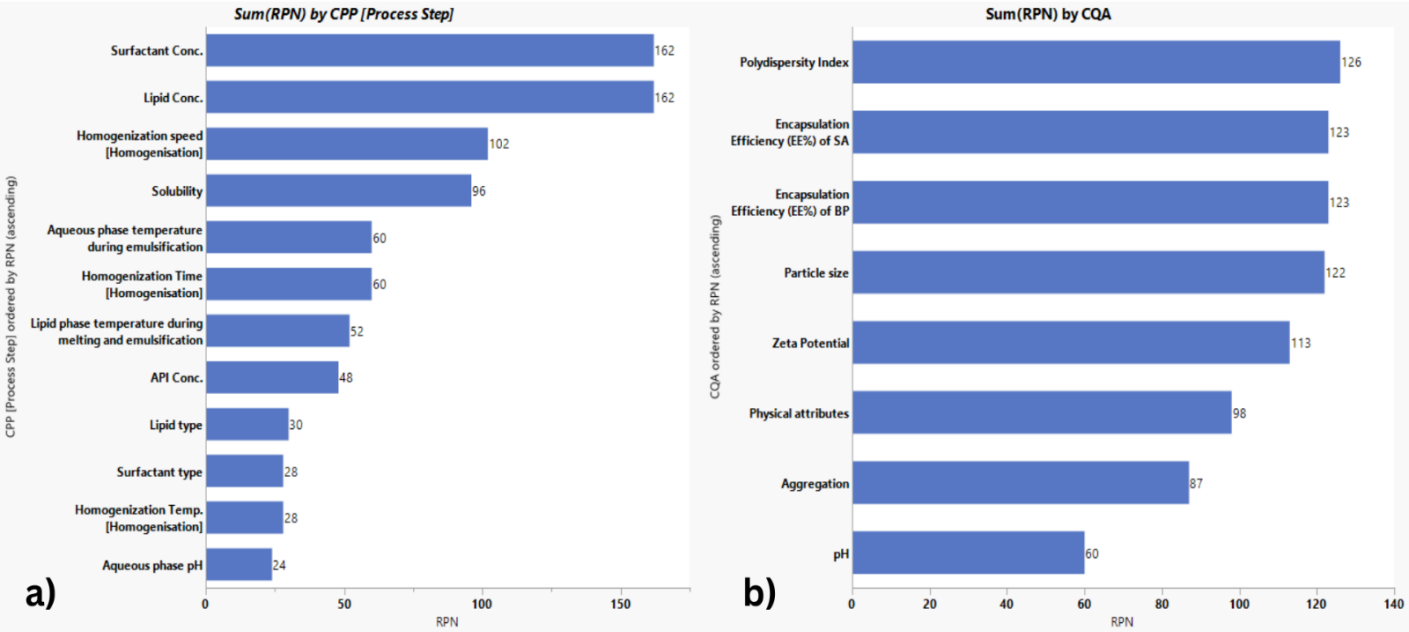


Figure 3: Pareto Analysis: a) Material/Process attributes b) CQA.

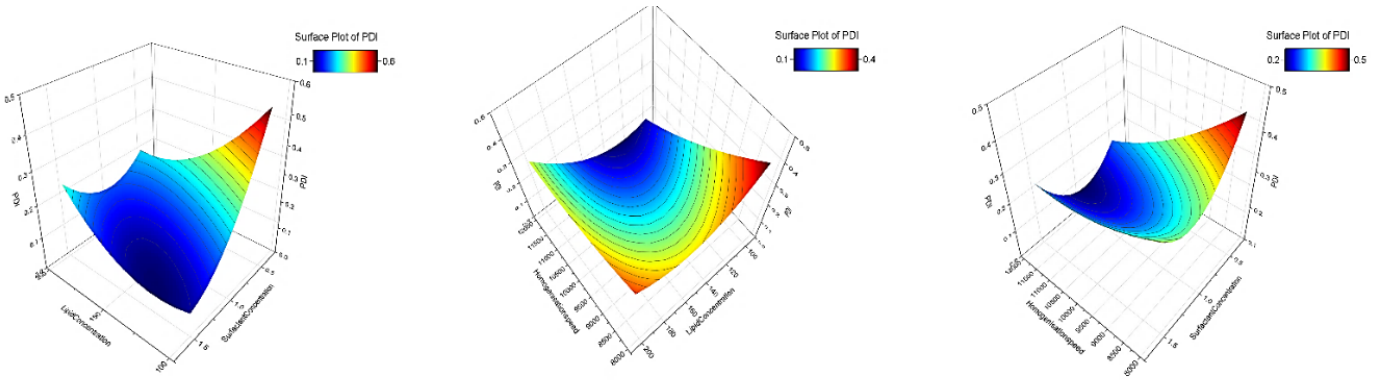


Figure 4: BBD effect on PDI. (A) Response surface plot for PDI on lipid conc. vs. %surfactant, lipid conc. vs. homogenisation speed and %surfactant vs. homogenisation speed and (B) Bar graphs of PDI vs. lipid conc., %surfactant and homogenisation speed.

$$A^2) + 0.3716666667(B^2) + 7.6041667e\text{-}10\ (C^2)$$

where A, B and C represent the coded values for the lipid concentration, surfactant concentration and homogenisation speed, respectively.

A PDI range of 0.3 or below indicates that a uniform particle distribution is achieved as the surfactant reduces the interfacial tension and forms a protective layer around the surface of the lipid particles and prevents aggregation.[38] Based on the polynomial equation, the PDI showed a negative correlation with surfactant concentration with a large negative coefficient (-2.200). When the surfactant ranged between 0.5 and 1.5 %, the optimal PDI values were found to be 0.14 - 0.82 as shown in Figure 4. Although 75% of formulations showed a PDI value below 0.3. Furthermore, a weak negative correlation was observed between the lipid concentration and homogenisation speed. As depicted in box plots, concentration of lipid demonstrated an inverse relationship with the PDI. At a lower concentration of 100 mg, the formulations showed a highly erratic PDI with a wide distribution. When the lipid concentration was increased to 150 and 200 mg, the PDI slightly decreased, and the same was observed for the homogenisation speed, although their individual impact was much smaller than that of the surfactant.[39] Overall, the box plots suggests a usage of higher homogenisation speeds ranging 10000-12000 rpm with a surfactant concentrations of 1.0-1.5 %. In this study, a PDI of 0.173 was achieved with a surfactant concentration of 1 % at 12000 rpm.

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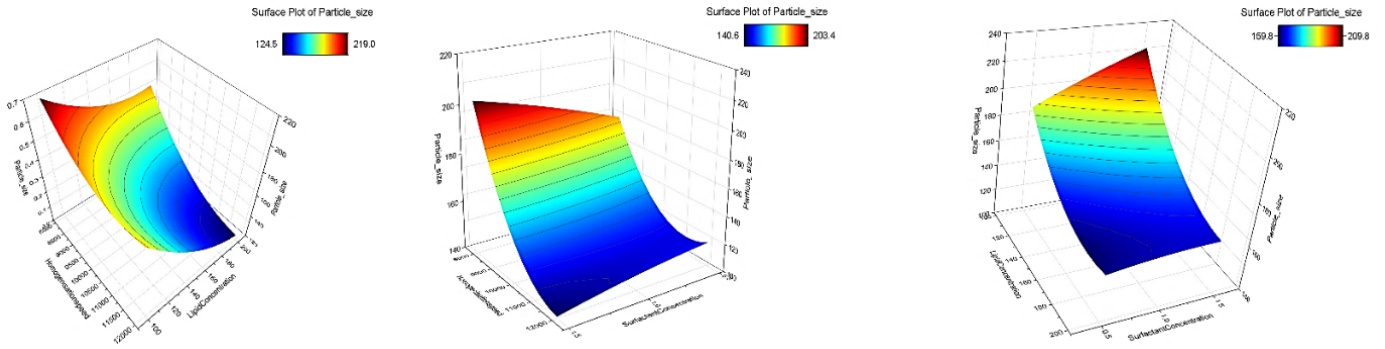


Figure 5: BBD effect on particle size. (A) Response surface plot for particle size on lipid conc. vs. %surfactant, %surfactant vs. homogenisation speed and lipid conc. vs. homogenisation speed and (B) Bar graphs of particle size vs. lipid conc. %surfactant and homogenisation speed.

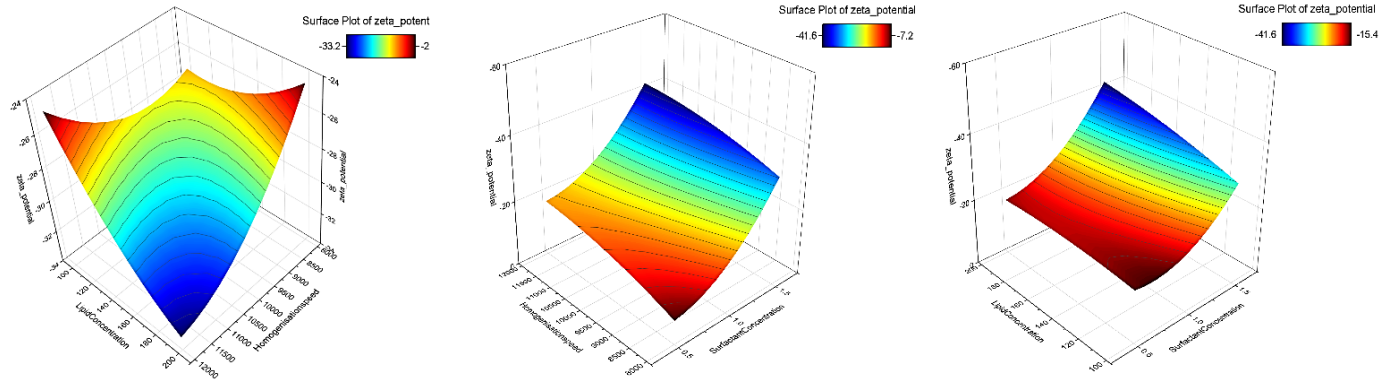


Figure 6: BBD effect on zeta potential. (A) Response surface plot for particle size on lipid conc. vs. %surfactant, %surfactant vs. homogenisation speed and lipid conc. vs. homogenisation speed and (B) Bar graphs of particle size vs. lipid conc. %surfactant and homogenisation speed.

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Effects on Particle Size

Particle size is crucial in dual drug-loaded solid lipid nanoparticles (SLNs) because it directly impacts drug loading, release kinetics, stability, bioavailability, and therapeutic efficacy. The optimal particle size ranges for high drug entrapment efficiency and controlled release is generally between 100 and 320 nm. Smaller particles (approximately 100-200 nm) tend to enhance bioavailability owing to their increased surface area and efficient tissue penetration, whereas larger particles (above 300 nm) may reduce absorption and result in slower drug release but can improve long-term stability in some cases.[40]

The effects of lipid concentration, surfactant concentration and homogenisation speed on particle size were observed in the present study. The significant F-value of 110.70 suggested a statistically significant quadratic model, where the *p*-value is also found to be significant with a value of 0.001 ($p<0.05$). The R^2 value was 0.9950 with an adjusted R^2 of 0.9860.[41] The ultimate goal was to obtain a reduced particle size with high drug release and entrapment efficiency. The polynomial equation for the particle size is as follows:

$$Y2 \text{ (Particle size)} = 576.5 - 0.645(A) + 109.3333(B) - 0.06704(C) - 0.18(AB) - 0.00009(AC) - 0.007(BC) + 0.00423(A2) - 3.6666(B2) + 0.00000377(C2)$$

The above equation shows a strong negative correlation of particle size with lipid concentration and a positive correlation with surfactant concentration and homogenisation speed. The surfactant concentration was a dominant factor (109.33), suggesting that particle size increases on increasing surfactant concentration due to the formation of secondary micelles or overcoating of the lipid matrix. Yet another negative quadratic term for the surfactant (-3.66) shows a diminished rate of particle size increase after a saturation point. Homogenisation speed showed a consistent and decrease in particle size with increasing speed due to higher mechanical shear and effective breaking down of lipid droplets into small monodisperse units as shown in Figure 5. A weak negative correlation was observed for lipid concentration for particle size and after the saturation point, the viscosity of the sample increases leading to a large particle size. Hence, the BBD design space suggested a lipid concentration of 180 mg and a homogenisation speed of 11,000 rpm to achieve an optimised SLN.

Effect on Zeta Potential

The zeta potential expresses the stability of the SLN for maintaining particle dispersion through repulsive forces reflections. Along with the particle size and PDI, it serves as an indicator for determining the short and long-term stability of SLNs. A zeta potential of approximately ±30 mV or higher indicates strong electrostatic repulsion between particles, helping prevent aggregation due to van der Waals attractive forces and promoting stable dispersion over time.[42] The model for the zeta potential was statistically significant, with an F-value of 55.007. Additionally, the *p*-value was found to be significant 0.0002 ($p<0.05$) with an R^2 value of 0.99 and an adjusted R^2 of 0.972. The quadratic equation for zeta potential is as follows:

Y3 (Zeta Potential) = -14.7375 + 0.25325(A) + 33.4083(B) - 0.00565(C) - 0.094(AB) - 0.00002825(AC) + 0.000375(BC) + 0.000403(A2) - 21.7667(B2) + 4.3333 e10-7(C2)

Almost 53% of formulations exhibited a high zeta potential (F1, F3, F7, F8, F9, F10, F13, and F14) when the % surfactant concentration ranged from 1 to 1.5%. Furthermore, from the above F3 and F8 showed an excellent zeta potential of -38.6 and -38.2 when the surfactant concentration was 1.5% Figure 6. This was supported by the polynomial equation, in which the surfactant concentration was the most influential variable (33.408), indicating a strong positive linear correlation.[43] This suggests that when the surfactant levels are increased, the zeta potential shifts towards a less negative value, because increasing the surfactant concentration thickens the steric adsorption layer surrounding the nanoparticles, which consequently masks the inherent negative surface charge of the lipid core. Lipid concentration showed a weak positive correlation (0.253), indicating that an increase in lipid concentration slightly modified the surface charge of the particle owing to the viscosity of the lipid matrix. The negative interaction between the surfactant and lipid (-0.094) depicts a synergistic effect, where the combination of both helps maintain a robust negative charge compared to when both are individually present. The model suggests a lipid concentration of 182 mg and a surfactant concentration of approximately 1.25% to achieve a greater zeta potential to ensure long-term formulation stability. The optimised formulation F14 exhibited a zeta potential of -23.3 mV maintaining an electro steric stabilisation which effectively prevents particle coalescence.

Effect on % entrapment efficiency

Entrapment efficiency (%) measures the amount of drug entrapped in the nanoparticulate drug delivery systems to quantify the dose of a particular drugs in the prescribed formulation. In the present study, %EE of both salicylic acid and benzoyl peroxide were studied through the effects of two factors and one factor was kept constant as shown in Figure 7a and 7b. The %EE model for both drugs (SA and BP) was statistically significant, with model F-values of 13.772 and 11.7646, respectively. The *p*-values for both SA and BP were significant, with values of 0.0373 and 0.0205 ($p<0.05$). In addition, the model was statistically insignificant for the lack of fit. The coefficient R^2 was found to be 0.9085 and 0.9463, respectively and was very close to the predicted R^2 values. Similarly, the adjusted R^2 were 0.9958 and 0.9962 respectively for SA and BP. The polynomial equations for both drugs are as follows:

Y4 (%EE of SA) = 349.1125 - 1.01095(A) - 30.96(B) - 0.036955(C) + 0.1815(AB) + 0.000043775(AC) - 0.0022775(BC) + 0.0012945(A2) + 12.925(B2) + 1.6565625 e10-6(C2)

Y5 (%EE of BP) = 363.60125 - 0.83955(A) - 31.63417(B) - 0.04388(C) + 0.0479(AB) + 0.000028(AC) - 0.00205(BC) + 0.001667(A2) + 21.28333(B2) + 2.15396 e10-6(C2)

The scatter plot shows the correlation between the factors and responses based on the BBD design of experiments. It illustrates a positive correlation between the % entrapment efficiency of SA and BP, with a correlation coefficient of 0.9. This implies that the independent parameters, most likely the lipid-to-surfactant ratio or the lipid concentration, influence the entrapment efficiency of both APIs in the SLN structure in a coordinated manner. Additionally, a high positive correlation was established between particle size and the PDI, as indicated by a correlation value of 0.5. This implies that as the SLN particles increased in size, the distribution of the particle diameter decreased, perhaps as a result of particle coalescence or non-uniform lipid crystallisation during the cooling phase of SLN formation.

A low correlation value of 0.1 was obtained between particle size and EE % of both SA and BP, indicating that the drug loading efficiency of the SLN nanoparticles was independent of particle size, implying that the chemical affinity between the APIs and the lipid core (GMS) was more dominant than the surface area or volume of the nanoparticles. A moderate positive correlation was observed between the PDI and Zeta Potential, with a correlation value of 0.4. This could be attributed to the fact that as the particle size distribution became more heterogeneous, the surface charge density became more heterogeneous, thereby influencing the Zeta Potential of the nanoparticles. A weak negative correlation was obtained between Zeta Potential and EE % of the SLN nanoparticles, with a correlation value of -0.2.

Validation of SA-BP SLN

The objectives of optimisation were to obtain a minimised particle size with an optimum PDI by maximising the entrapment efficiencies of salicylic acid and benzoyl peroxide with a sufficiently negative zeta potential for the stability of SLN. The overall desirability function of 0.251 suggested the optimum experimental conditions around 160 to 165 mg lipid, concentration of surfactant from 0.85 to 0.90% and

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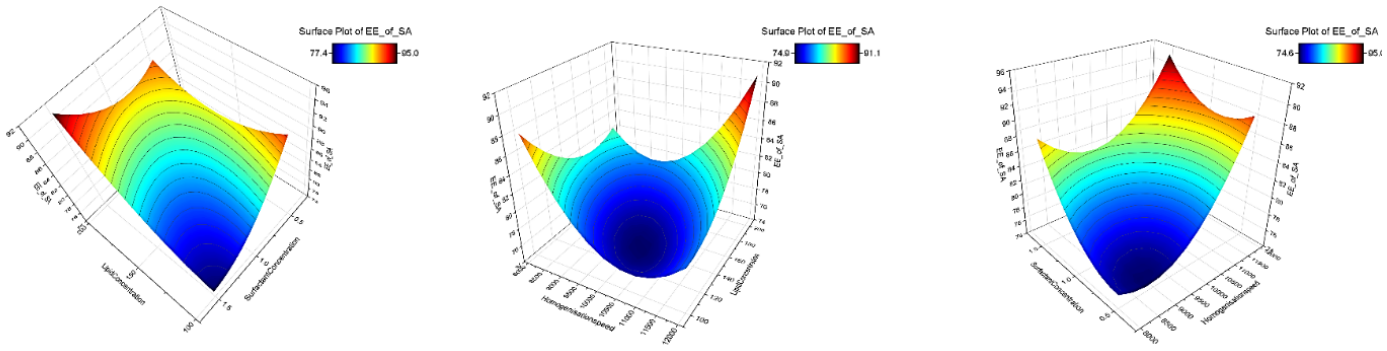


Figure 7: BBD effect on %entrapment efficiency. (a) Response surface plot for %Entrapment efficiency of Salicylic Acid on lipid conc. vs. %surfactant, %surfactant vs. homogenisation speed and lipid conc. vs. homogenisation speed, and (b) response surface plot for %Entrapment efficiency of Benzoyl peroxide on lipid conc. vs. %surfactant, %surfactant vs. homogenisation speed and lipid conc. vs. homogenisation speed.

homogenisation speed of 12000 rpm. The validation batch was prepared at the software-derived optimum and tested three times to verify the predictive performance of the BBD models. The predicted values were closely matched the experimental values of the particle size and PDI, demonstrating the ability of the models to predict the influence of the factor settings on these important parameters. The experimental entrapments of the SA and BP were slightly lower than the theoretical predictions. They indicated efficient dual drug loading and small prediction error comparable to those reported for other QbD optimised nanoparticle systems.[44] The zeta potential of the optimised SLNs was moderately negative (-23.3 mV), which is consistent with previous studies that found similar zeta potential values related to physically stable lipid nanoparticle dispersions for dermal or transdermal application.[45] However, while the validation batch confirmed the models mathematical accuracy, experimental entrapments of SA and BP were slightly lower than theoretically predicted. Upon evaluating the broader explored design space, the formulations from run 14 with a lipid concentration of 200 mg, 1% Tween 80 and a homogenisation speed of 12000 rpm yielded the smallest particle size of 133 nm which is practically superior for a dual loaded nanocarrier system. It also resulted in lowest PDI of 0.171 with a stable zeta potential of -23.3 mV, suggesting a good colloidal stability as shown in Figures 8a and 8b. The entrapment efficiencies of both SA and BP were 90.8% and 88.2%. Therefore, the formulation F-14 is the ideal formulation.

Characterisation of SA&BP SLN

SEM Analysis

Optimised dual drug loaded SLN were subjected to investigation of their morphological characteristics using Scanning Electron Microscopy (SEM). The observed particles were smooth and spherical with a uniform size distribution as shown in Figure 9a. The particle size was found to be less than 200 nm. The particle sizes of the samples were correlated with the data of dynamic light scattering.

Differential Scanning Calorimetry

Thermal behaviours of individual drugs and the BP-SA SLN was studied using DSC to analyse the phase transitions and solid-state compatibility. BP-SA SLN showed three endothermic peaks corresponding to melting points of GMS at 55-60°C, BPO at 103-110°C and SA at 159-165°C. The initial endotherm, which is relatively small for GMS reveals that the lipid excipient preserves its polymorphic form within the mixture.[46] The second endothermic peak with a higher magnitude coincided with the reported melting ranges of BPO, suggesting a weak interaction without any crystalline phase. The third peak with intense endotherm between 159-165°C, which is specific to high pure crystalline drug with a large fusion enthalpy remained unaffected by coformulations. The individual drugs DSC profiles confirmed a low melting nature of GMS, and the crystalline nature of benzoyl peroxide without any amorphization with GMS or SA.[47] Also, the single extended flat baseline at 158°C confirmed the crystalline nature of salicylic acid as shown in Figure 9b.

FTIR Analysis

Functional groups were detected using FTIR for pristine drugs, blank SLN and SA-BP loaded SLN formulations (Figure 9c). The Salicylic acid IR spectrum revealed an O-H stretching vibration at 3238 cm^{-1}, C=O bands at 1661 cm^{-1} (COOH) and C-H aromatic stretching at 3008 cm^{-1}. Similarly, for benzoyl peroxide a strong band was observed at 1758 cm^{-1} (Ester carbonyl) followed by C-O stretching at 1175.92 cm^{-1} and aromatic bending at 846 cm^{-1}. The SA-BP SLN spectrum retained the major peaks of both drugs and no additional peaks for new functional groups were observed. Similarly, the physical mixture produced an individual spectrum with retained characteristic bands. Overall, a perfect compatibility was found among the drugs and only minor peak shifts were observed among all spectra, which may be due to the microenvironmental changes and hydrogen bonding, rather than the formation of new compounds. No additional peaks or new carbonyl peaks corresponding to benzoyl peroxide proving that no decomposition products such as benzoic acid, were formed

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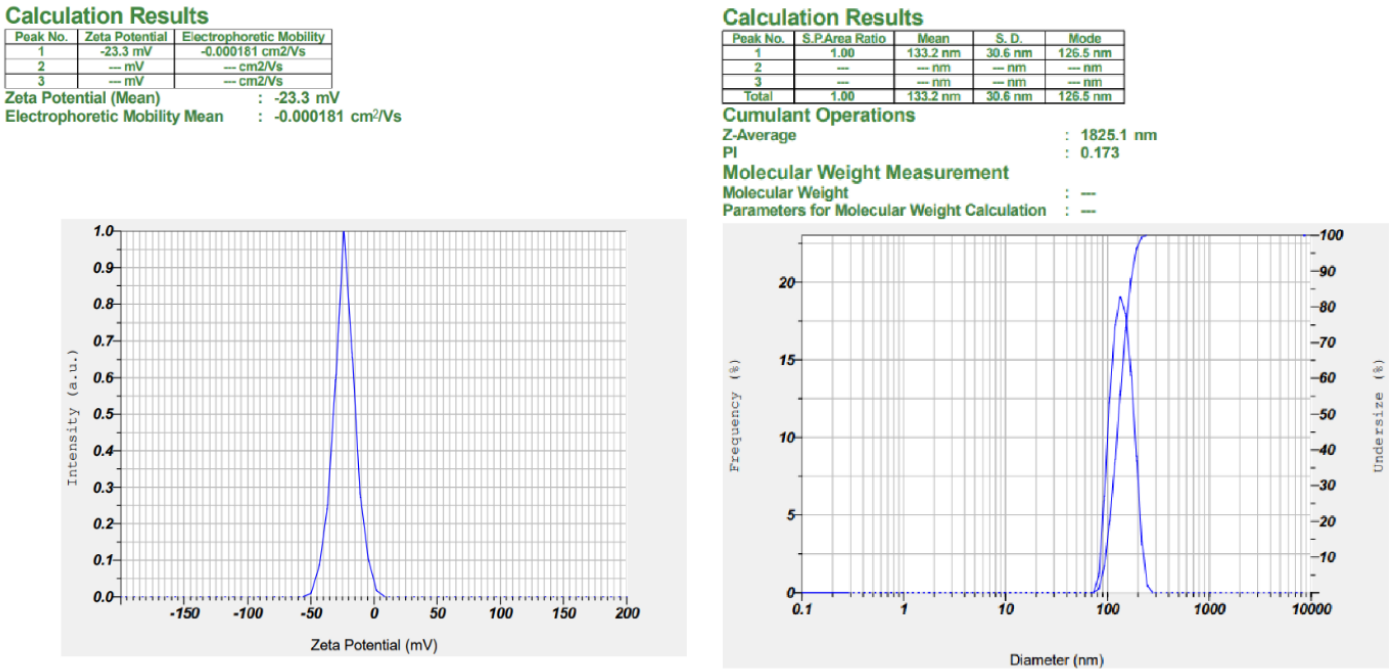


Figure 8: a) Zeta potential of optimised SA-BP SLN b) Particle size of optimised SA-BP SLN.

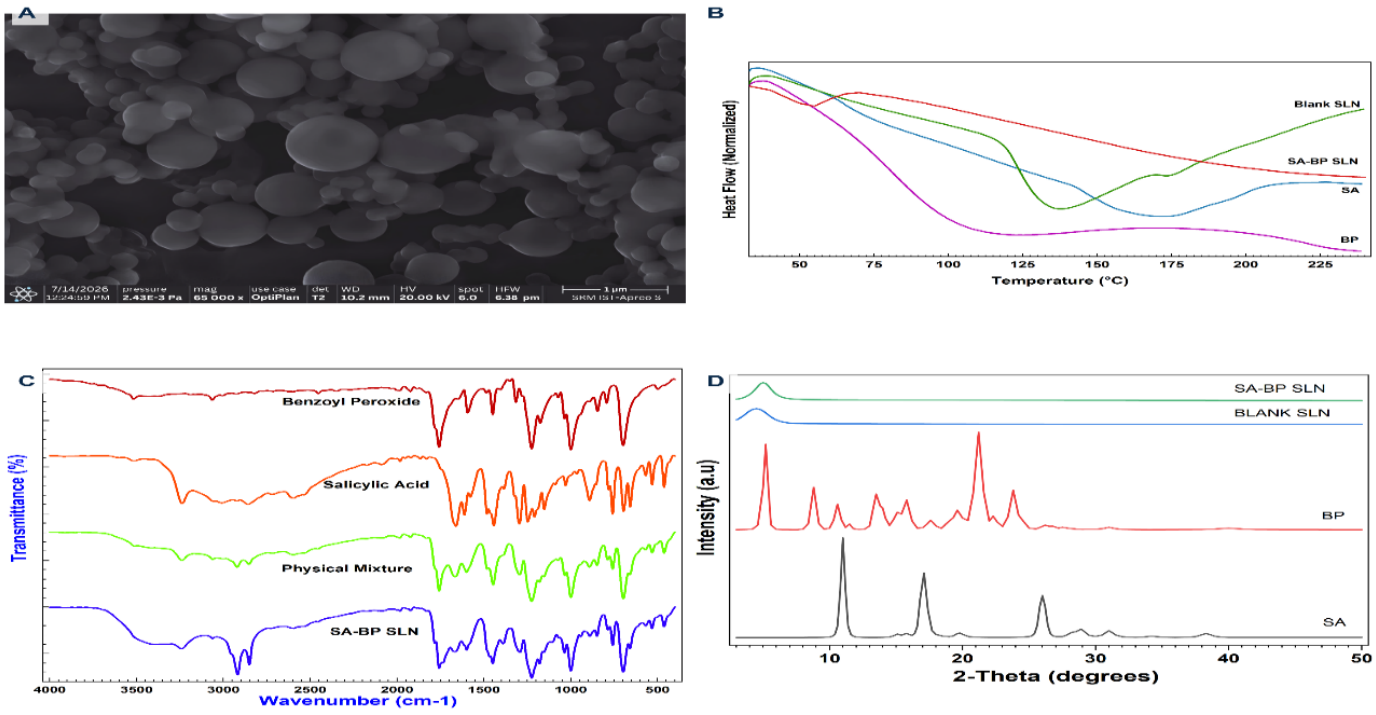


Figure 9: Characterisation of optimised drug-loaded SLN (A) SEM, (B) DSC, (C) FTIR, (D) XRD SLN: Solid lipid nanoparticles; BP: Benzoyl peroxide; SA: Salicylic acid, Blank SLN: Blank Solid lipid nanoparticles.

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under these conditions. A small broad shift was observed in SA-BP and lipid esters owing to the weak hydrogen bonding between them. Broadened O-H and C-H bands were observed in SLNs due to the additional hydrogen bonding and van der Waals interactions between GMS and cholesterol. This is consistent with the previous studies reported for various SLNs where drugs are encapsulated in a solid lipid core without interactions, preserving structural integrity and enabling controlled drug release.

X-ray Powder Diffraction Analysis

The crystallinities of pristine salicylic acid, benzoyl peroxide, blank SLN and SA-BP loaded SLNs were studied using XRD. The diffractograms are shown in Figure 9 d. Pristine SA demonstrated sharp and intense peaks, confirming the crystalline nature of salicylic acid. The prominent reflections at 10.8°, 17.2°, 19.0°, 25.5°, and 27.1° (2θ) match the reported crystalline structure of SA. Similarly, BP exhibited many sharp peaks within the 5-25° (2θ) range, signifying its crystalline polymorphic nature. In the SA-BP SLN peak, complete absence of either drug peaks suggests that they are no longer detectable as separate crystalline phases but are in amorphous form within the GMS matrix.[48] Additionally, no new crystalline phases are observed during the preparation of nanoparticles, further confirming the good physicochemical compatibility between the drugs and the lipid carrier. The decrease in crystallinity of GMS matrix is beneficial for drug incorporation because defects in the crystal lattice and void spaces can more readily provide space for the drug molecules. High encapsulation efficiency with minimal drug leakage can be achieved using imperfect lipid structures.[49] This is further exemplified by the amorphization of salicylic acid and benzoyl peroxide resulting in improved dissolution profiles and extended drug release from SLNs. Taken together, the findings underscore the conversion of both drugs from crystalline to amorphous is complemented by the increased stability of SA-BP SLN to improve topical drug delivery.

In vitro Drug Release Studies and Release Kinetics

The dialysis bag method was employed to study the *in vitro* drug release of SA-BP dual drug loaded SLNs and their corresponding raw drugs. The % drug release (cumulative) was plotted against time ranging from 0-24 hr for both drugs in pristine form and in dual-drug loaded SLNs as shown in Figures 10a and 10b. A rapid release of 85.10% within 24 h was observed for pristine salicylic acid, suggesting complete availability of the drug due to its moderate aqueous solubility and partial ionisation. Conversely, SA release from the dual drug SLN was clearly sustained with only 60.24% showing a complete lipid matrix retention of drug and acting as a controlled release medium.[50] Pristine benzoyl peroxide showed a comparatively slower and unfinished release with 28.57% in 24 h, indicating its lipophilic nature, poor aqueous solubility and crystalline nature leading to constricted diffusion

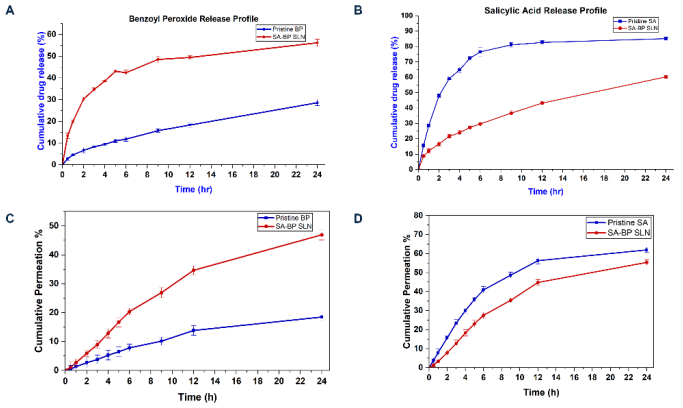


Figure 10: *In vitro* dissolution profiles of (A) pristine BP and optimised dual-loaded SLN formulation and (B) pristine SA and optimised dual-loaded SLN formulation. *Ex vivo* permeation studies of (C) Pristine BP and optimised dual-loaded SLN formulation and (D) pristine SA and optimised dual-loaded SLN formulation.
SLN: Solid lipid nanoparticles; BP: Benzoyl peroxide; SA: Salicylic acid.

across the dialysis membrane. Whereas BP incorporated in dual-drug-loaded SLNs enhanced the overall release by 56.15% at 24 h, due to decrease in particle size with increased surface area in presence of surfactants, promoting wetting and solubilisation of the drug. SA-BP-loaded SLN provided sustained release of SA and coherently improves the release of BP compared to its pristine form.

The release mechanism was evaluated using the *in vitro* release data of SA and BPO from pristine forms and SA-BP dual drug-loaded SLNs were fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas models. The best fit was assessed based on correlation coefficients (R^2). A higher linearity was observed for SA release from dual drug-loaded SLNs with an R^2 of 0.9947 compared to zero and first order fits. This suggests a diffusion-controlled release from the heterogeneous lipid matrix.[51] The Korsmeyer-Peppas model yielded an exponent *n* of SA is 0.551, suggesting non-Fickian (anomalous) transport, where release of drug is mediated by a combination of Fickian diffusion and erosion of the lipid matrix. As the salicylic acid is moderately soluble and partially ionised, it is distributed both at the lipid-water interface and the lipid matrix, resulting in an initial burst release followed by a sluggish diffusion via denser lipid network.[11] Similarly, benzoyl peroxide had the best fit for the SLNs with a R^2 of 0.9491 enhancing a diffusion-supported release of lipid particles. The Korsmeyer-Peppas exponent *n* of BP is 0. 473, with a quasi-Fickian diffusion due to diffusion of high lipophilic drug from a deep lipid core of the SLN. This results in a slower and incomplete release under pristine conditions, which is enhanced when compared to the SLN system. The gradual desorption and diffusion of BP provide a controlled release and irritant reduction associated with conventional formulations.[10]

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Ex vivo Skin Permeation Studies

The *Ex vivo* permeation behaviour of Salicylic Acid (SA) and Benzoyl Peroxide (BP) from the dual drug-loaded Solid Lipid Nanoparticles (SA-BP SLN) was evaluated using excised skin mounted on Franz diffusion cells. The cumulative permeation profiles of both drugs from the SLN formulation were compared with those of their respective pristine drug suspensions over a 24 h period as shown in Figure 10c and 10 d. The cumulative permeation profiles showed evident permeation for both drugs with respect to their physicochemical properties and their interaction with a GMS lipid matrix.

For salicylic acid, the pristine drug exhibited relatively rapid permeation during the initial stages of the study, reaching a cumulative permeation of approximately 61.9% after 24 h. In contrast, the SA-BP SLN displayed a slower and more controlled permeation profile, with approximately 55.4% permeation over the same period. This reduced permeation rate from the SLN formulation is attributed to the encapsulation of salicylic acid within the GMS matrix, which acts as a diffusion barrier and provides sustained drug release. This finding is consistent with the *in vitro* release study, in which the SLN formulation also showed a slower release profile than the pristine drug. The controlled permeation indicates that the lipid nanoparticles function as a drug reservoir, maintaining prolonged drug availability at the skin surface while potentially reducing irritation associated with the rapid penetration of salicylic acid.[52] In contrast, benzoyl peroxide in its pristine form demonstrated limited permeation, with only 18.5% cumulative permeation after 24 h, consistent with its poor aqueous solubility and highly lipophilic crystalline nature. Incorporation into the SLN formulation markedly enhanced permeation, yielding a cumulative permeation of approximately 46.9% at 24 h. This enhancement is likely attributable to the nanoscale particle size, increased surface area, improved wetting by the surfactant system, and molecular dispersion of benzoyl peroxide within the lipid matrix. Collectively, these factors promote greater partitioning of the drug into the stratum corneum and enhance its diffusion across the skin barrier.[53] XRD analysis showed the disappearance of the characteristic crystalline peaks of both salicylic acid and benzoyl peroxide after encapsulation, indicating conversion of the drugs into an amorphous state within the GMS matrix. This structural transformation is known to increase drug thermodynamic activity and promote release from lipid carriers. In addition, the reduced crystallinity of GMS introduces imperfections within the lipid matrix that improve drug accommodation and enable gradual diffusion during topical application. SLNs form a thin occlusive film on the skin surface that reduces transepidermal water loss and increases hydration of the stratum corneum.[54] Enhanced hydration disrupts the highly ordered lipid bilayers of the stratum corneum, thereby decreasing barrier resistance and facilitating drug transport. Moreover, the nanoparticles accumulate within hair follicles and skin furrows, creating localised drug depots that may be particularly beneficial for targeting the pilosebaceous units involved in acne vulgaris. Therefore, the enhanced permeation observed in this study serves as a biophysical proof of concept, and future studies on follicular deposition and localised epidermal skin retention of the dual-loaded SLNs will establish the formulation's clinical superiority for acne therapy.

Stability Studies

The optimised formulation of SA-BP SLN (F14) was selected for stability studies. The formulation was stored at 4°C in a refrigerator, at room temperature (25±2°C) and at 37 ± 2 °C in a hot air oven over two months and reported in Table S2. The formulation was found to retain its colour and homogeneous physical state at all three storage conditions without any degradation.[55] No visible changes were observed in the pH at different time intervals of 0, 1, and 2 months across all storage conditions. This range is consistent with the physiological skin pH and falls within the mildly acidic window and is favourable for acne, with reduced *Cutibacterium acnes* proliferation and improved barrier function.[47] Particle size remained constant across all storage conditions, ranging from 131-135 nm, compared with an initial value of 133±2.1 nm. Similarly, the PDI remained tightly clustered between 0.171 and 0.174 throughout the study, well below the 0.3 threshold generally used to define an acceptable narrow size distribution. The stability of particle size and PDI suggests that Tween-80 provides a robust electrostatic stabilisation sufficient to resist thermal driven particle growth over the tested period. A consistent zeta potential of -22.7 to -24.8 mV across all conditions over 2 months shows the absence of particle aggregation with either storage durations or temperatures. The optimised SA-BP SLN was stable across all conditions.

CONCLUSION

This study reports the formulation and optimisation of salicylic acid and benzoyl peroxide dual-drug-loaded solid lipid nanoparticles by successful implementation of QbD and risk assessment. All the critical attributes and process parameters were optimised using BBD to ensure that the defined parameters of the study objectives are achieved. The 3D surface plots and corresponding equations established the effects of independent variables on dependent variables. In addition, no peaks of SA and BP were observed on the DSC peaks of SA-BP SLN, confirming the encapsulation of the drug inside the SLN. *In vitro* drug release from SLN for SA was sustained, and it was improved for BP because of the SLN composition structure. The application of both drugs in a synergistic combination encapsulated in SLN will support its potential as a topical delivery platform for acne management. The present study laid a critical foundational proof of concept for the SLN delivery system. Moreover, it is necessary

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to conduct a thorough evaluation of this novel combination across different *in vitro* cell lines and *in vivo* models to fully validate it in a single SLN system for acne treatment.

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ABBREVIATIONS

SA: Salicylic Acid; **BP**: Benzoyl Peroxide; **SLN**: Solid Lipid Nanoparticle; **QbD**: Quality by Design; **DoE**: Design of Experiments; **BBD**: Box-Behnken Design; **QTTP**: Quality Target Product Profile; **CQA**: Critical Quality Attributes; **CMP**: Critical Method Parameter; **CPP**: Critical Process Parameter; **FMEA**: Failure Mode Effect Analysis; **GMS**: Glyceryl Monostearate; **FTIR**: Fourier Transform Infrared Spectroscopy**; SEM:** Scanning Electron Microscopy**; XRD**: X-ray Diffraction; **DSC**: Differential Scanning Calorimetry; **PDI**: Polydispersity Index; **ANOVA**: Analysis of Variance; **rpm**: rotation per minutes.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING DISCLOSURE

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AI DISCLOSURE

During the preparation of this work, the author(s) used Paperpal to check the grammar and FigureGuild to create graphical abstract. After using these tools, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the published work.

AUTHOR CONTRIBUTIONS (CREDIT TAXONOMY)

S.K.S.K.: Conceptualization, Methodology, Writing Original Draft, data analysis. **A.S.:** Supervision. **B.R.S.:** Supervision, Writing- Review and Editing. All authors reviewed and approved the final manuscript.

ETHICS APPROVAL AND CONSENT

No Humans/Animals were used in this study.

DATA AVAILABILITY

All the data associated with this work are provided within the manuscript.

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