

PREPARATION AND EVALUATION OF NANO FORMULATION CONTAINING THIOCOLCHICOSIDE FOR TOPICAL USE

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Abstract

Thiocolchicoside, a muscle relaxant with limited aqueous solubility, was formulated into nano lipid carriers (NLCs) to enhance solubility, stability, and controlled drug delivery. Preliminary physicochemical characterization confirmed the drug's purity, identity, and suitability for formulation, with a melting point of 191 °C, pH of 7.1, and λ_{max} at 376 nm. NLCs were prepared using heat homogenization and sonication, employing stearic acid, oleic acid, Tween 80, and sodium lauryl sulfate. Five formulations (F1–F5) were evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, morphology, and in vitro drug release. Formulation F1 exhibited the most favorable characteristics, including the smallest particle size (42.53 nm), narrow polydispersity index (27.4%), zeta potential of –13.8 mV, and highest entrapment efficiency (94.56%). SEM analysis confirmed spherical, homogeneous Nanoparticles. In vitro studies demonstrated sustained drug release up to 16 hours, with F1 achieving 93.43% cumulative release, indicating controlled and efficient drug delivery. These findings suggest that thiocolchicoside-loaded NLCs, particularly F1, offer a promising strategy for improving bioavailability, therapeutic efficacy, and patient compliance, supporting their potential for future in vivo and clinical applications.

Keywords: *Thiocolchicoside, Nano lipid carriers (NLCs), Pre-formulation study, Entrapment efficiency, Particle size*

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1. Introduction

Thiocolchicoside is a semi-synthetic derivative of colchicine widely used as a centrally acting muscle relaxant with anti-inflammatory and analgesic properties for the management of painful muscle spasms and musculoskeletal disorders (DrugBank, 2025; Wikipedia, 2025). It exerts its effect primarily through interaction with inhibitory neurotransmitter systems, including competitive antagonism at γ -aminobutyric acid (GABA)_A and glycine receptors, which contributes to muscle relaxation, though its exact mechanism remains complex and associated with potential convulsant activity in susceptible patients (DrugBank, 2025; Wikipedia, 2025). After oral administration, thiocolchicoside shows limited systemic availability due to extensive first-pass metabolism; comparative studies demonstrate significantly lower bioavailability via oral routes than intramuscular administration, reflecting challenges in achieving optimal therapeutic levels (Artusi et al., 2003; Wikipedia, 2025). This pharmacokinetic limitation, combined with its relatively high molecular weight and lipophilicity considerations, underscores the need for advanced formulation strategies to enhance its solubility, permeability, and overall therapeutic performance (Artusi et al., 2003; Singh et al., 2013).

Nanostructured lipid carriers (NLCs) represent a second-generation lipid-based nanocarrier system engineered to overcome the constraints of conventional formulations, especially for poorly soluble and lipophilic drugs. NLCs are composed of a mixture of solid and liquid lipids that create an irregular lipid matrix, enhancing drug loading capacity, minimizing drug expulsion, and enabling controlled and sustained release profiles compared to rigid crystalline systems such as solid lipid nanoparticles (SLNs) (Patil et al., 2025; Prema et al., 2025; Jeyaprabha & Saravanan, 2025). These carriers improve the physical stability of formulations, protect encapsulated drugs from degradation, and can enhance absorption through various administration routes including oral, topical, transdermal, and parenteral pathways (Prema et al., 2025; Latifah et al., 2025; Adv Pharm Bull, 2020). Moreover, NLCs leverage biocompatible and biodegradable lipids, facilitating better interaction with biological membranes and potentially increasing therapeutic efficacy while reducing systemic side effects (Patil et al., 2025; Prema et al., 2025; Asian J Pharm Educ Res, 2024).

In this study, thiocolchicoside was subjected to comprehensive pre-formulation characterization followed by incorporation into NLCs to assess the potential of this nanocarrier system in enhancing drug solubility, stability, encapsulation, and in vitro release

behaviour, thereby addressing inherent challenges in delivering thiocolchicoside through conventional dosage forms.

2. Preliminary physicochemical analysis of drug

To determine whether thiocolchicoside was suitable for NLC formulation, a preliminary physicochemical investigation was performed. Melting point and organoleptic characteristics were assessed for purity and identification. FTIR and UV-visible spectroscopy were used to establish structural integrity and determine λ_{max} , while solubility was examined in a variety of solvents and lipids. For the creation of NLC, these research supplied baseline data (Camp et al., 2015).

2.1 Sensory Evaluation of Thiocolchicoside

During the pre-formulation stage, a sensory evaluation was carried out to determine the initial quality of thiocolchicoside. According to typical descriptions, the substance was found to be a pale-yellow crystalline powder with no distinctive smell upon visual and olfactory inspection. Despite being qualitative, this quick and affordable evaluation aids in verifying raw material integrity and identifying potential contamination or degradation prior to additional analysis and formulation (Gautam et al., 2010).

2.2 Solubility determination of the drug

To aid in dissolving, excess thiocolchicoside was added to distilled water, ethanol, methanol, and certain lipids. The mixture was then well shaken. After the combinations were filtered to eliminate any medicine that had not dissolved, the clear filtrates were appropriately diluted and their solubility was assessed using UV-visible spectrophotometry. The best solvents for formulation development were found in this study (Veseli et al., 2019).

2.3 pH determination of the drug

To create a homogenous solution or suspension, a known quantity of thiocolchicoside was dissolved in distilled water and gently agitated. After readings had stabilized, the pH was measured using a digital pH meter that had been calibrated using standard buffers and appropriate electrode washing. This evaluation aided in determining the drug's pH environment, which is crucial for both formulation appropriateness and stability (Samuelsen et al., 2021).

2.4 Melting point determination

The capillary tube method was used to determine the melting point of thiocolchicoside. A sealed capillary tube containing finely powdered medication was put within a melting point

equipment. The melting range was determined by gradually raising the temperature and recording the beginning and end of melting. The identification and purity of the medication were verified using this analysis (Mao et al., 2016).

2.5 Determination of Maximum Wavelength (λ_{\max})

➤ **Preparation of Thiocolchicoside standard stock solution in methanol**

To find the maximum wavelength (λ_{\max}) of thiocolchicoside, a standard solution was made in methanol. Following an appropriate dilution, the solution was scanned across the 200–400 nm range using a UV–Visible spectrophotometer, using methanol as the blank. For additional spectrophotometric investigation, the wavelength exhibiting the highest absorption was noted as λ_{\max} .

➤ **Lambda max analysis**

Using methanol as the blank, a diluted methanolic solution of thiocolchicoside was scanned over 200–400 nm in a UV–visible spectrophotometer to estimate the drug's λ_{\max} . For precise spectrophotometric quantification in further analyses, the wavelength that corresponded to the greatest absorbance peak was noted as λ_{\max} (De et al., 2016).

➤ **Calibration curve analysis**

To create a calibration curve for thiocolchicoside, a methanolic stock solution was serially diluted to create standard solutions. Using methanol as the blank, the absorbance of each concentration was measured at the predefined λ_{\max} . The calibration equation and correlation coefficient (R^2) were obtained by doing linear regression analysis on a concentration versus absorbance plot. In later formulation and dissolving investigations, this curve was used as a reference to quantify thiocolchicoside (De et al., 2016).

2.6 Functional group identified by FTIR analysis

The structural integrity of thiocolchicoside was evaluated using FTIR spectroscopy. The medication was combined with dry KBr, compacted into a pellet, and scanned between 4000 and 400 cm^{-1} . To verify the drug's identity, functional groups, and chemical consistency, the distinctive absorption peaks were examined and contrasted with reference spectra (Enders et al., 2021).

3. Formulation of Nano lipid carriers

The heat homogenization process was used to create NLCs laden with barbaloin and berberine. The medications were dissolved in ethanol, combined with a lipid phase of oleic and stearic acid in acetone, and then gradually added to an aqueous phase that contained sodium lauryl sulfate and Tween 80. After homogenizing and sonicating the liquid at 85 °C to create NLCs, the mixture was cooled to solidify the lipids. To create a stable NLC powder, the dispersion was lyophilized. Table 3 displays formulation details (Kim et al., 2019; Arora et al., 2017).

Table: 3 Composition of Nano lipid carriers

S. No	Formulation code	Drugs (100 mg)	Ethanol (ml)	Acetone (ml)	Stearic acid (mg)	Oleic acid (%)	Tween 80 (%)	Sodium lauryl sulfate (mg)	Temperature (°C)
1.	NLC F 1	1:1	10.0	10.0	50	0.2	0.1	50.0	85 °C
2.	NLC F 2	1:1	10.0	10.0	100	0.2	0.2	50.0	85 °C
3.	NLC F 3	1:1	10.0	10.0	150	0.2	0.3	50.0	85 °C
4.	NLC F 4	1:1	10.0	10.0	200	0.2	0.4	50.0	85 °C
5.	NLC F 5	1:1	10.0	10.0	250	0.2	0.5	50.0	85 °C

4. Evaluation parameter of drug loaded nano lipid carriers

4.1 Physical appearance

Drug-loaded NLCs were visually inspected for color, homogeneity, clarity, and any indications of phase separation or aggregation. Turbidity, sedimentation, or visible particles were the main subjects of observations made in normal lighting. Good physical stability of the formulation was indicated by a uniform, smooth, milky, or transparent dispersion without precipitation (Mishra et al., 2016).

4.2 Particle size analysis

Using a particle size analyzer and dynamic light scattering (DLS), the drug-loaded NLCs' particle size was ascertained. The mean particle size and polydispersity index (PDI) of diluted samples were measured at room temperature. The homogeneity, stability, and possible

bioavailability of nanoparticles—all essential for consistent NLC performance—were evaluated using particle size and PDI (Shekunov et al., 2007).

4.3 Zeta potential analysis

The stability and surface charge of drug-loaded NLCs were evaluated using zeta potential analysis. A Malvern Zetasizer, which monitored particle mobility under an applied electric field, was used to examine diluted samples. The degree of electrostatic repulsion between particles and, consequently, the formulation's stability were assessed by the zeta potential values. Reliability was ensured by taking many measurements and reporting the mean value (Shekunov et al., 2007).

4.4 Scanning Electron Microscopy (SEM) analysis

The surface morphology of drug-loaded NLCs was investigated using scanning electron microscopy (SEM). To create high-resolution photographs, dried materials were covered with a thin conductive layer and examined under a concentrated electron beam. SEM confirmed the nanoscale structure and general quality of the NLC formulation by providing qualitative data on particle shape, surface texture, size uniformity, and aggregation (Mohammed & Abdullah, 2018).

4.5 Entrapment efficiency determination

The degree of berberine and barbaloin incorporation into NLCs was assessed using entrapment efficiency (EE). UV-visible spectrophotometry with verified calibration curves was used to examine the supernatant after formulations were ultracentrifuged to separate free drug from nanoparticles. EE, a metric of drug loading essential for controlled release, efficacy, and NLC stability, was computed by comparing the initial drug amount with the unencapsulated drug (Sadeghi et al., 2023).

5. *In vitro* drug release study

The dialysis bag diffusion method was used to investigate the *in vitro* drug release of thiocolchicoside-loaded NLCs. The NLC formulation was submerged in 100 mL of PBS (pH 7.4) at 37 ± 0.5 °C while being agitated at 100 rpm in a dialysis membrane. Samples were taken out at prearranged intervals, refilled with new PBS, and spectrophotometrically measured at the λ_{max} of thiocolchicoside. To assess the NLC formulation's sustained-release profile, cumulative drug release was computed using calibration curves and plotted over time

(Paswan and Saini 2021).

6. RESULTS AND DISCUSSION

6.1 Pre-formulation study of drug

Table 4: Sensory Evaluation of Thiocolchicoside

Drug	Organoleptic properties	Observation
Thiocolchicoside	Color	Yellow to yellowish powder
	Odor	Odorless or faint characteristic odor
	Appearance	Fine crystalline or amorphous powder
	State Thiocolchicoside	Solid

Description

Thiocolchicoside was found to be a yellow to yellowish, fine, crystalline or amorphous powder that was solid, odorless, or had a faint, distinct fragrance. These organoleptic characteristics are consistent with conventional descriptions and offer crucial first data for medication identification, formulation, and quality evaluation.

6.1.1 Solubility study

Table 5: Solubility study of Thiocolchicoside

Drug	Solvents	Observation/Inference
Thiocolchicoside	Water	Slightly soluble
	Ethanol	Freely soluble
	Methanol	Freely soluble
	Chloroform	Sparingly soluble
	DMSO	Freely soluble

Description

Thiocolchicoside shown moderate solubility in chloroform but good solubility in organic solvents such ethanol, methanol, and DMSO. These findings aid in the selection of solvents and formulation techniques that enhance the drug's therapeutic efficacy and bioavailability.

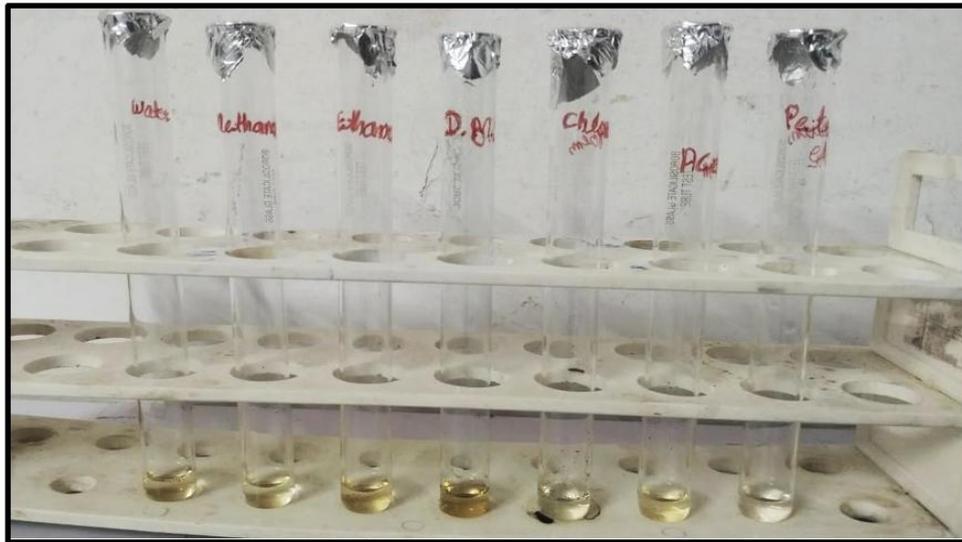


Figure 7: Solubility study of Drug

6.1.2 pH determination

Table 6: pH of Thiocolchicoside

Drug	Observed Range	Reference Range
Thiocolchicoside	7.1	6.0 to 7.5

Description

Within the standard range of 6.0–7.5, the pH of the thiocolchicoside solution was 7.1, indicating near-neutrality. The drug's chemical stability, compatibility with biological tissues, and appropriateness for formulation without significant pH change are all supported by this advantageous pH.



Figure 8: digital pH meter

6.1.3 Melting point

Table 7: Melting point of Thiocolchicoside

Drugs	Observed	Reference
Thiocolchicoside	191 °C	190 -198 °C

Description

Thiocolchicoside's purity, identity, and thermal stability were confirmed by its melting point of 191 °C, which fell within the reference range of 190–198 °C. This consistency shows that the medication is free from serious contamination or deterioration and is appropriate for additional formulation development.



Figure 9: Melting point Apparatus

6.1.4 Lambda max of Thiocolchicoside

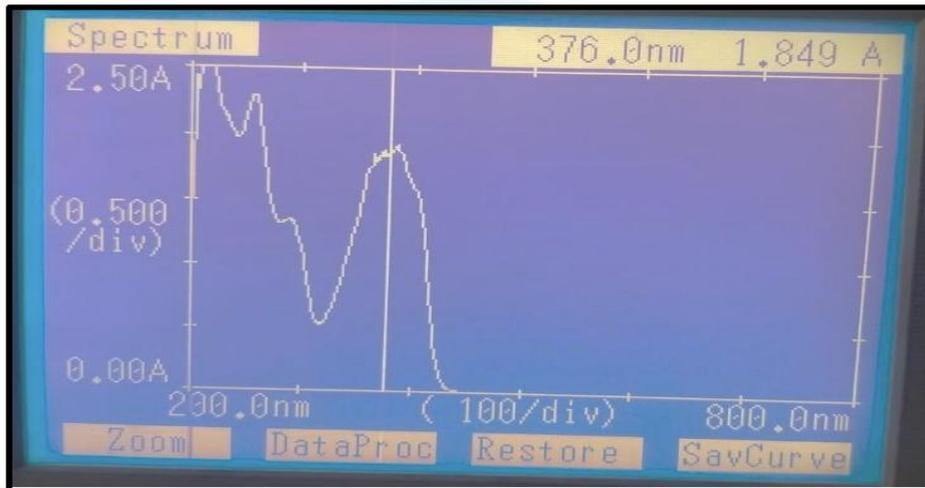


Figure 10: Lambda max of Thiocolchicoside (376.0 nm)

Table 8: Lambda max

S. No	Drug	UV absorption maxima (Lambda max)
1.	Thiocolchicoside	376.0 nm

Description

Thiocolchicoside's UV-visible measurement revealed a maximum absorbance (λ_{max}) at 376 nm (Figure 10, Table 8). For sensitive and precise quantitative drug determination in formulations and dissolving experiments, this wavelength is ideal.

6.1.5 Calibration curve of Thiocolchicoside

Table 9: Calibration curve

Concentration ($\mu\text{g/ml}$)	Absorbance
5	0.255
10	0.365
15	0.548
20	0.741
25	0.954
30	1.090
Mean	0.6588
SD	0.329084

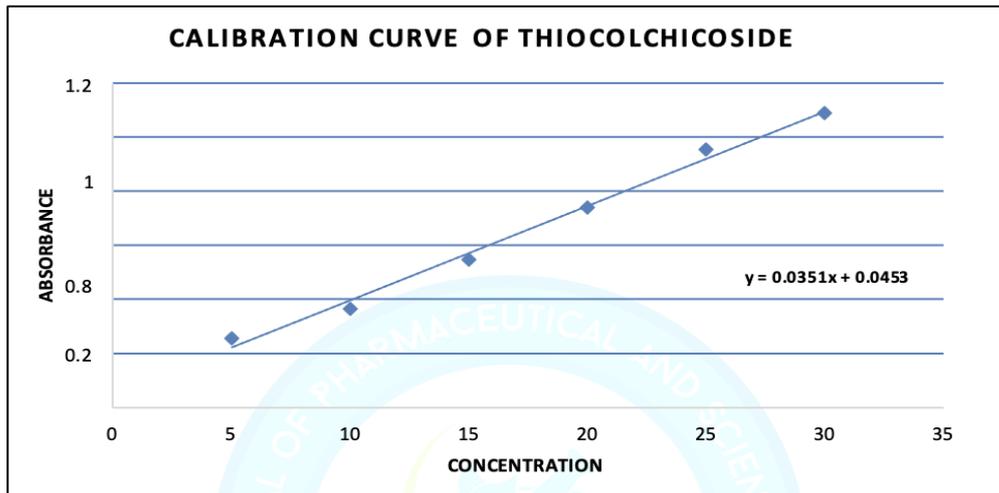


Figure 11: Calibration curve of Thiocolchicoside

Standard solutions ranging from 5 to 30 µg/mL were used to create the thiocolchicoside calibration curve. According to Table 9 and Figure 11, absorbance rose in direct proportion to concentration, exhibiting a strong linear relationship ($y = 0.0351x + 0.0453$, $R^2 = 0.993$). This linearity validates the precision and dependability of the approach, making it appropriate for accurate thiocolchicoside quantification in a variety of pharmaceutical formulations and analytical investigations.

6.1.6 Functional group identified by Infra-Red spectroscopy

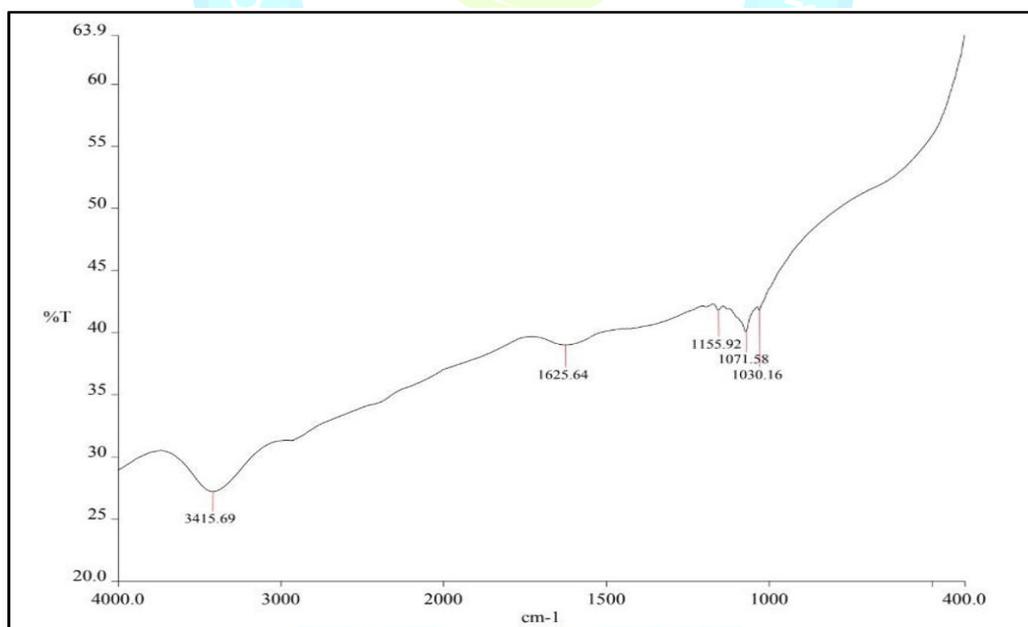


Figure 12: FTIR study of Thiocolchicoside

6.2 Characterization of NLCs formulation

6.2.1 Physical appearance

Table 10: Physical appearance

S. No	Parameter	Result
1.	Colour	Pale yellow
2.	Odour	Mild characteristic odour
3.	Appearance	Clear and transparent
4.	Homogeneity	Uniform, no visible particles

6.2.2 Particle size determination

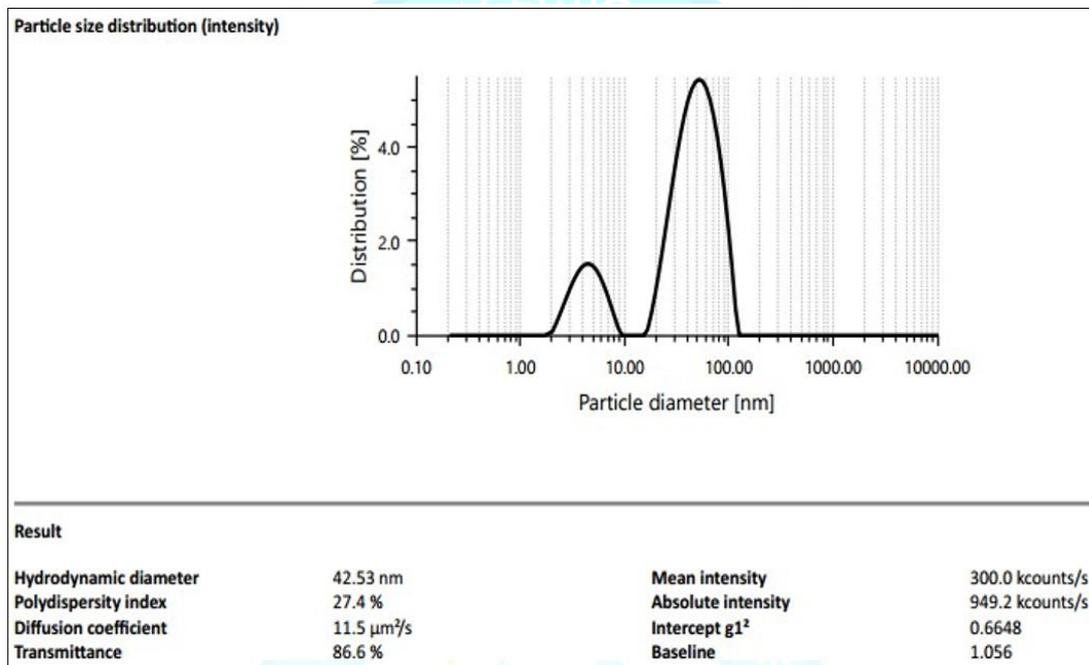


Figure 13: Particle size (F1)

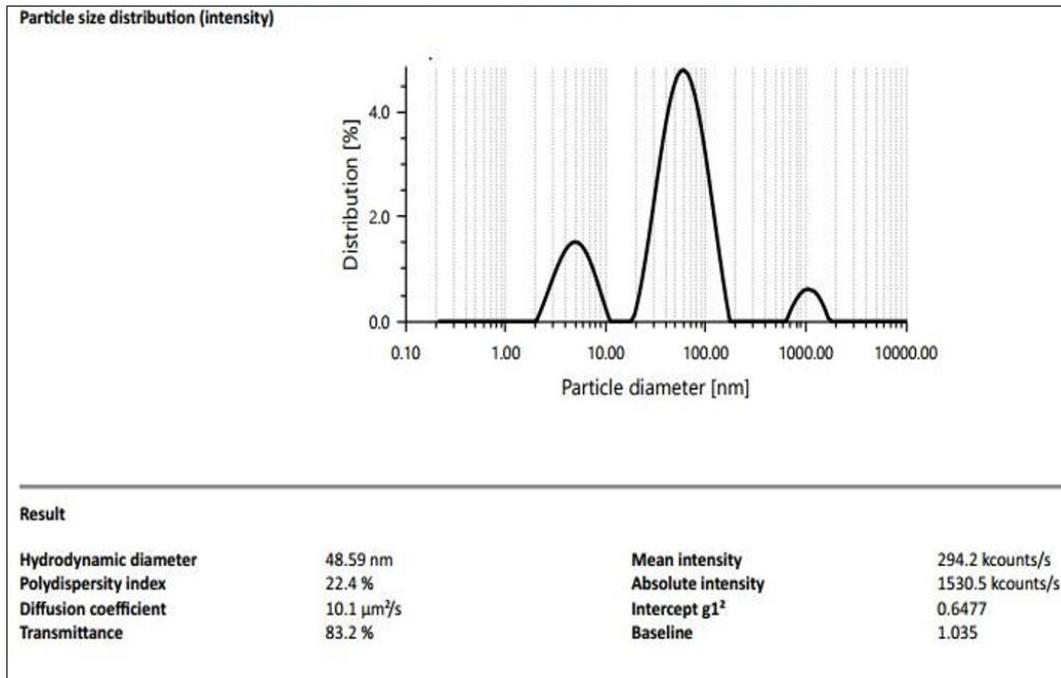


Figure 14: Particle size (F2)

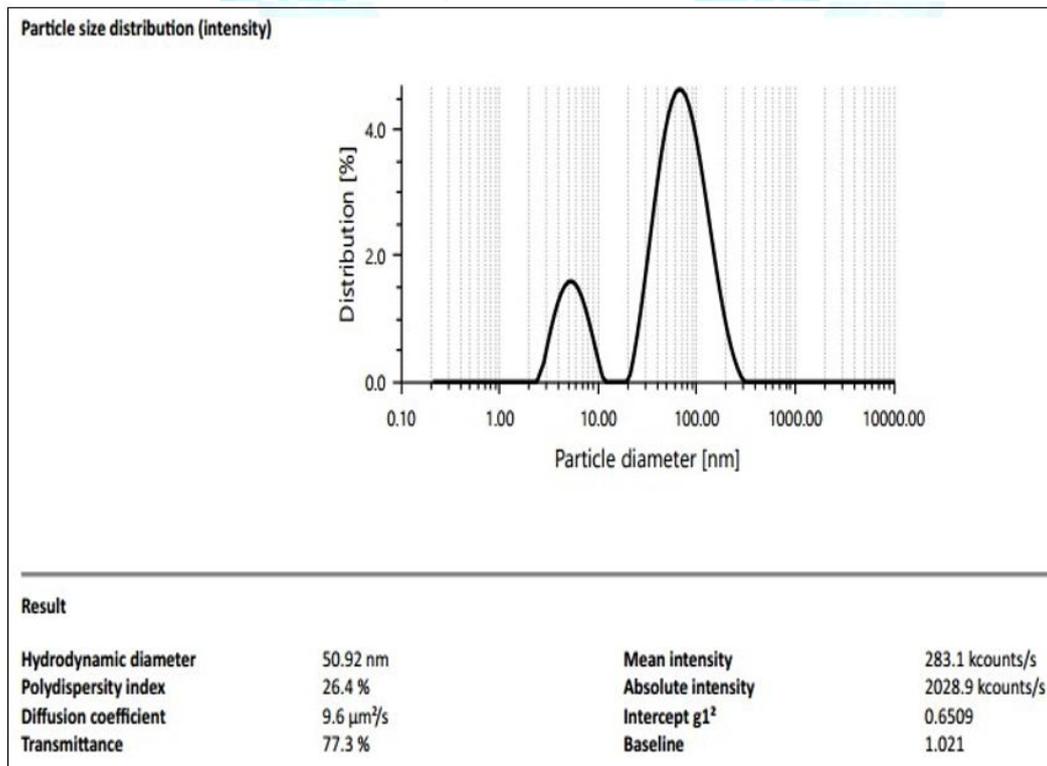


Figure 15: Particle size (F3)

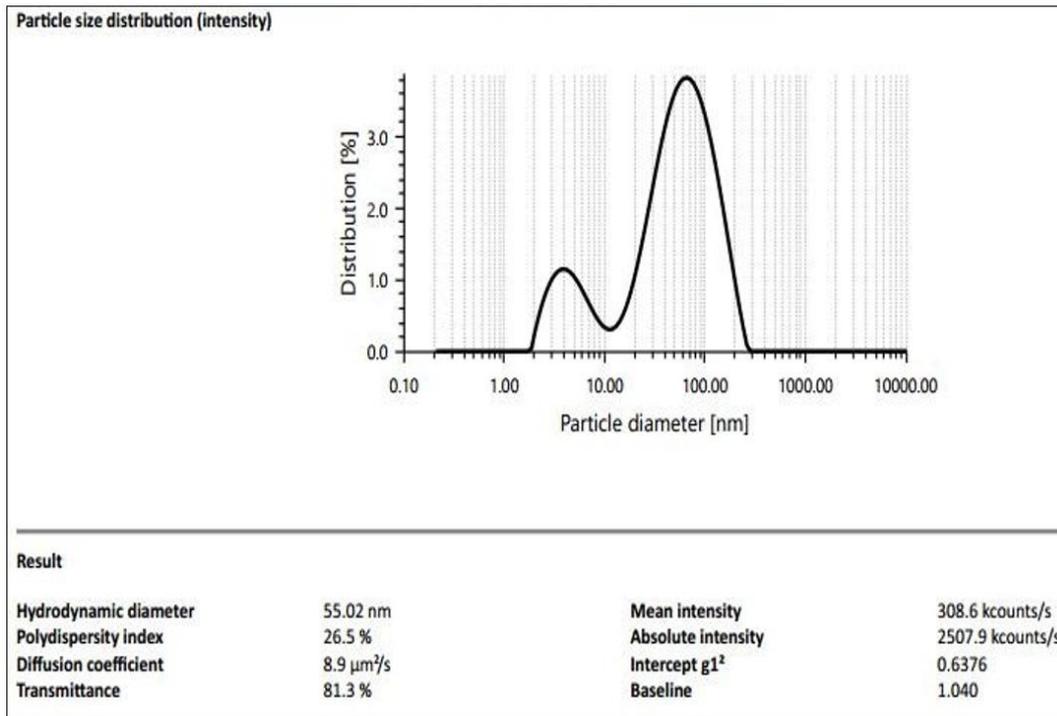


Figure 16: Particle size (F4)

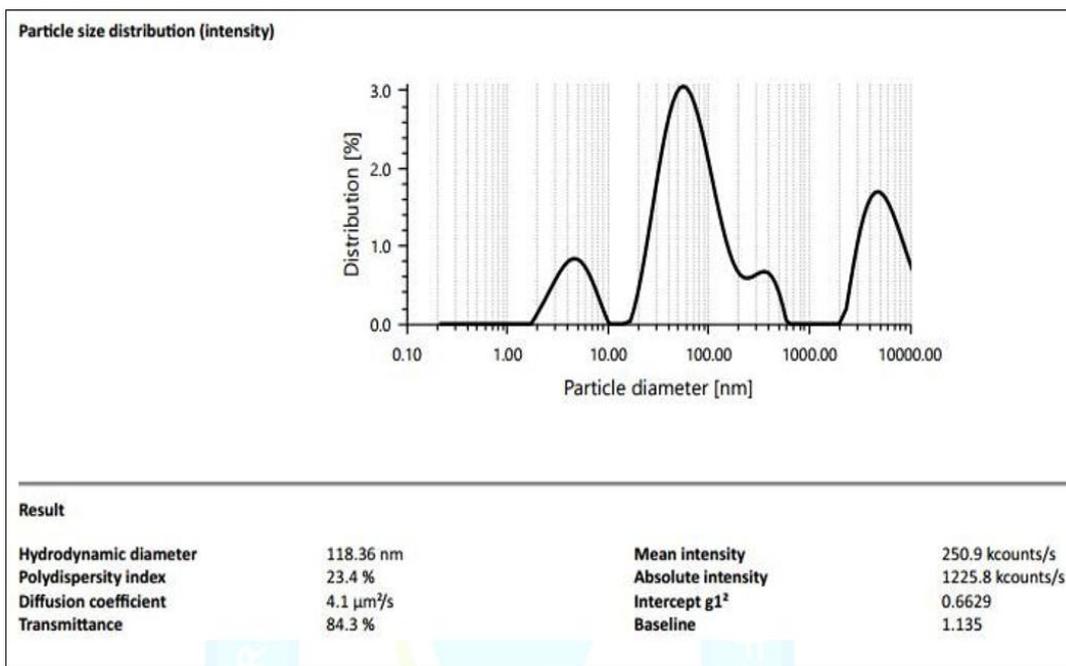


Figure 17: Particle size (F5)

Table 11: Particle size of drug loaded nano lipid carrier formulation

S. No	Formulation code	Particle size (nm)	PI Value %
1.	NLCS (F1)	42.53	27.4
2.	NLCS (F2)	48.59	22.4
3.	NLCS (F3)	50.92	26.4
4.	NLCS (F4)	55.02	26.5
5.	NLCS (F5)	118.36	23.4

Description

The drug-loaded NLC formulations (F1–F5) had particle sizes ranging from 42.53 to 118.36 nm, and size homogeneity was reflected in the corresponding polydispersity index (PI) values (Table 13). With a PI of 27.4% and the smallest particle size (42.53 nm), Formulation F1 demonstrated good homogeneity and a narrow distribution, which can improve skin penetration, stability, and solubility. The biggest size (118.36 nm) was displayed by F5, which can have an impact on performance. Because of its favorable nanoscale size and homogeneity, which enable enhanced therapeutic efficacy, F1 was deemed ideal overall.

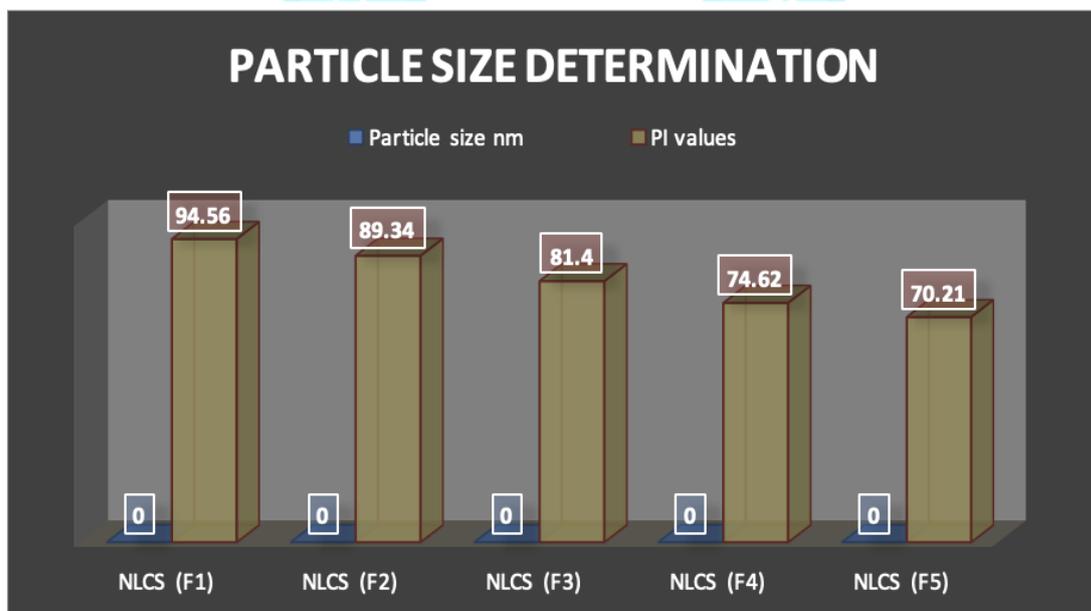


Figure 18: Graphical Data of Particle Size Determination

6.2.3 Zeta potential determination

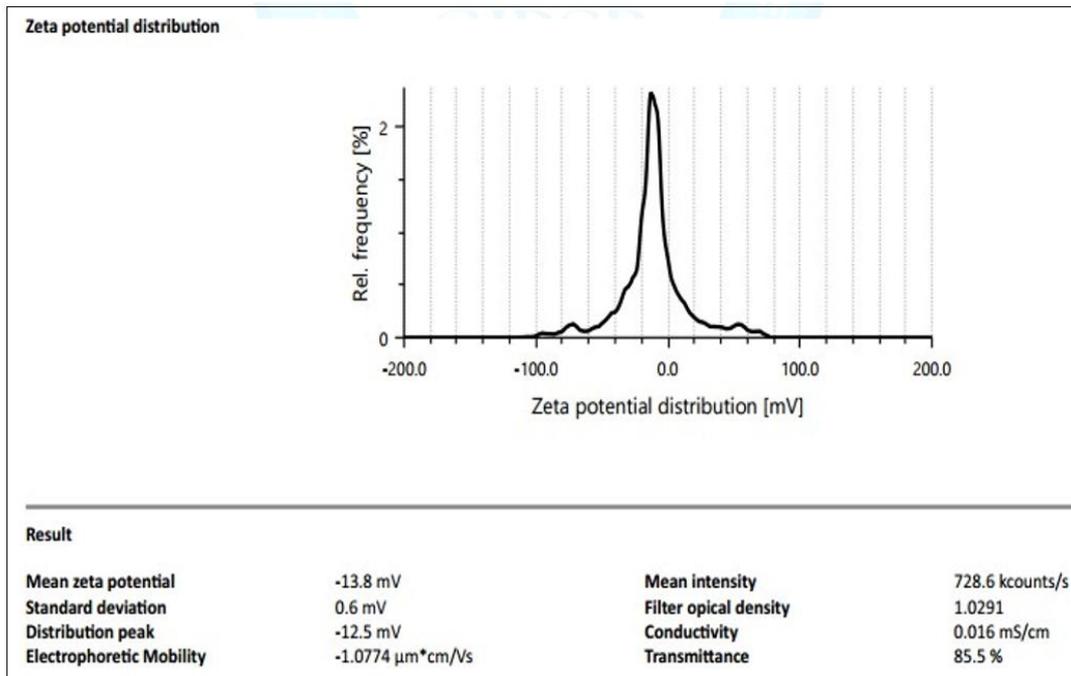


Figure 19: Zeta potential (F1)

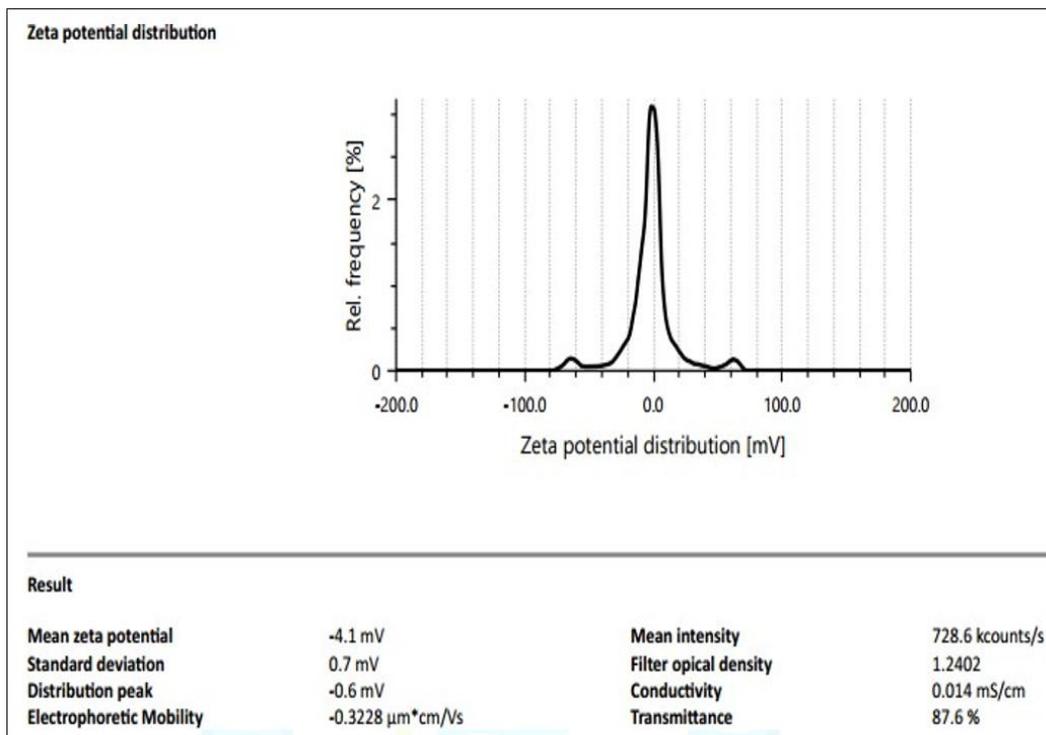


Figure 20: Zeta potation (F2)

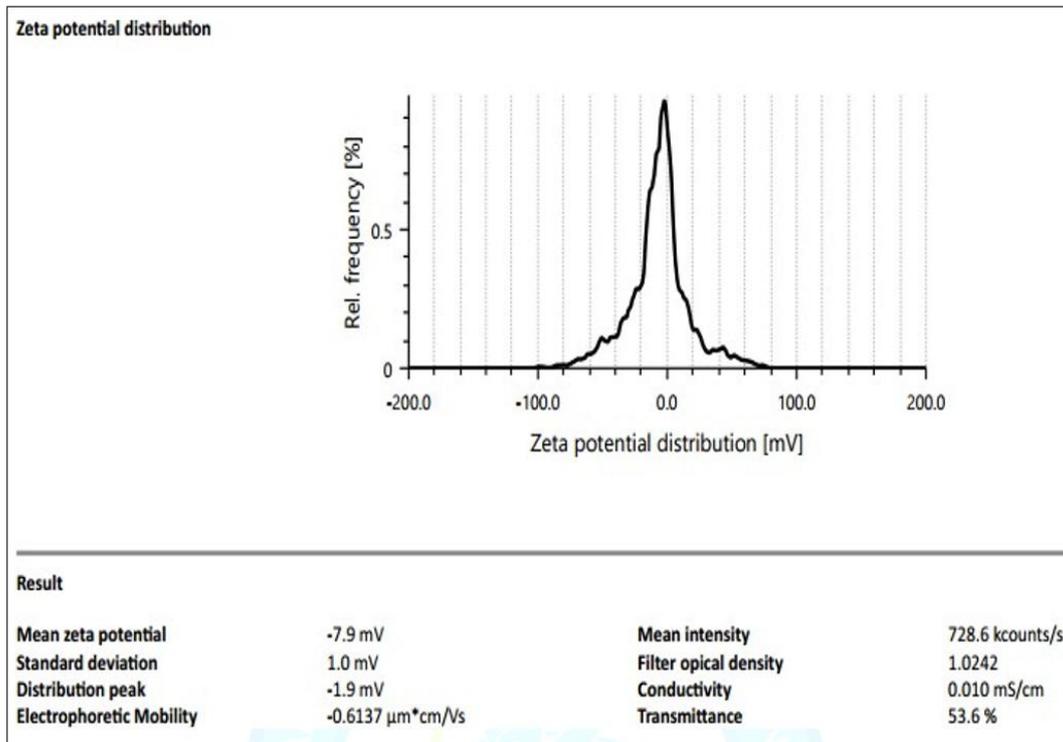


Figure 21: Zeta potation (F3)

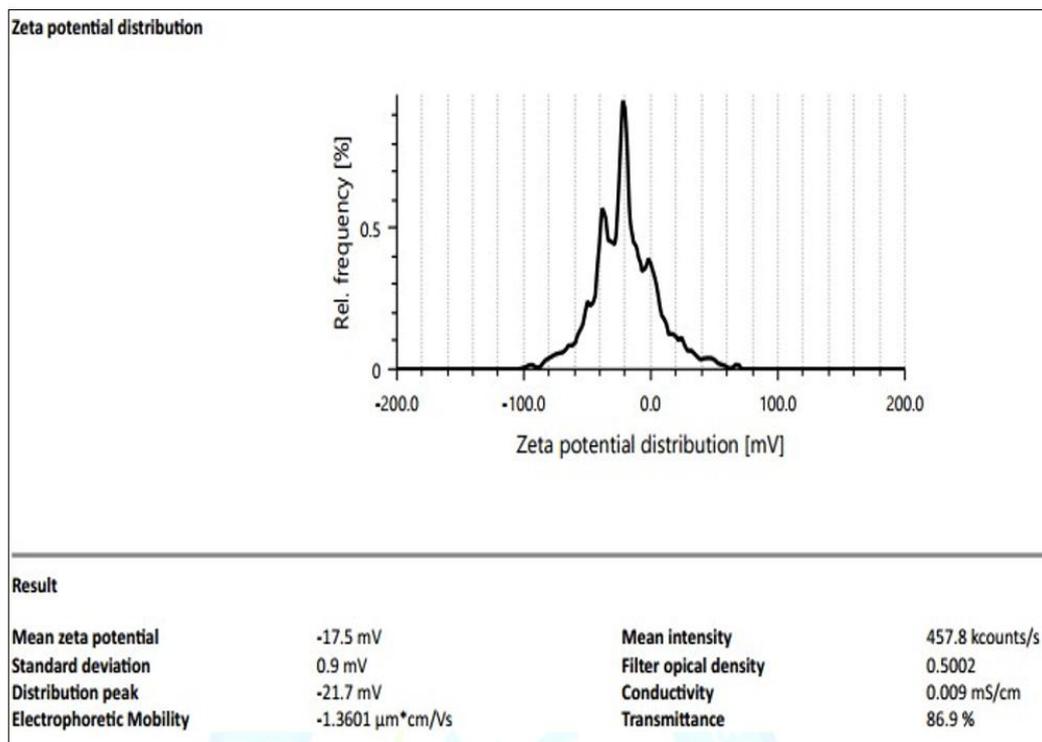


Figure 20: Zeta potation (F4)

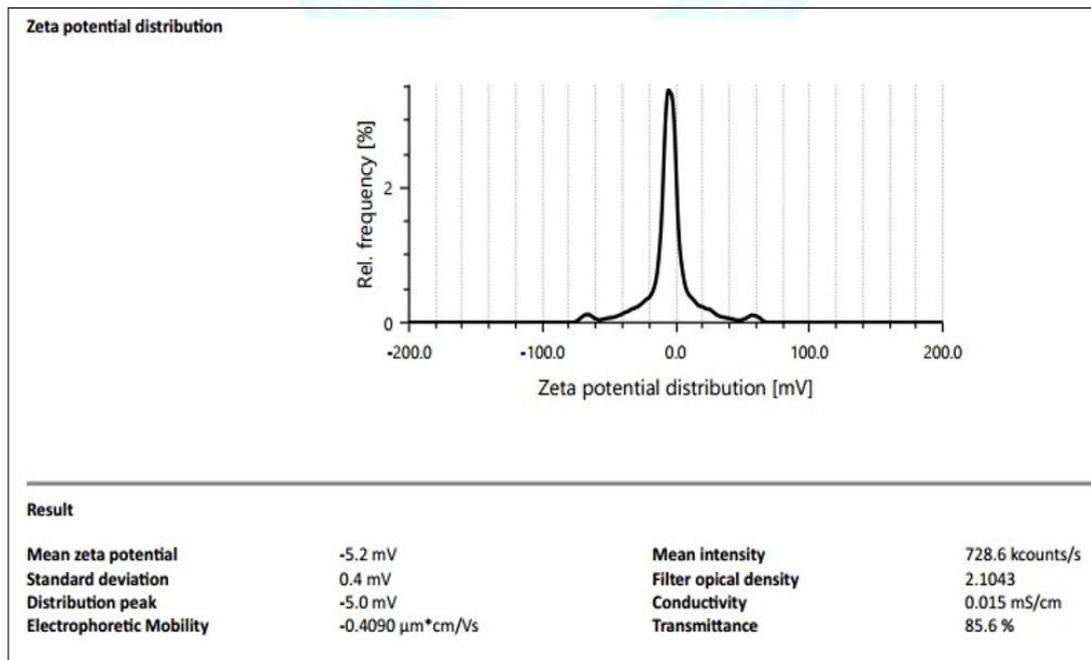


Figure 22: Zeta potation (F5)

Table 12: Zeta potential of drug loaded nano lipid carrier formulation

S. No	Formulation code	Zeta potential
1.	NLCS (F1)	-13.8 mV
2.	NLCS (F2)	-4.1 mV
3.	NLCS (F3)	-7.9 mV
4.	NLCS (F4)	-17.5 mV
5.	NLCS (F5)	-5.2 mV

Description

Zeta potential investigation of NLC formulations (F1–F5) revealed values between –4.1 mV and –17.5 mV (Table 14). With a zeta potential of –13.8 mV, F1 demonstrated moderate stability and enough electrostatic repulsion to stop aggregation. F1 offered the best balance of particle size, charge, and dispersion stability, although F4 had a slightly larger negative value (-17.5 mV). Weaker repulsion and aggregation potential are suggested by lower zeta potentials in F2, F3, and F5. F1 was therefore judged to be the most stable formulation, supporting improved performance and long-term suspension stability.

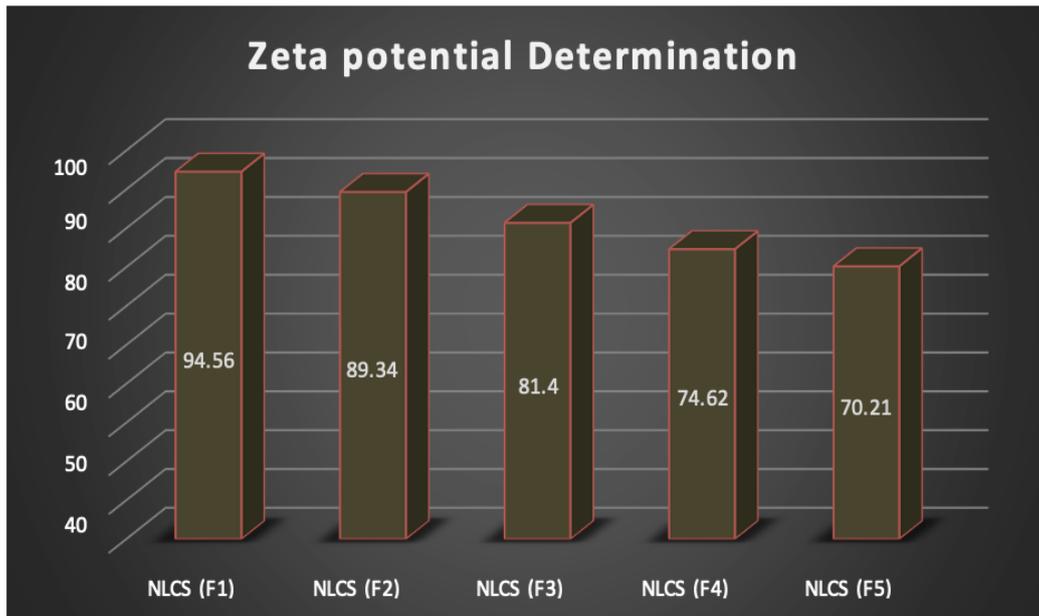


Figure 24: Graphical Data of Particle Size Determination

6.2.4 Entrapment efficacy determination

Table 13: Entrapment efficacy

S. No	Formulations (F1-F5)	Entrapment efficacy (%)
1.	NLCS (F1)	94.56
2.	NLCS (F2)	89.34
3.	NLCS (F3)	90.40
4.	NLCS (F4)	74.62
5.	NLCS (F5)	70.21

Description

Table 13 illustrates the variation in the entrapment efficiency of NLC formulations (F1–F5). With an entrapment efficiency of 94.56%, F1 demonstrated the best drug incorporation and the least amount of loss during formulation. F4 and F5, on the other hand, demonstrated lower efficiencies of 74.62% and 70.21%, most likely as a result of structural instability or poorer lipid–drug interactions. All things considered, F1 was the best formulation, promoting enhanced therapeutic efficacy and prolonged drug release.

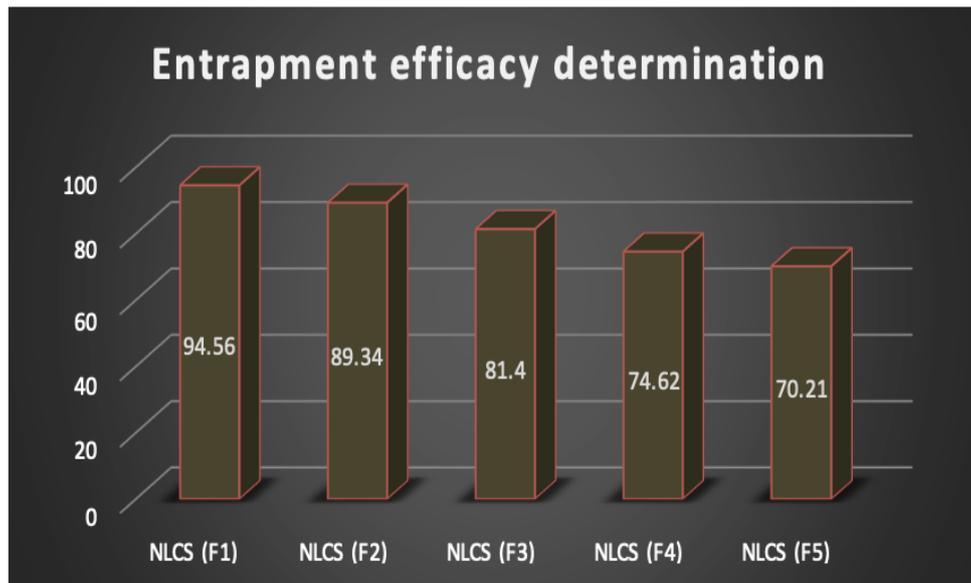


Figure 25: Graphical Data of Entrapment efficacy

6.2.5 Scanning electron microscope (SEM)

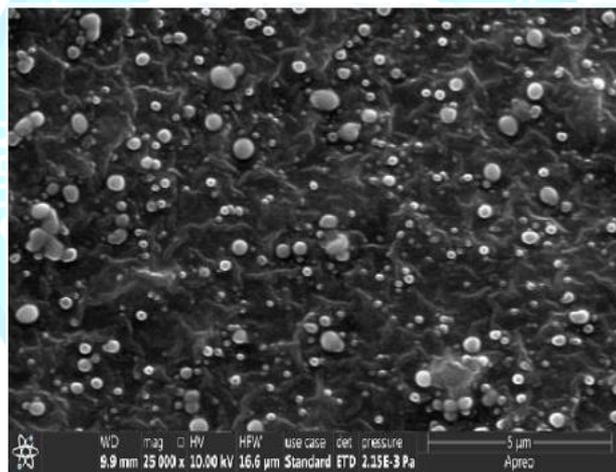


Figure 26: Scanning electron microscope (SEM)

Description

Excellent physical stability and homogeneity were demonstrated by the SEM analysis of the prepared NLCs, which showed homogenous, spherical particles with smooth surfaces and no indications of aggregation. Predictable release profiles and effective drug encapsulation are supported by the uniform particle size and shape. These NLCs' eligibility for pharmaceutical applications is confirmed by the smooth lipid matrix shown, which is advantageous for regulated drug delivery and may improve circulation time, targeted distribution, and overall therapeutic efficacy.

6.3 In-vitro drug release of all

formulation Table 14: In-vitro drug release studies

S. No	Ti me (hr s)	Cumulative % drug release study				
		NLCs F 1	NLCs F 2	NLCS F 3	NLCs F 4	NLCs F 5
1	0	0	0	0	0	0
2	2	28.82	15.53	20.65	22.63	26.36
3	4	37.56	29.86	32.72	30.75	33.21
4	6	44.80	30.76	33.39	35.29	39.14
5	8	60.19	47.57	40.52	42.60	57.55
6	10	68.72	60.80	63.35	66.31	67.89
7	12	79.41	73.29	71.23	69.25	75.17
8	14	84.11	81.25	81.21	82.21	82.41
9	16	93.43	88.94	91.11	90.19	92.25

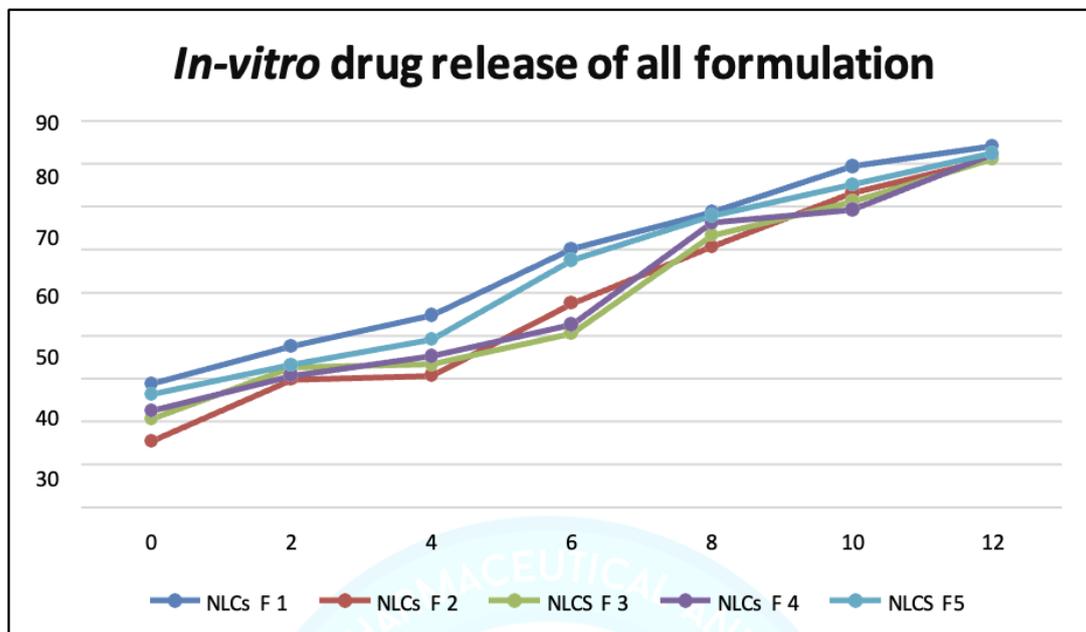


Figure 27: In-vitro drug release studies of all formulation

Description

Out of all the NLCs, formulation F1 had the best profile, according to the in vitro drug release analysis (Table 14, Figure 26). F1 showed a continuous release that reached 93.43% cumulative release at 16 hours after an initial burst release of 28.82% at 2 hours, facilitating a quick therapeutic onset. Effective drug encapsulation and regulated diffusion from the lipid matrix are reflected in this pattern. F1 outperforms other formulations because to its balanced rapid and delayed release phases, underscoring its promise as an optimal NLC for efficient drug delivery.

7. Conclusion

The present study successfully developed and characterized thiocolchicoside-loaded nano lipid carriers (NLCs) to improve solubility, stability, and controlled drug release. Pre-formulation studies confirmed the drug's identity, purity, and favorable properties, including a melting point of 191 °C, near-neutral pH of 7.1, and λ_{max} at 376 nm, with good solubility in ethanol, methanol, and DMSO. NLCs prepared via heat homogenization and sonication exhibited particle sizes ranging from 42.53 to 118.36 nm, polydispersity indices between 22.4–27.4%, and zeta potentials from -4.1 to -17.5 mV, indicating moderate stability. Formulation F1 showed the most promising profile with the smallest particle size (42.53 nm), highest entrapment efficiency (94.56%), and sustained in vitro drug release of 93.43% over 16 hours. SEM analysis confirmed homogeneous, spherical nanoparticles with smooth surfaces. Overall, thiocolchicoside-loaded NLCs, particularly F1, demonstrate excellent potential for enhanced therapeutic efficacy, controlled drug delivery, and improved patient compliance, providing a strong foundation for future in vivo and clinical evaluations.

8. Acknowledgment

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9. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research.

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