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FORMULATION OPTIMIZATION AND IN VITRO EVALUATION OF NLCS FOR TOPICAL DELIVERY OF ANDROGRAPHOLIDE AND THIOCOLCHICOSIDE

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ABSTRACT

Nanostructured lipid carriers (NLCs) are advanced lipid-based nanocarriers that offer significant advantages for topical drug delivery, including improved drug loading, enhanced stability, controlled release, and better skin penetration. The present study focuses on the design, formulation optimization, and in vitro evaluation of nanostructured lipid carriers for topical delivery of a combination of andrographolide and thiocolchicoside using a Box–Behnken design (BBD) approach. Andrographolide, a bioactive diterpenoid with anti-inflammatory and antioxidant properties, and thiocolchicoside, a muscle relaxant, were selected to achieve a synergistic therapeutic effect. NLCs were prepared using an appropriate lipid blend consisting of solid and liquid lipids along with suitable surfactants. A three-factor, three-level Box–Behnken design was employed to optimize formulation variables, with particle size and entrapment efficiency considered as critical responses. Design-Expert® software was used to evaluate the effect of independent variables and to predict the optimized formulation. The optimized NLC formulation exhibited nanoscale particle size, high entrapment efficiency, and acceptable zeta potential, indicating good physical stability. The optimized NLCs were further characterized for drug content, in vitro drug release, and stability studies. In vitro release studies demonstrated a sustained and controlled release pattern of both drugs, suggesting improved topical performance. Stability studies confirmed that the formulation remained stable under accelerated storage conditions. Overall, the results indicate that the developed NLC-based topical formulation is a promising carrier system for the effective delivery of andrographolide and thiocolchicoside, with potential applications in the management of inflammatory and musculoskeletal conditions.

KEYWORDS: Nanostructured lipid carriers; Topical drug delivery; Andrographolide; Thiocolchicoside; Box–Behnken design; Formulation optimization; In vitro evaluation.

INTRODUCTION

Novel Drug Delivery Systems (NDDS) have emerged as an advanced approach in pharmaceutical research to overcome the shortcomings of conventional drug delivery methods. Traditional systems often suffer from poor bioavailability, lack of target specificity, frequent dosing requirements, and undesirable side effects, which ultimately affect therapeutic outcomes and patient compliance. NDDS aim to resolve these issues by delivering drugs in a controlled, targeted, and efficient manner, thereby enhancing therapeutic efficacy while minimizing toxicity.

NDDS encompass a wide range of delivery platforms, including nanoparticles, liposomes, micelles, polymeric implants, transdermal systems, gene delivery carriers, and 3D-printed dosage forms. Nano-based systems such as liposomes and polymeric nanoparticles allow drugs to reach diseased tissues with greater precision due to their small size and surface modification capabilities. Controlled-release systems, including polymeric implants and transdermal patches, provide sustained drug delivery over extended periods, reducing dosing frequency and improving patient adherence. Additionally, gene delivery systems enable the transport of genetic material into target cells, offering new therapeutic possibilities in cancer and genetic disorders.[1-6]

The development and optimization of NDDS are strongly supported by statistical tools such as **Design-Expert® software**, particularly the **Box–Behnken Design (BBD)**. BBD is a response surface methodology that evaluates the relationship between formulation variables and experimental outcomes using a limited number of experimental runs. This design efficiently predicts optimal formulation conditions by analyzing linear, quadratic, and interaction effects of independent variables, thereby reducing development time, cost, and experimental workload.

Nanoparticles used in NDDS are broadly classified into organic and inorganic types. Organic nanoparticles, including liposomes, micelles, and dendrimers, are biodegradable and biocompatible, making them suitable for targeted drug delivery applications. Inorganic nanoparticles, such as metal and metal oxide-based systems, possess unique physicochemical properties like high surface area and enhanced reactivity, which can be exploited for diagnostic and therapeutic purposes.[7-9]

Among lipid-based nanocarriers, **Nanostructured Lipid Carriers (NLCs)** represent a significant advancement over solid lipid nanoparticles. NLCs are composed of a mixture of solid and liquid lipids, resulting in an imperfect and less-ordered lipid matrix. This structural imperfection allows higher drug loading, improved stability, and reduced drug expulsion during storage. NLCs are particularly advantageous for dermal delivery, as they enhance skin hydration, reinforce the lipid barrier, and improve drug penetration. Beyond dermal applications, NLCs are also explored for oral, nasal, ocular, pulmonary, and parenteral delivery.

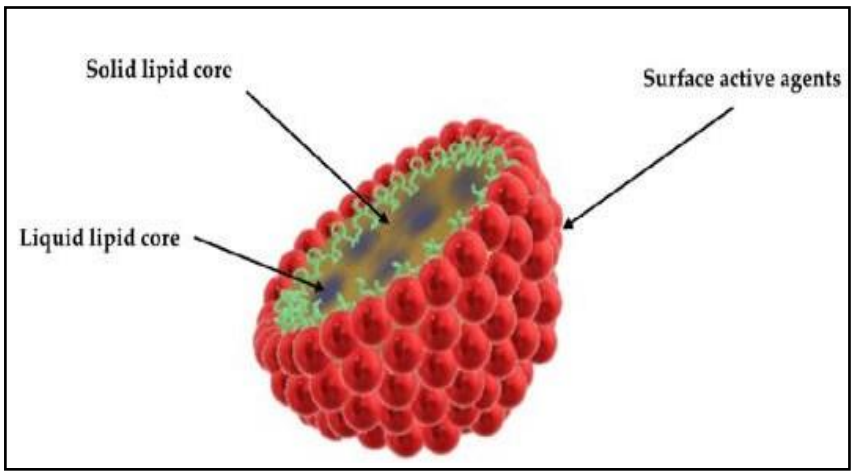


Fig. 1: Nanostructured lipid carriers (NLC).

Various methods such as hot and cold high-pressure homogenization, microemulsion techniques, solvent-based methods, and hot melt extrusion are employed to prepare NLCs. Each method is selected based on drug stability, lipid characteristics, and desired particle size. NLCs are further classified into imperfect crystal, multiple (oil-in-fat-in-water), and amorphous types, depending on lipid composition and internal structure.[10-12]

Overall, NDDS—and particularly nanostructured lipid carriers—offer a versatile, safe, and effective platform for modern drug delivery. Their ability to improve drug stability, control release profiles, enhance bioavailability, and provide targeted delivery makes them highly promising systems for future pharmaceutical, cosmetic, and biomedical applications.

Recent studies (2024–2025) demonstrate the broad potential of **nanostructured lipid carriers (NLCs)** for enhancing drug delivery across multiple therapeutic areas, including antibacterial, antifungal, anticancer, antidiabetic, ocular, pulmonary, nasal, dermal, and nutraceutical applications. NLCs consistently showed **nanoscale particle size, high entrapment efficiency, controlled/sustained drug release, and improved stability**, leading to enhanced bioavailability, permeability, and therapeutic efficacy compared to conventional formulations. Advanced approaches such as **Box–Behnken Design (QbD), surface modification (e.g., chitosan, transferrin), in situ gels, and targeted delivery mechanisms** further improved clinical performance. Overall, these findings confirm NLCs as a **versatile, efficient, and promising nanocarrier system** for targeted and sustained drug delivery with reduced side effects and improved patient outcomes.[14-18]

Andrographolide
Chemical name: 3-[2-{Decahydro-6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene-1-naphthalenyl}ethylidene]-2,5-dihydro-4-hydroxyfuran-2-one.
Source: *Andrographis paniculata* (Family: Acanthaceae).
Category: Diterpenoid lactone.
Molecular formula: $C_{20}H_{30}O_5$.
Molecular weight: 350.45 g/mol.

Pharmacological actions
Andrographolide exhibits **anti-inflammatory, antioxidant, antimicrobial, anticancer, antidiabetic, hepatoprotective, and immunomodulatory** activities. It inhibits inflammatory mediators such as TNF-α, IL-6, and NF-κB signaling pathways.

Therapeutic applications
Used in the management of **inflammatory disorders, diabetes, infections, cancer, liver disorders, and cardiovascular diseases**.

Biopharmaceutical limitations
- Poor aqueous solubility
- Low oral bioavailability
- Rapid metabolism
- Limited permeability

Rationale for NDDS/NLCs
Encapsulation of andrographolide in nanostructured lipid carriers improves **solubility, stability, skin permeation, sustained release, and bioavailability**, making it suitable for topical and targeted delivery.

Thiocolchicoside
Chemical name: (S)-N-deacetylthiocolchicine-2-O-β-D-glucopyranoside
Category: Semi-synthetic derivative of colchicoside
Molecular formula: $C_{27}H_{33}NO_{10}S$
Molecular weight: 563.62 g/mol

Pharmacological actions
Thiocolchicoside is a **centrally acting muscle relaxant** with **anti-inflammatory and analgesic** properties. It acts as a **GABA-A and glycine receptor agonist**, leading to muscle relaxation.

Therapeutic applications
Used in the treatment of **muscle spasms, musculoskeletal disorders, back pain, cervical and lumbar pain, and inflammatory conditions.**[20-21]

Biopharmaceutical limitations
- Low water solubility
- Short half-life
- Gastrointestinal side effects upon oral administration
- Dose-related toxicity

Rationale for NDDS/NLCs
Formulation in nanostructured lipid carriers enhances **localized delivery, sustained drug release, reduced systemic exposure, improved skin penetration, and minimized side effects**, especially for topical therapy.

Justification for Combined Delivery
The combination of **andrographolide (anti-inflammatory, antioxidant)** and **thiocolchicoside (muscle relaxant, analgesic)** offers **synergistic therapeutic benefits** for inflammatory and musculoskeletal conditions. Incorporation into **NLC-based topical delivery systems** can improve efficacy, safety, and patient compliance.

This study focused on the development and optimization of **nanostructured lipid carriers (NLCs)** for the topical co-delivery of **andrographolide** and **thiocolchicoside** to achieve enhanced treatment of inflammatory and musculoskeletal conditions. The combination was selected due to their complementary anti-inflammatory, antioxidant, immunomodulatory, and muscle-relaxant properties, which together provide a synergistic therapeutic effect. Both drugs exhibit poor aqueous solubility and limited skin permeability, restricting their topical efficacy.

To overcome these limitations, NLCs were formulated to improve drug solubility, dermal bioavailability, and sustained release. The formulation was optimized using **Box–Behnken design**, evaluating critical variables such as lipid-to-drug ratio, surfactant concentration, and homogenization speed. The optimized NLCs showed efficient co-encapsulation, suitable particle size and stability, sustained drug release, and significantly enhanced skin permeation compared to conventional formulations.

Overall, the findings establish NLCs as an effective and promising nanocarrier system for the topical delivery of poorly soluble phytoconstituents, offering improved therapeutic efficacy with localized action and minimal systemic exposure.

MATERIAL AND METHODS

Pre-formulation Studies of Drugs
Pre-formulation studies were conducted on **andrographolide** and **thiocolchicoside** to evaluate their physicochemical properties and suitability for incorporation into nanostructured lipid carriers (NLCs). These studies helped in understanding drug behavior, stability, and compatibility with formulation excipients, thereby guiding rational formulation development for effective topical delivery.

Organoleptic Properties
The organoleptic evaluation included assessment of appearance, color, odor, taste, and texture of both drugs. The observations confirmed acceptable physical characteristics and ensured the identity, purity, and quality of the raw materials prior to formulation.

Solubility Studies
Solubility studies were performed in distilled water, organic solvents, and buffers of varying pH to determine the dissolution behavior of andrographolide and thiocolchicoside. Both drugs showed limited aqueous solubility, highlighting the need for a lipid-based carrier system. The results guided the selection of suitable solvents, lipids, surfactants, and formulation techniques to enhance drug solubility and topical bioavailability.

pH Determination

pH stability studies evaluated the behavior of both drugs under acidic, neutral, and alkaline conditions. The drugs exhibited pH-dependent stability, making pH optimization critical for formulation development. These findings assisted in selecting appropriate excipients and pH modifiers to maintain drug stability and efficacy in the final NLC formulation.

Melting Point Determination

Melting point analysis was carried out using the open capillary tube method to assess purity and thermal stability. The observed melting ranges confirmed the suitability of both drugs for formulation processes involving heat, such as hot homogenization.

Determination of Maximum Wavelength (λmax) and Calibration Curve

Standard stock solutions of andrographolide and thiocolchicoside were prepared in methanol and analyzed using UV–Visible spectrophotometry. The λmax values were identified in the UV region for andrographolide and in the visible region for thiocolchicoside. Calibration curves constructed at respective λmax values showed good linearity, enabling accurate and reliable quantitative analysis during formulation and evaluation studies.

FTIR Analysis

FTIR spectroscopy was used to identify characteristic functional groups and confirm the chemical integrity of both drugs. The spectra matched reported literature values, indicating no structural modification and suitability for further formulation studies.

Functional group identified by Infra-Red spectroscopy

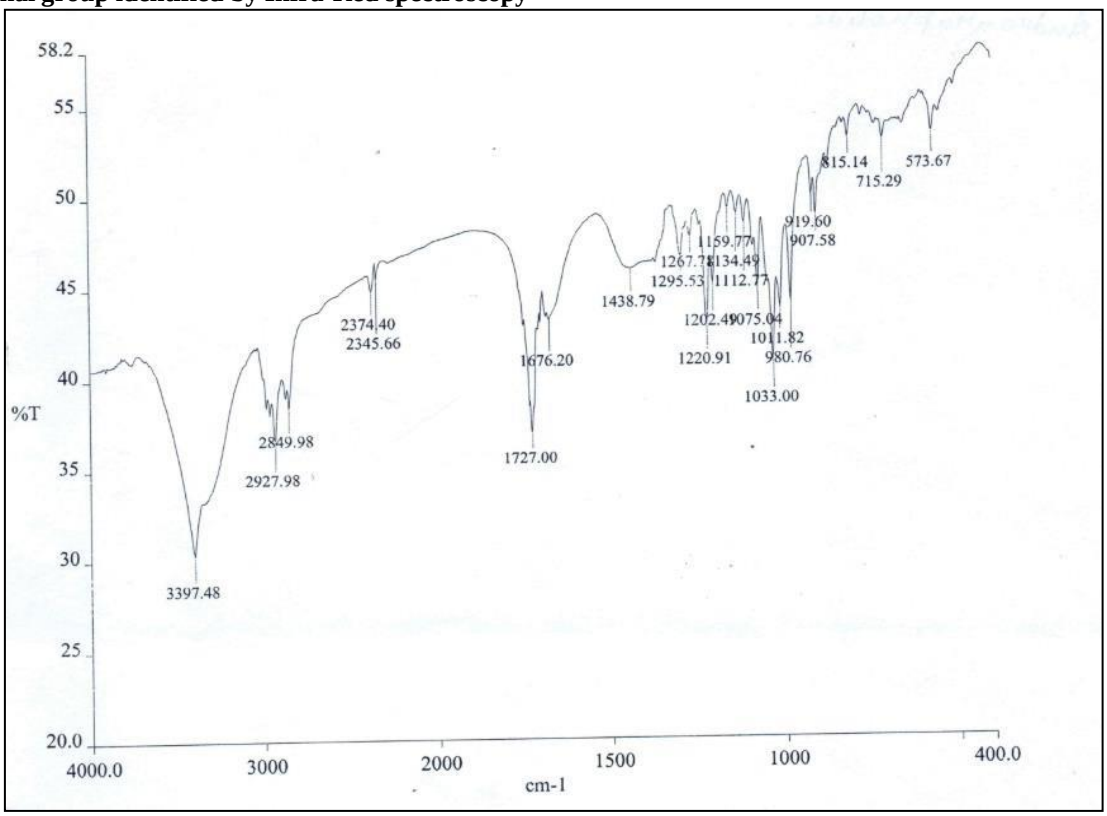


Figure 2: FTIR study of Andrographolide.

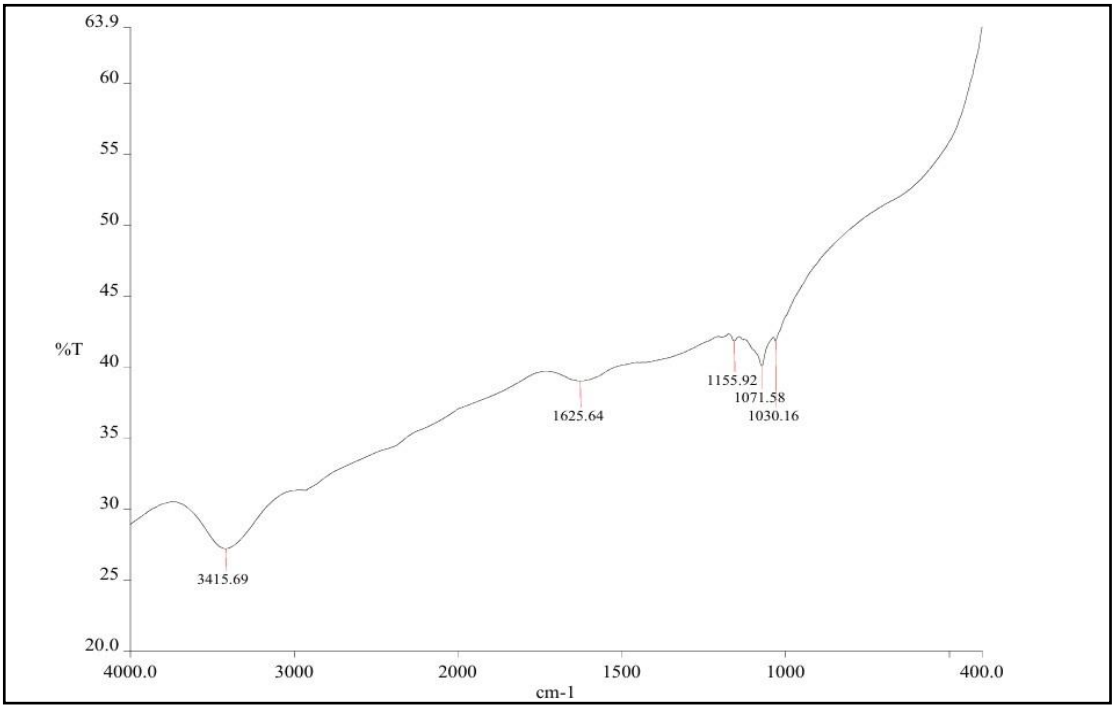


Figure 3: FTIR study of Thiocolchicoside.

Formulation of Drug-Loaded Nanostructured Lipid Carriers

Drug-loaded NLCs were prepared using the **hot homogenization technique**. Andrographolide and thiocolchicoside were dissolved in organic solvents and incorporated into a lipid phase containing solid lipid and oleic acid. This lipid phase was emulsified into an aqueous phase containing surfactants under controlled temperature, stirring, and sonication. Subsequent homogenization and cooling resulted in the formation of stable nanosized lipid carriers. The final NLC dispersion was lyophilized and stored for further characterization and evaluation.

Table 1: Composition of Nano lipid carriers.

S. No	Formulation code	Drugs (0.25% and 0.025% mg)	Ethanol (ml)	Acetone (ml)	Steari c acid Solid lipid (mg)	Oleic acid (%)	Surfactant Tween 80 (%)	Sodium lauryl sulfate (mg)	Tempe rature (ºC)	Sonication time (min.)
1.	NLCs 1	1:1	15.0	15.0	250	0.1	0.65	48.0	80 ºC	10
2.	NLCs 2	1:1	15.0	15.0	150	0.1	0.65	48.0	80 ºC	6.5
3.	NLCs 3	1:1	15.0	15.0	50	0.1	0.65	48.0	80 ºC	3
4.	NLCs 4	1:1	15.0	15.0	150	0.1	1	48.0	80 ºC	10
5.	NLCs 5	1:1	15.0	15.0	150	0.1	0.3	48.0	80 ºC	3
6.	NLCs 6	1:1	15.0	15.0	250	0.1	0.3	48.0	80 ºC	6.5
7.	NLCs 7	1:1	15.0	15.0	50	0.1	0.3	48.0	80 ºC	6.5
8.	NLCs 8	1:1	15.0	15.0	50	0.1	0.65	48.0	80 ºC	10
9.	NLCs 9	1:1	15.0	15.0	150	0.1	1	48.0	80 ºC	3
10.	NLCs 10	1:1	15.0	15.0	250	0.1	1	48.0	80 ºC	6.5
11.	NLCs 11	1:1	15.0	15.0	150	0.1	0.3	48.0	80 ºC	10
12.	NLCs 12	1:1	15.0	15.0	50	0.1	1	48.0	80 ºC	6.5
13.	NLCs 13	1:1	15.0	15.0	250	0.1	0.65	48.0	80 ºC	3

Physical Appearance Evaluation

Physical appearance evaluation served as a preliminary quality assessment of drug-loaded NLCs. The formulations were visually examined for color uniformity, surface texture, odor, and the presence of visible aggregates or phase separation. The absence of clumping, discoloration, and unusual odor indicated good physical stability and acceptable formulation quality. Consistent visual characteristics supported the suitability of the NLCs for further physicochemical evaluation.

Particle Size and Polydispersity Index Analysis

Particle size analysis was performed using Dynamic Light Scattering to determine the average particle size and polydispersity index (PDI) of the NLC formulations. Particle size plays a critical role in drug release, skin penetration, and formulation stability, while PDI reflects the uniformity of particle distribution. The NLCs

exhibited nanoscale particle size with low PDI values, indicating a narrow size distribution and homogeneous formulation. Such characteristics are essential for predictable drug release and enhanced topical bioavailability.

Table 2: Variables operating range for nanostructured lipid carrier's formulation.

Name	Goal	Lower Limit	Upper Limit	Importance
A: Solid lipid	is in range	50	250	3
B: Surfactant	is in range	0.3	1	3
C: Sonication time	is in range	3	10	3
Particle size	none	204.36	780.04	3
Entrapment efficiency	none	66.23	95.07	3

Zeta Potential Analysis

Zeta potential measurement was carried out to evaluate the surface charge and colloidal stability of the NLCs. Adequate positive or negative zeta potential values suggested strong electrostatic repulsion between particles, minimizing aggregation and enhancing dispersion stability. The results confirmed that the formulated NLCs possessed sufficient surface charge to ensure long-term physical stability and shelf life.

Scanning Electron Microscopy (SEM) Analysis

SEM analysis was used to examine the surface morphology and structural characteristics of the drug-loaded NLCs. The micrographs revealed well-defined, spherical particles with smooth surfaces and minimal aggregation. SEM findings corroborated particle size analysis results and confirmed the nanoscale nature and uniformity of the formulation, supporting its suitability as an efficient drug delivery system.

Entrapment Efficiency Determination

Entrapment efficiency was determined to evaluate the ability of NLCs to encapsulate andrographolide and thiocolchicoside. The method involved separating free drug from the NLC dispersion and quantifying the unencapsulated drug. High entrapment efficiency values indicated effective drug incorporation within the lipid matrix, ensuring sustained drug release and enhanced therapeutic performance.

***In-vitro* Drug Release Study**

In-vitro drug release studies were conducted using the dialysis bag diffusion method in phosphate buffer (pH 7.4) at 37 ± 2 °C. The optimized NLC formulations exhibited sustained and controlled drug release profiles. Release kinetics were analyzed using zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to understand the release mechanism. The results suggested diffusion-controlled and concentration-dependent release behavior, confirming the ability of NLCs to provide prolonged drug release suitable for topical therapy.

Table 3: Actual Value and Predicted Value of all formulations.

Formulations	Actual Value of Particle size	Predicted Value of Particle size
NLCs 1	204.36	191.68
NLCs 2	496.23	492.00
NLCs 3	780.04	792.33
NLCs 4	220.75	258.71
NLCs 5	692.35	725.29
NLCs 6	417.84	424.97
NLCs 7	648.36	597.60
NLCs 8	350.26	364.31
NLCs 9	647.07	686.73
NLCs 10	417.22	386.41
NLCs 11	278.11	297.28
NLCs12	597.36	559.03
NLCs 13	646.09	619.70

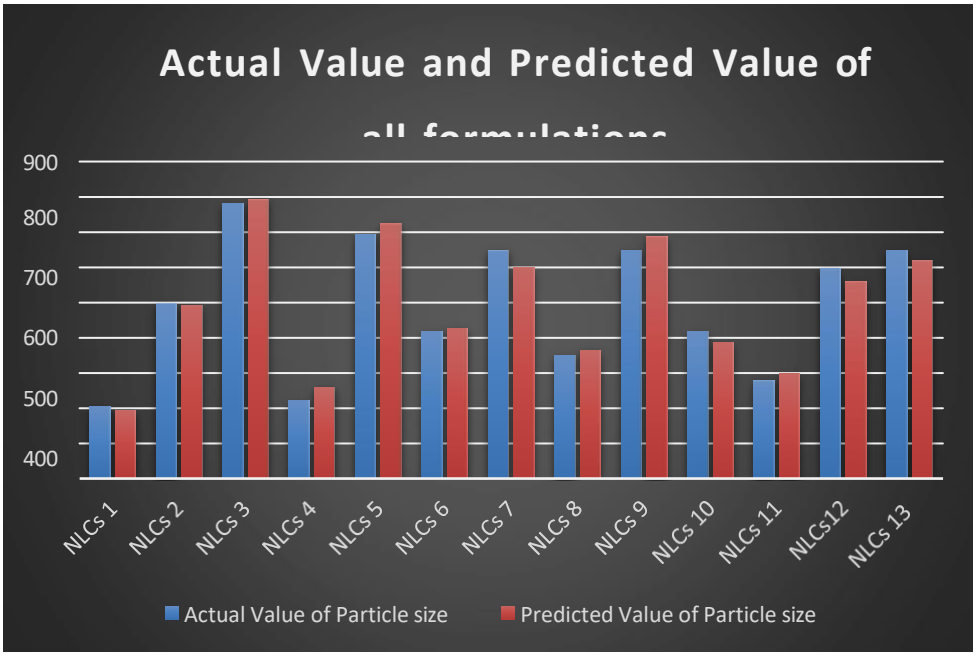


Fig. 4: Graphical representation of actual and predicted value.

Stability Studies

Stability studies were performed in accordance with ICH Q1A guidelines to assess the physical and chemical stability of the optimized NLC formulation. Samples were stored at accelerated conditions and evaluated at predetermined intervals for physical appearance, particle size, and entrapment efficiency. The formulation remained stable throughout the study period, with no significant changes observed in the evaluated parameters. These findings confirmed the robustness and stability of the NLC system during storage.

RESULT AND DISCUSSION

Pre-formulation Evaluation of Andrographolide and Thiocolchicoside

Organoleptic Evaluation

Organoleptic evaluation confirmed the identity and quality of both drugs. Andrographolide appeared as a white to off-white crystalline solid with no detectable odor, while Thiocolchicoside was observed as a yellow to yellowish fine crystalline or amorphous powder with a faint or no odor. Both drugs were in solid state, consistent with reported literature and pharmacopeial standards, confirming their suitability for formulation development.

Solubility Studies

Solubility studies revealed poor aqueous solubility for both drugs, with improved solubility in organic solvents such as methanol and DMSO. Thiocolchicoside showed relatively higher solubility compared to Andrographolide. These findings highlighted the need for a lipid-based carrier system to enhance solubility and topical bioavailability.

pH Determination

Andrographolide exhibited an acidic pH within the reference range, whereas Thiocolchicoside showed a slightly basic pH. Understanding their pH behavior was essential for selecting compatible excipients and maintaining drug stability in the final formulation.

Melting Point Determination

The observed melting points of both drugs closely matched reported reference values, confirming their purity and thermal stability. This indicated that both drugs are suitable for formulation processes involving heat, such as hot homogenization.

UV Spectrophotometric Analysis

Determination of λmax and Overlay Spectrum

UV analysis showed characteristic absorption maxima at **223 nm for Andrographolide** and **376 nm for Thiocolchicoside**. Overlay spectra confirmed no spectral interference, enabling simultaneous estimation of both drugs.

Calibration Curve Analysis

Calibration curves for individual drugs and their combination demonstrated excellent linearity over the concentration range studied, confirming adherence to Beer–Lambert's law. This validated methanol as a suitable solvent and confirmed the reliability of the analytical method for quantitative studies.

FTIR Analysis

FTIR spectra confirmed the presence of characteristic functional groups corresponding to the known chemical structures of both drugs. The absence of peak shifts or disappearance indicated chemical integrity and compatibility, supporting their suitability for formulation.

Optimization of NLC Formulation Using DOE
Experimental Design and Variables
A Box–Behnken design was employed to optimize the formulation using solid lipid concentration, surfactant concentration, and sonication time as independent variables, with particle size and entrapment efficiency as responses. This approach minimized experimental runs while enabling evaluation of variable interactions.

Effect on Particle Size
Statistical analysis indicated that particle size was significantly influenced by solid lipid concentration and sonication time. The developed model showed good predictability, confirming the suitability of the design for formulation optimization.

Effect of Formulation Variables on Entrapment Efficiency
Entrapment efficiency was significantly affected by surfactant concentration and sonication time. The optimized model demonstrated high predictive accuracy, indicating effective drug incorporation within the lipid matrix.

Optimized NLC Formulation
The optimized formulation showed high desirability with nanoscale particle size and maximum entrapment efficiency, confirming the success of the DOE-based optimization approach.

Characterization of Optimized NLC Formulation
Physical Appearance
The optimized NLC formulation was pale yellow, clear, homogeneous, and free from visible particles, indicating good physical stability.

Particle Size
The actual particle size closely matched the predicted value, confirming the reliability of the optimization model and suitability for topical delivery.

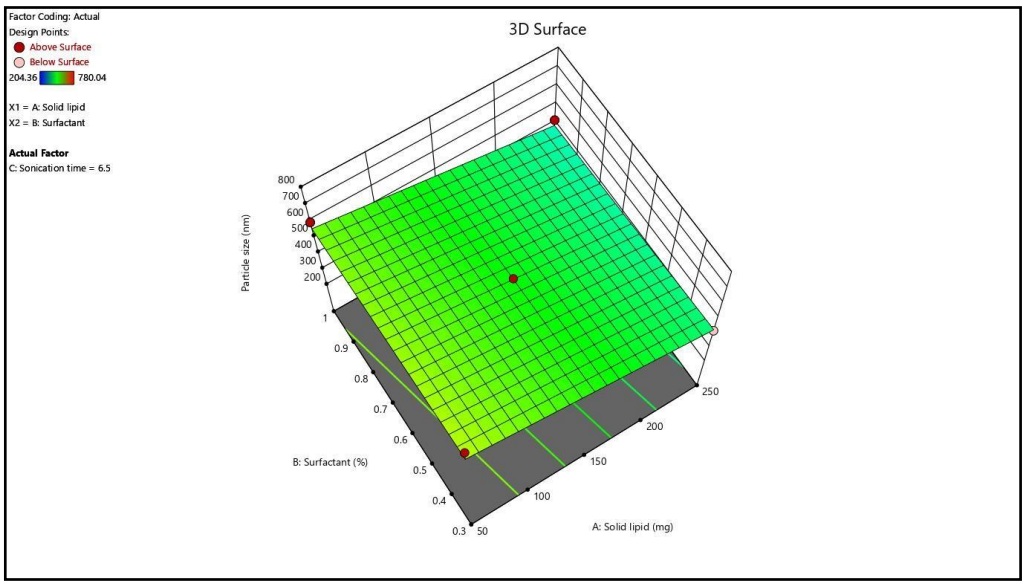
Zeta Potential
A high negative zeta potential indicated excellent colloidal stability and reduced risk of particle aggregation.

Entrapment Efficiency
High entrapment efficiency confirmed effective drug loading, supporting sustained release and improved therapeutic performance.

SEM Analysis
SEM images showed uniform, well-dispersed nanoparticles with smooth surfaces, validating the nanoscale structure and formulation stability.

In Vitro Drug Release Study
The optimized NLCs exhibited sustained drug release over time. Kinetic modeling indicated that the release followed **zero-order kinetics**, with diffusion playing a secondary role. This controlled release behavior is desirable for prolonged topical therapeutic action.

Stability Studies
Stability studies conducted under ICH conditions demonstrated negligible changes in particle size and entrapment efficiency over 90 days. The formulation remained physically and chemically stable under both normal and accelerated storage conditions, confirming its suitability for long-term use.



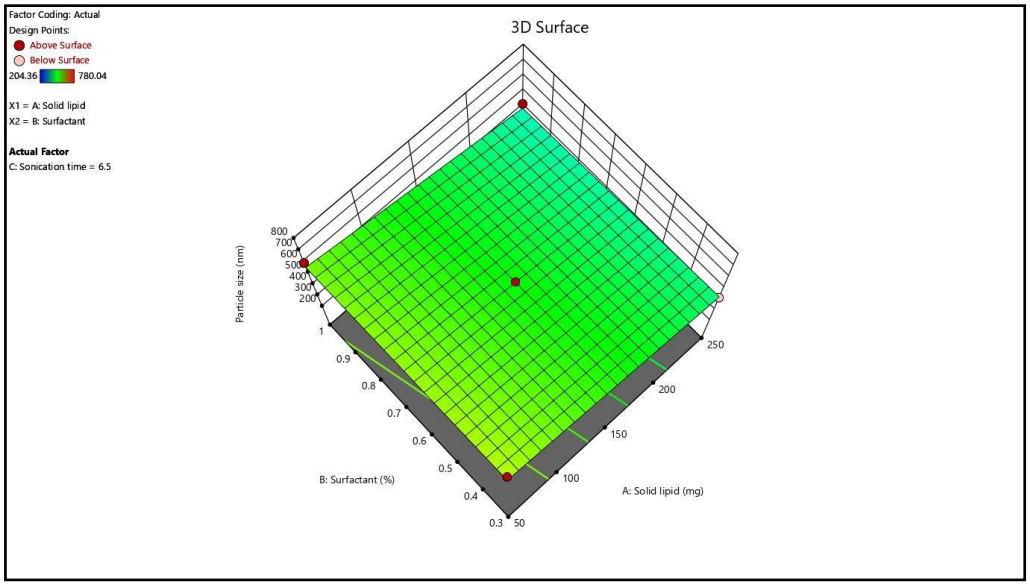


Figure 5: Response surface plot showing combined effect of polymer and surfactant on particle size of NLCs.

A 2FI model was discovered to be significant for particle size with an F value of 119.87and a P value of < 0.0001 when the outcomes of formulations were installed in accordanc.

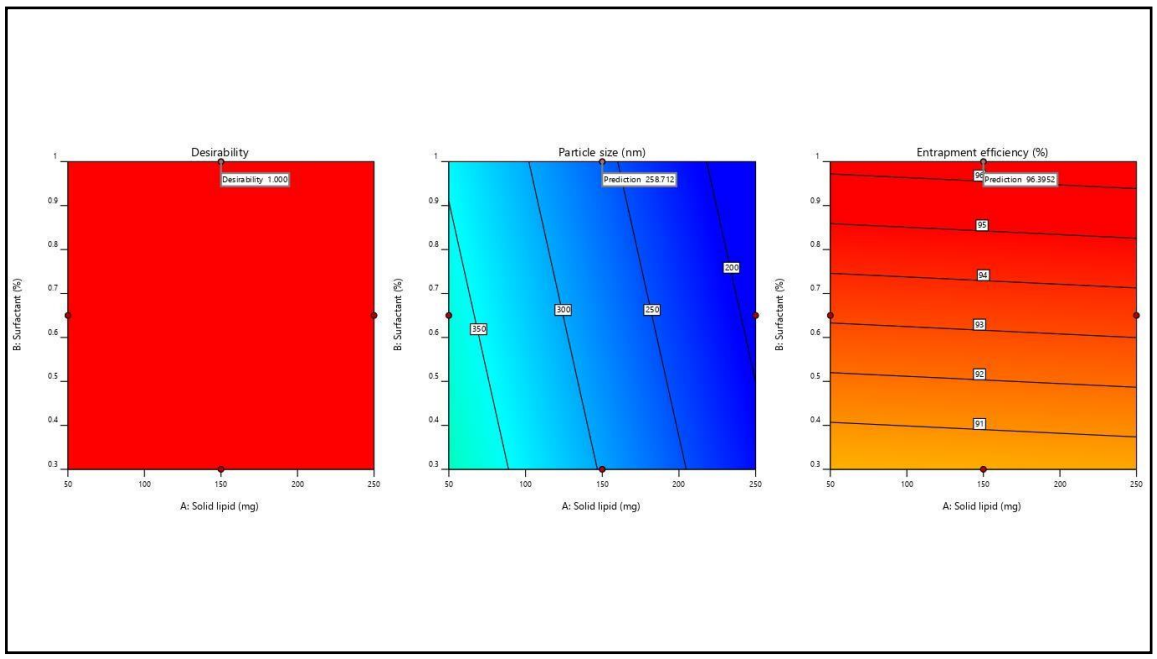


Figure 6: Response surface plot showing prediction data for optimization.

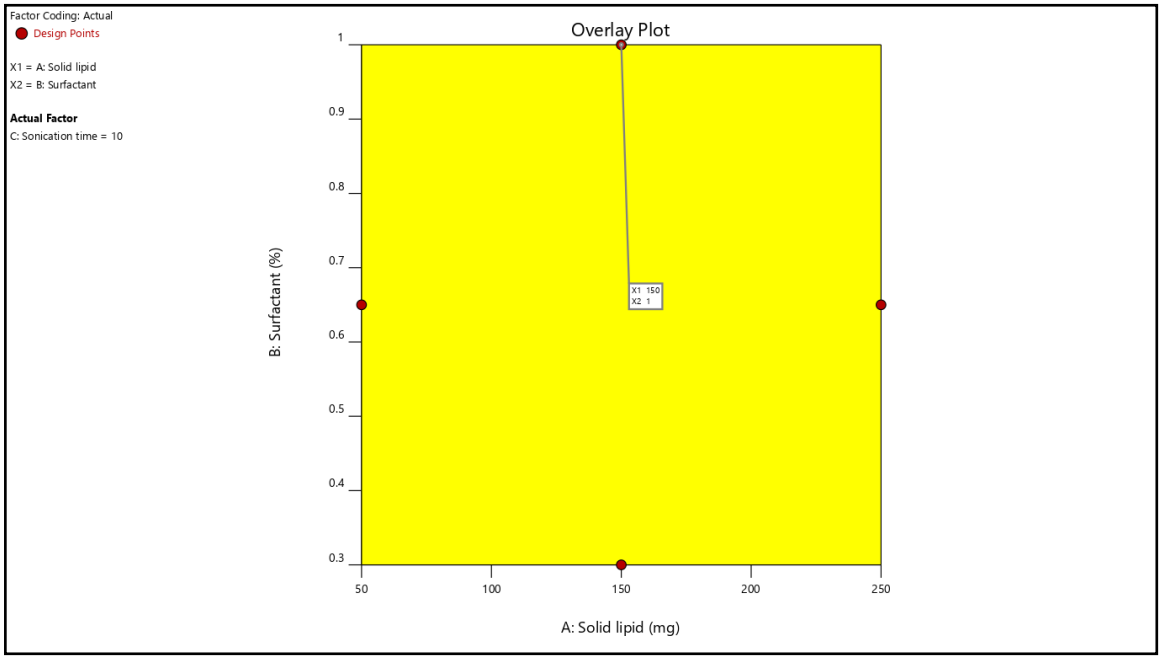


Figure 7: Overlay plot for optimization formulation.

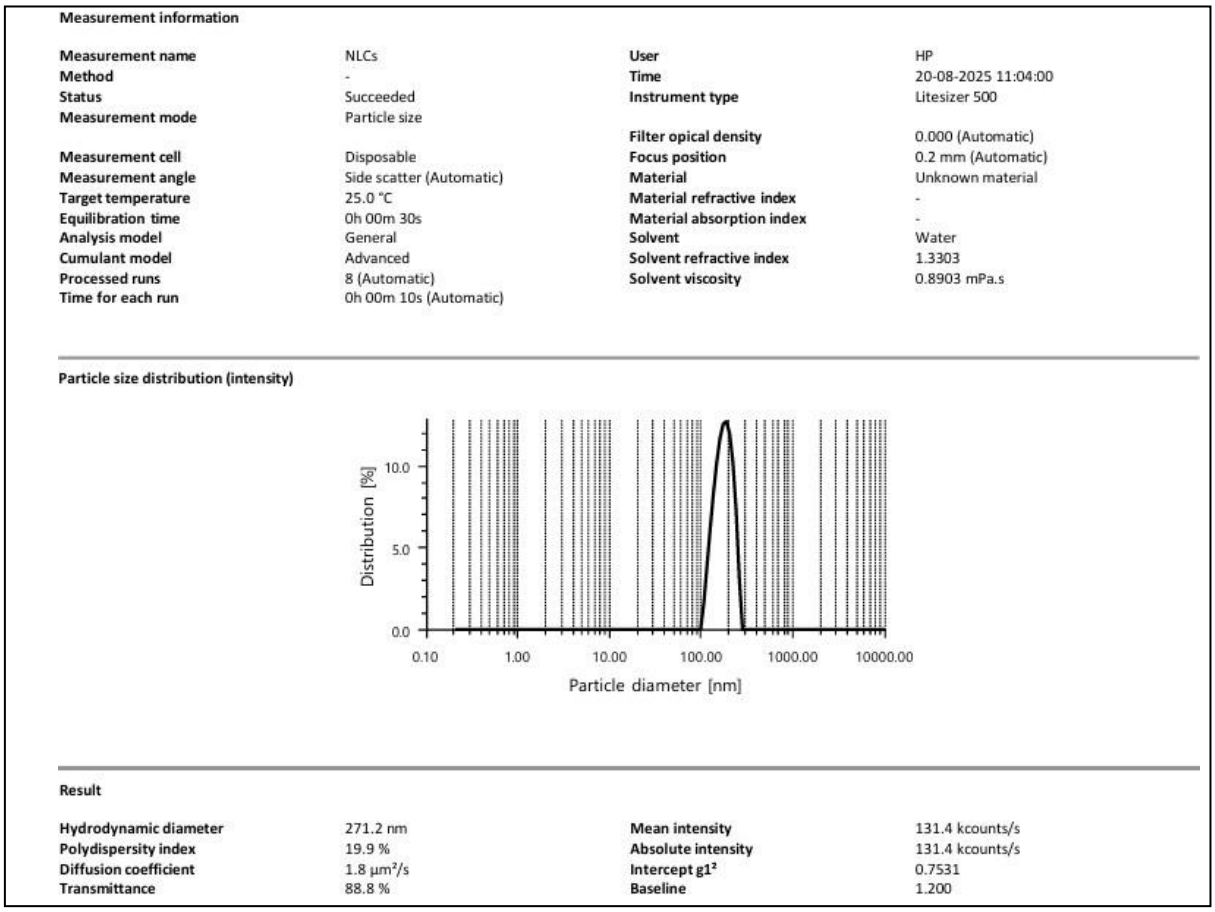


Figure 8: Particle Size.

Particle size

S. No	Formulation	Particle size (Predicted value)	Particle size (Actual value)
1.	NLCs	258.7 nm	271.2 nm

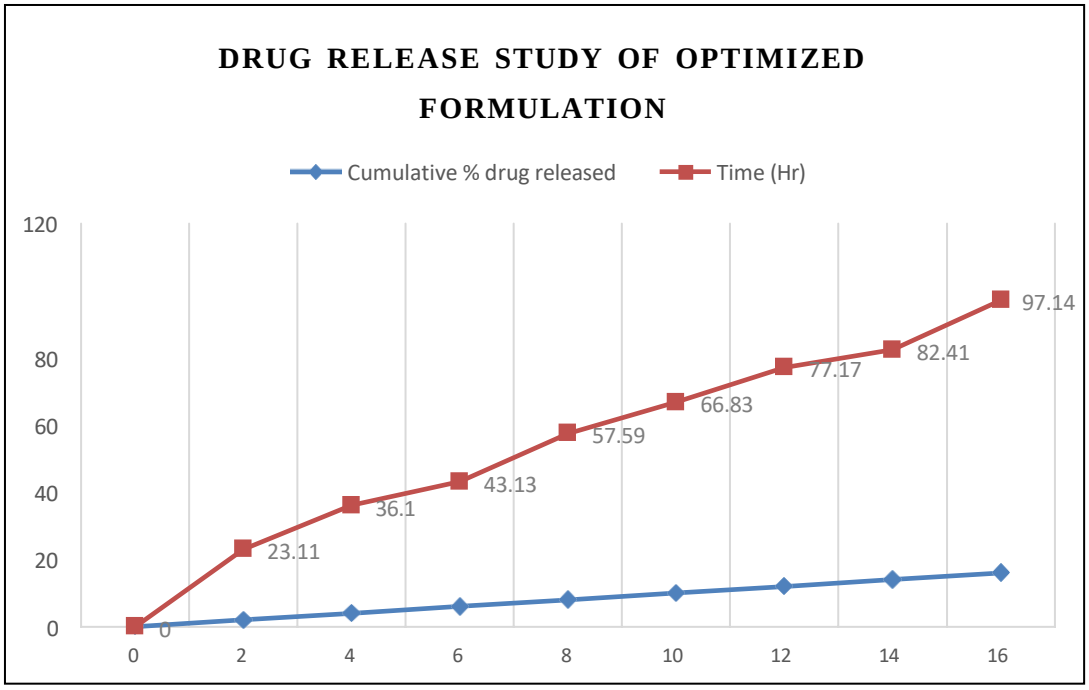


Figure 9: Cumulative % drug released.

SUMMARY

This study aimed to develop and optimize nanostructured lipid carriers (NLCs) for the topical delivery of a combination of Andrographolide and Thiocolchicoside, two pharmacologically active compounds with poor water solubility. Pre-formulation characterization confirmed their organoleptic properties, solubility profiles, pH range, melting points, and UV absorbance peaks, supporting their suitability for nanoparticulate delivery. Using Box-Behnken design (a response surface methodology), the effects of formulation variables solid lipid concentration, surfactant concentration, and sonication time on critical parameters such as particle size and entrapment efficiency were systematically studied. A total of 13 NLCs formulations were evaluated, and statistical analysis identified a quadratic model as the best fit for predicting particle size and a linear model for entrapment efficiency. The optimized formulation contained 150 mg of solid lipid, 1% surfactant, and 10 minutes of sonication, resulting in nanoparticles with a particle size of 271.2 nm (predicted 258.7nm), zeta potential of −59.4 mV indicating excellent stability, and an entrapment efficiency of 95.12% (predicted 96.39%). *In vitro* drug release studies revealed a sustained release over 16 hours, best fitting the zero-order kinetic model, suggesting a concentration-independent release mechanism. Morphological analysis via SEM showed uniform, spherical particles, while stability testing over 90 days under standard and accelerated conditions confirmed the formulation's physical and chemical stability.

CONCLUSION

The results of this study demonstrate the successful formulation of a stable and efficient nanostructured lipid carrier system for the topical co-delivery of Andrographolide and Thiocolchicoside. The use of Box-Behnken design enabled a systematic and cost-effective optimization process, highlighting the significant influence of sonication time and solid lipid concentration on particle size and drug entrapment. The optimized NLCs exhibited desirable nanoscale characteristics, excellent drug loading capacity, and controlled release properties, making them suitable for prolonged therapeutic action at the site of application. The high zeta potential and sustained drug release behavior reinforce the formulation's potential to overcome the limitations associated with the poor solubility of the active agents. Collectively, the findings support the potential of NLCs-based topical formulations as an advanced strategy for enhancing the therapeutic efficacy and bioavailability of hydrophobic drug combinations in localized drug delivery systems.

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