

# “Development Of Phenothiazine-Loaded Nanostructured Lipid Carriers (NLCS) For Improved Bioavailability and Stability”

Pranav Kumar<sup>1\*</sup>, Mahadev Kanere<sup>2\*</sup>, A.K. Singhai<sup>3\*</sup>

<sup>1</sup>Student- School of Pharmacy, LNCT University, Bhopal (M.P.)

<sup>2</sup>Associate Professor - School of Pharmacy, LNCT University, Bhopal (M.P.)

<sup>3</sup>Director- School of Pharmacy, LNCT University, Bhopal (M.P.)

Department of Pharmacology, School of Pharmacy, LNCT University, Bhopal Madhya Pradesh, 462042

Corresponding author

Date of Submission: 28-04-2026

Date of Acceptance: 07-05-2026

## ABSTRACT

The graphical abstract of the study represents the systematic design and evaluation of phenothiazine-loaded nanostructured lipid carriers (NLCs) developed to enhance bioavailability and stability. Initially, preformulation studies confirmed that phenothiazine is a crystalline, hydrophobic drug with poor aqueous solubility but good solubility in organic solvents, a near-neutral pH (6.78), and a melting point within the standard range, indicating purity and suitability for formulation. Analytical characterization using UV spectroscopy ( $\lambda_{max}$  314.0 nm), calibration curve, and FTIR confirmed the identity and structural integrity of the drug. Based on these findings, NLC formulations (NLC 1– NLC 5) were prepared, showing uniform appearance and homogeneity. Among them, NLC 3 emerged as the optimized formulation with the smallest particle size (~188 nm), high zeta potential (–31.8 mV) indicating good stability, and maximum entrapment efficiency (96.40%), supported by SEM images showing spherical, smooth, and non-aggregated nanoparticles. *In vitro* drug release studies demonstrated sustained and controlled release over 13 hours, with NLC 3 achieving the highest drug release (95.11%), indicating improved dissolution and bioavailability. Furthermore, stability studies conducted under ICH conditions for 90 days revealed negligible changes in particle size and entrapment efficiency, confirming excellent formulation stability. Overall, the study concludes that the optimized NLC system (NLC 3) significantly enhances the bioavailability, stability, and controlled release of phenothiazine, making it a promising lipid-based nanocarrier for effective drug delivery.

**Keywords:** Phenothiazine, Nanostructured Lipid Carriers (NLCs), stability, Zeta potential,

Entrapment efficiency, *In-vitro* release, FTIR, UV spectroscopy.

## I. INTRODUCTION

Nanostructured lipid carriers (NLCs) are drug delivery system comprising a mixture of solid and liquid lipids as a core matrix. Furthermore, NLCs are second-generation lipid nanoparticles that have an unstructured matrix with high drug loading capacity, which are suitable for drug delivery system (Ghasemiyeh and Mohammadi-Samani, 2018). Due to these unique characteristics, several studies have investigated NLCs as alternate carriers for the dermal delivery of pharmaceuticals, particularly natural active ingredients. Among the associated benefits discovered include biocompatible ingredients, drug release modification, adhesion to the skin, film-forming ability with hydration of the superficial skin layers, as well as increased penetration and permeation into deeper skin layers (Firmansyah *et al.*, 2026).

The use of NLCs requires certain qualities and properties for effective topical or transdermal administration. For instance, NLCs for cutaneous delivery of drugs typically have particles in the submicron size ranging from 40 to 1000 nm, based on the composition of lipids. A smaller particle ensures close contact with the stratum corneum (SC) to improve the skin penetration of the loaded active compound. When used topically, NLCs should be biocompatible and skin-safe, without causing irritation or other unpleasant effects (Chauhan *et al.*, 2020). In addition to size and safety considerations, NLCs should enable high drug loading to ensure a sufficient amount of the active ingredient is encapsulated for therapeutic efficacy. Drug loading is improved by optimizing formulation

parameters, such as the types and concentrations of lipids, surfactants, and co-surfactants (Garg *et al.*, 2022). Generally, NLCs have a higher drug-loading capacity than SLNs and encapsulate from 5% to over 20% active substances, accommodating 30% of some formulations. To guarantee stability, controlled release, and effective dermal distribution while avoiding potential side effects or irritation, the exact amount of the loaded drug should be optimized during the formulation process (Azar *et al.*, 2020).

Phenothiazine, a heterocyclic compound with a tricyclic structure, has been widely recognized for its diverse pharmacological activities, including antipsychotic, antihistaminic, antiemetic, antimicrobial, and anticancer properties (Posso *et al.*, 2022). Despite its therapeutic potential, the clinical application of phenothiazine is often limited due to poor aqueous solubility, low bioavailability, rapid metabolism, and instability under physiological conditions. These challenges necessitate the development of an advanced drug delivery system that can enhance its solubility, protect it from degradation, and ensure sustained release for improved therapeutic outcomes (Singh *et al.*, 2025).

The development of phenothiazine-loaded NLCs involves careful selection of lipids, surfactants, and preparation techniques such as high-pressure homogenization, solvent emulsification, or ultrasonication. Optimization of formulation parameters plays a critical role in achieving desirable characteristics such as particle size, polydispersity index, zeta potential, entrapment efficiency, and drug release behavior. These parameters directly influence the stability, bioavailability, and therapeutic performance of the developed system (Danaei *et al.*, 2018).

Therefore, the present study focuses on the development, optimization, and characterization of phenothiazine-loaded nanostructured lipid carriers with the aim of improving its bioavailability and stability. This approach is expected to provide a novel and efficient drug delivery platform that maximizes the therapeutic potential of phenothiazine while minimizing its limitations, thereby contributing to advancements in pharmaceutical nanotechnology and drug formulation science.

## II. MATERIAL AND METHODS

### 2.1 Chemicals

Phenothiazine were obtained from Triveni Interchem Pvt. Ltd. Swadesh India Chemical Pvt.

Ltd provided the Oleic Acid / Caprylic Triglyceride Stearic Acid. Headquarters provided the Stearic Acid while India Glycols Limited (IGL) supplied Tween 80. Chloroform was supplied by Genteq Laboratories Pvt. Ltd. Methanol were obtained from Vinati Organics and Bajaj Hindusthan Sugar Ltd. provided the Ethanol. HiMedia Laboratories provided the Potassium dihydrogen phosphate.

### 2.2 Preliminary physicochemical analysis of drug

Preliminary physicochemical studies of phenothiazine were performed to assess its suitability for incorporation into the nanostructured lipid carrier system. These studies provided essential baseline information for development of phenothiazine-loaded NLCs (Camp *et al.*, 2015).

#### 2.2.1 Visual evaluation

The evaluation of phenothiazine was carried out to assess its organoleptic properties, including color, odor and physical appearance. These observations were used to confirm the identity, quality, and consistency of phenothiazine to its incorporation into nanostructured lipid carrier system (Visht *et al.*, 2010).

#### 2.2.2 Solubility determination

The solubility of phenothiazine was determined to assess its compatibility with various solvents and lipid components for formulation development. An excess amount of drug was added to different solvents, including water, ethanol, methanol, DMSO and selected solid and liquid lipids, and the mixtures were subjected to continuous stirring until equilibrium was achieved. The samples were then filtered, and concentration of dissolved phenothiazine was analyzed using UV-Visible spectrophotometry. Using the solubility data, appropriate solvents and lipid matrices were chosen in order to produce nanostructured lipid carriers loaded with phenothiazine (Veseli *et al.*, 2019).

#### 2.2.3 pH determination

For pH determination of phenothiazine, a weighed sample was dissolved in distilled water to form a uniform solution, and the pH was measured using a calibrated digital pH meter with a glass electrode (Samuelsen *et al.*, 2021).

#### 2.2.4 Melting point determination

The melting point of phenothiazine was determined using capillary method to evaluate the purity and identity (Mao *et al.*, 2016).

#### 2.2.5 Determination of Maximum Wavelength ( $\lambda_{max}$ )

To determine the maximum wavelength of absorption ( $\lambda_{max}$ ) of phenothiazine, a stock solution was prepared in methanol and further diluted to obtain a suitable working concentration. The

solution was scanned in a UV-Vis spectrophotometer over 200–400 nm using methanol as blank, and the wavelength showing maximum absorbance was recorded as  $\lambda_{max}$  for further analysis.

➤ **Lambda max analysis**

A standard solution of phenothiazine in methanol was scanned in a UV-Visible spectrophotometer over 200–400 nm using methanol as the blank. The absorbance spectrum was recorded, and the wavelength showing the highest peak was identified as  $\lambda_{max}$ , which was used for further analysis and calibration curve preparation(De Luca *et al.*, 2016).

➤ **Calibration curve analysis**

A series of phenothiazine standard solutions (5–30  $\mu\text{g/mL}$ ) were prepared from a methanolic stock and analyzed at the predetermined  $\lambda_{max}$  using methanol as blank. The absorbance values were recorded and plotted against concentration to obtain a calibration curve, showing a linear relationship. Linear regression analysis provided the equation and correlation coefficient ( $R^2$ ), confirming Beer-Lambert's law and enabling estimation of unknown sample concentrations(Enders *et al.*, 2021).

**2.2.6 Functional group identified by FTIR analysis**

FTIR spectroscopy was performed to evaluate the structural integrity of phenothiazine. The drug was mixed with dry KBr, compressed into a pellet, and scanned over 4000–400  $\text{cm}^{-1}$ . The obtained spectrum was analyzed to identify characteristic functional group peaks and confirmed the identity and purity of the drug by comparison with reference data(Bang *et al.*, 2019).

**2.3 Formulation of Nano lipid carriers**

Phenothiazine-loaded nanostructured lipid carriers were prepared by hot homogenization. Stearic acid and oleic acid were melted at 85 °C to form the lipid phase, and phenothiazine dissolved in ethanol-acetone was incorporated. A hot aqueous phase containing Tween 80 and sodium lauryl sulfate was added under stirring to form a coarse emulsion, followed by high-shear homogenization to obtain a nanoemulsion. On cooling, lipid recrystallization led to formation of NLCs, which were collected and stored for further evaluation(Mishra *et al.*, 2016).

**Table 1: Composition of Nano lipid carriers**

Formulation code	Drugs (25 mg)	Ethanol (ml)	Acetone (ml)	Stearic acid (mg)	Oleic acid (%)	Tween 80 (%)	Sodium lauryl sulfate (mg)	Temperature (°C)
NLC 1	25	10.0	10.0	50	0.2	0.1	50.0	85 °C
NLC 2	25	10.0	10.0	100	0.2	0.2	50.0	85 °C
NLC 3	25	10.0	10.0	150	0.2	0.3	50.0	85 °C
NLC 4	25	10.0	10.0	200	0.2	0.4	50.0	85 °C
NLC 5	25	10.0	10.0	250	0.2	0.5	50.0	85 °C

**2.4 Evaluation parameter of drug loaded nano lipid carriers**

**2.4.1 Physical appearance**

The physical appearance of phenothiazine-loaded nanostructured lipid carriers was assessed by visual inspection to evaluate uniformity and preliminary stability of formulations(Shekunov, 2007).

**2.4.2 Particle size analysis**

The particle size of phenothiazine-loaded nanostructured lipid carriers was determined using dynamic light scattering with particle size analyzer or zeta sizer(Mohammed and Abdullah, 2018).

**2.4.3 Zeta potential analysis**

To assess the surface charge and colloidal stability of nanoparticle dispersion, zeta potential of the nanostructured lipid carriers loaded with phenothiazine was evaluated(Sadeghi *et al.*, 2023).

**2.4.4 Scanning Electron Microscopy (SEM) analysis**

Scanning Electron Microscopy (SEM) was employed to study the morphology of phenothiazine-loaded NLCs. The sample was dried, mounted on a stub, and coated with a thin conductive metal layer before imaging. SEM micrographs revealed particle shape, surface characteristics, and approximate size, confirming the structural integrity of the formulation(Paswan, 2021).

**2.4.5 Entrapment efficiency determination**

Entrapment efficiency of phenothiazine-loaded NLCs was determined by separating the free drug from the nanoparticles using ultracentrifugation. The supernatant containing untrapped drug was analyzed at  $\lambda_{max}$  using a UV-Vis spectrophotometer. The amount of entrapped drug was calculated by subtracting free drug from the total drug, and entrapment efficiency (%) was then determined accordingly(González-Barcia *et al.*, 2022).

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

This method allows indirect estimation of drug encapsulation by quantifying the untrapped portion, providing insight into effectiveness of lipid carrier in retaining phenothiazine.

### 2.5 In vitro drug release study

The in vitro drug release of phenothiazine-loaded NLCs (NLC1–NLC5) was studied using the dialysis bag method. Each formulation was placed in a pre-soaked dialysis membrane and immersed in phosphate buffer (pH 7.4) at  $37 \pm 0.5$  °C with constant stirring. Samples were withdrawn at set intervals, replaced with fresh medium, and analyzed at  $\lambda_{\text{max}}$  using a UV–Vis spectrophotometer. The cumulative drug release (%) was calculated using the calibration curve and plotted against time to compare release profiles(D’Souza, 2014).

### 2.6 Stability studies

Stability studies of the optimized phenothiazine-loaded NLCs were conducted under accelerated conditions ( $25 \pm 2$  °C/ $60 \pm 5$  % RH and  $40 \pm 2$  °C/ $70 \pm 5$  % RH) for 90 days as per ICH guidelines. Samples were analyzed at intervals (0, 30, 60, and 90 days) for parameters such as particle size and entrapment efficiency to assess any changes and confirm formulation stability(Cha *et al.*, 2011).

## III. RESULT AND DISCUSSION

### 3.1 Pre-formulation study of drug

Table 2: Organoleptic Evaluation of phenothiazine

Drug	Organoleptic properties	Observation
Phenothiazine	Color	Light green to greenish-yellow powder or crystalline form
	Odor	Slight or weak characteristic odor
	Appearance	Solid powder (crystalline)
	State	Solid

#### 3.1.1pH and Melting point determination

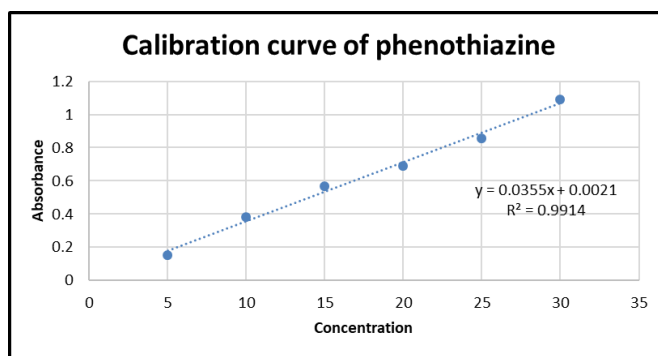
Table 3: pH and Melting pointof Phenothiazine

Drug	Observed Range (pH)	Reference Range (pH)	Observed (Melting point)	Reference (Melting point)
Phenothiazine	6.78	6 to 7	185 °C	183-188°C

#### 3.1.2 Calibration Curve of Phenothiazine

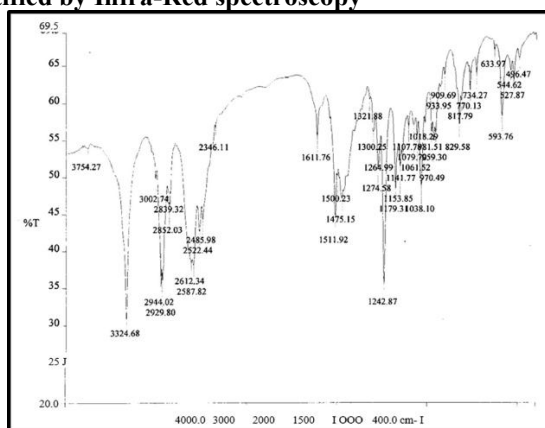
Table 4: Calibration Curve

Concentration (µg/ml)	Absorbance
5	0.154
10	0.384
15	0.568
20	0.690
25	0.854
30	1.090
<b>Mean</b>	<b>0.623333</b>
<b>SD</b>	<b>0.33348</b>



**Graph 1: Calibration Curve of Phenothiazine**

**3.1.3 Functional group identified by Infra-Red spectroscopy**



**Table 5: Interpretation of IR spectrum of Phenothiazine**

Peak obtained	Reference peak	Functional group	Name of functional group
3324.68	3500- 3400	N-H stretching	Primary amine
2929.80	3000-2840	C-H stretching	Alkane
2852.03	3100-3000	C-H stretching	Alkene
2485.98	2600-2550	S-H stretching	Thiol
1611.76	1620-1610	C=C stretching	$\alpha,\beta$ -unsaturated ketone
1511.92	1550-1500	N-O stretching	nitro compound
1321.88	1342-1266	C-H stretching	aromatic amine
1264.99	1275-1200	C-O stretching	alkyl aryl ether
1061.62	1070-1030	S=O stretching	sulfoxide
933.95	980-960	C=C bending	Alkene

**3.2 Characterization of NLCs formulation**

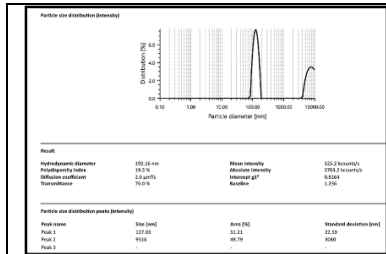
**3.2.1 Physical Appearance**

**Table 6: Physical Appearance**

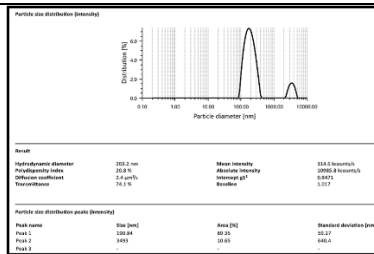
Parameter	NLC 1	NLC 2	NLC 3	NLC 4	NLC 5
Colour	Greenish yellow	Light green	Greenish yellow	Light green	Greenish yellow
Odour	Slight characteristic odour	Slight characteristic odour	Slight characteristic odour	Slight characteristic odour	Slight characteristic odour
Appearance	Solid crystalline powder	Solid crystalline powder	Solid crystalline powder	Solid crystalline powder	Solid crystalline powder
Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform

dispersion      dispersion      dispersion      dispersion      dispersion

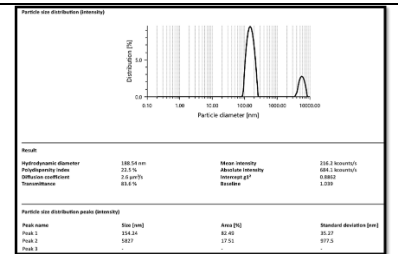
**3.2.2 Particle Size Determination**



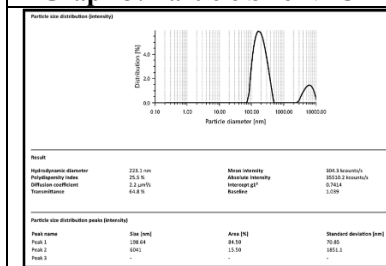
**Graph 3: Particle Size NLC 1**



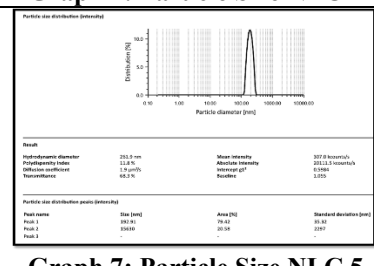
**Graph 4: Particle Size NLC 2**



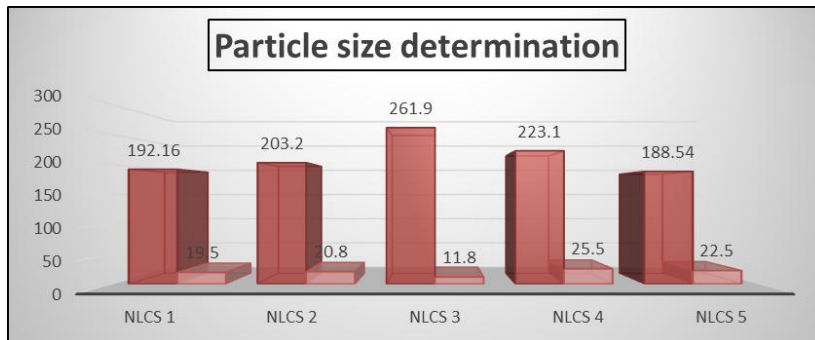
**Graph 5: Particle Size NLC 3**



**Graph 6: Particle Size NLC 4**

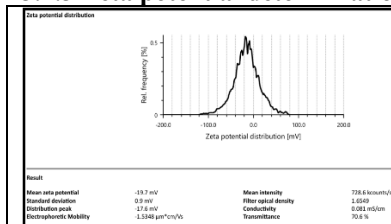


**Graph 7: Particle Size NLC 5**

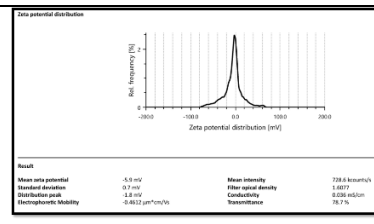


**Graph 8: Graphical Data of Particle Size Determination**

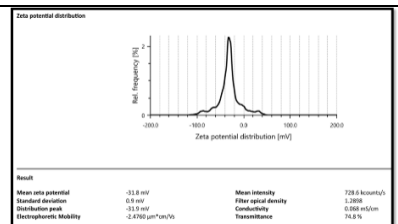
**3.2.3 Zeta potential determination**



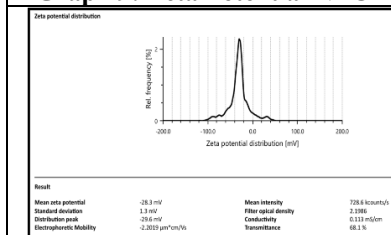
**Graph 9: Zeta Potential NLC 1**



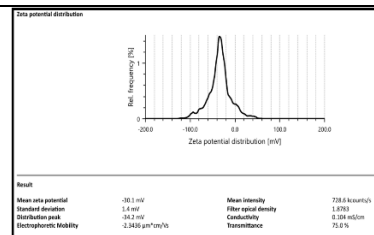
**Graph 10: Zeta Potation NLC 2**



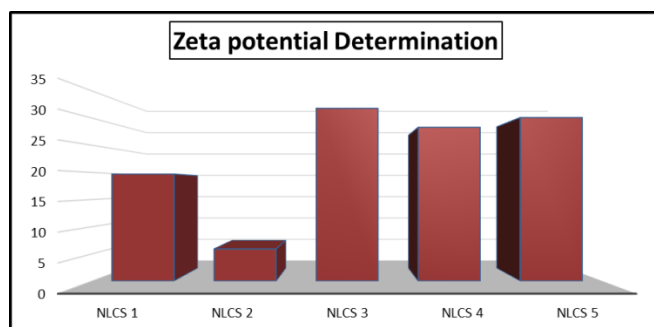
**Graph 11: Zeta Potation NLC 3**



**Graph 12: Zeta Potation NLC 4**



**Graph 13: Zeta Potation NLC 5**

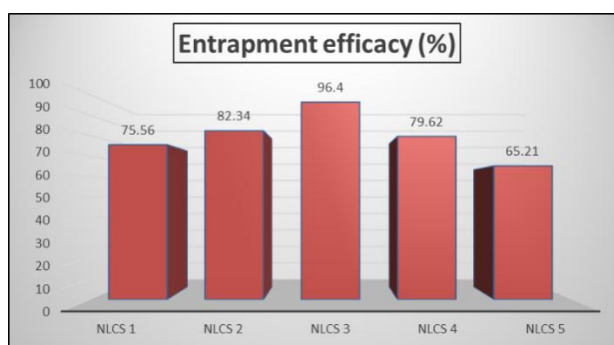


Graph 14: Graphical Data of Zeta potential Determination

### 3.2.4 Entrapment Efficacy determination

Table 7: Entrapment Efficacy

Formulations (F1-F5)	Entrapment efficacy (%)
NLC 1	75.56
NLC 2	82.34
NLC 3	96.40
NLC 4	79.62
NLC 5	65.21



Graph15: Graphical Data of Entrapment Efficacy

### 3.2.5 Scanning Electron Microscope (SEM)

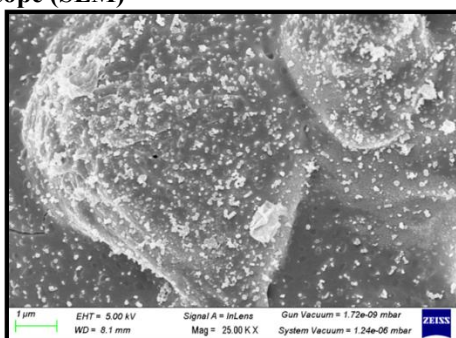


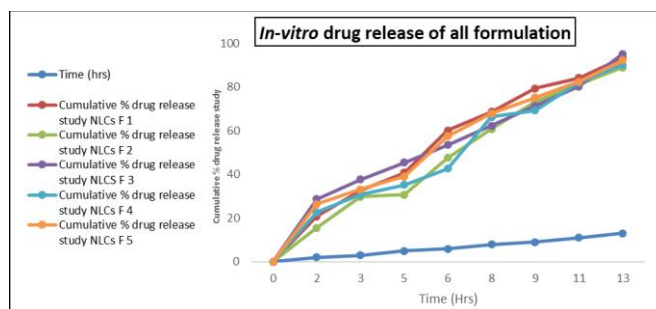
Figure 1: Scanning Electron Microscope (SEM)

### 3.3 In-vitro drug release of all formulation

Table 8: In-vitro drug release studies

Time (hrs)	Cumulative % drug release study				
	NLC 1	NLC 2	NLC 3	NLC 4	NLC 5
0	0	0	0	0	0
2	20.82	15.53	28.65	22.63	26.36
3	32.56	29.86	37.72	30.75	33.21

5	40.80	30.76	45.39	35.29	39.14
6	60.19	47.57	53.52	42.60	57.55
8	68.72	60.80	62.35	66.31	67.89
9	79.41	73.29	71.23	69.25	75.17
11	84.11	81.25	80.21	82.21	82.41
13	93.43	88.94	95.11	90.19	92.25



Graph 16: *In-vitro* drug release studies of all formulation

### 3.4 Stability Study

Table 9: Stability Study of optimized formulation (NLCs) (ICH Q1A guidelines)

Time (Days)	25°C±2 °C and 60 ± 5% RH		40°C±2 °C and 70 ±5% RH	
	Particle size	Entrapment efficacy (%)	Particle size	Entrapment efficacy (%)
0	188.54 nm	96.40%	188.54 nm	96.40%
30	188.48 nm	96.36%	188.51nm	96.43%
60	188.32nm	96.39%	188.47nm	96.38%
90	188.28nm	96.32%	188.40nm	96.30%

## IV. Discussion

The preformulation studies confirmed that phenothiazine possesses suitable physicochemical properties for formulation into nanostructured lipid carriers (NLCs), despite its poor aqueous solubility. The observed organoleptic characteristics, melting point, pH, and FTIR spectra verified the identity and purity of the drug. The UV-Visible analysis showed a  $\lambda_{max}$  at 314 nm with good linearity in the calibration curve, confirming the reliability of the analytical method based on Beer-Lambert's law.

Among the developed formulations, NLC 3 demonstrated optimal performance with the smallest particle size (~188 nm), acceptable polydispersity, and high zeta potential, indicating good stability and uniformity. It also exhibited the highest entrapment efficiency (96.40%), suggesting efficient drug incorporation within the lipid matrix. SEM analysis further confirmed spherical morphology and minimal aggregation, supporting structural integrity.

In vitro drug release studies revealed a sustained release pattern, with NLC 3 showing the highest cumulative release, indicating improved drug availability. Stability studies under ICH conditions showed negligible changes in particle size and entrapment efficiency over 90 days,

confirming robustness of the formulation. Overall, NLC 3 was identified as the optimized formulation, offering enhanced stability, controlled release, and potential for improved bioavailability of phenothiazine.

## V. CONCLUSION

The study successfully developed and evaluated phenothiazine-loaded Nano Lipid Carriers with desirable physicochemical and stability characteristics. Preformulation studies confirmed purity and suitability of phenothiazine for formulation development. Among the prepared formulations, NLC 3 emerged as optimized formulation due to its high entrapment efficiency (96.40%), suitable particle size (188.54nm), strong negative zeta potential (-31.8 mV), controlled and sustained drug release profile (95.11% at 13 hours), and excellent stability over 90 days under ICH-recommended storage conditions. The developed NLC system demonstrated its potential as effective drug delivery carrier for phenothiazine, offering improved drug encapsulation, enhanced stability, and sustained release characteristics. Therefore, this nano lipid carrier formulation may serve as promising approach for improving the therapeutic

performance of phenothiazine and considered for further *in-vivo* evaluation and potential pharmaceutical application.

#### REFERENCES

- [1]. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. *Research in pharmaceutical sciences*. 2018 Aug 1;13(4):288-303.
- [2]. Firmansyah F, Budiman A, Muchtaridi M, Chabib L, Syaputri FN, Afandi F, Elamin KM, Mohammed AF, Wathoni N. Film-Forming Gels for Topical Drug Delivery: A Systematic Review of the Effects of Formulation on Film Performance, Drug Release, and Skin Permeation. *Drug Design, Development and Therapy*. 2026 Dec 31:591505.
- [3]. Chauhan I, Yasir M, Verma M, Singh AP. Nanostructured lipid carriers: A groundbreaking approach for transdermal drug delivery. *Advanced pharmaceutical bulletin*. 2020 Feb 18;10(2):150.
- [4]. Garg J, Pathania K, Sah SP, Pawar SV. Nanostructured lipid carriers: a promising drug carrier for targeting brain tumours. *Future Journal of Pharmaceutical Sciences*. 2022 Apr 8;8(1):25.
- [5]. Azar FA, Pezeshki A, Ghanbarzadeh B, Hamishehkar H, Mohammadi M. Nanostructured lipid carriers: Promising delivery systems for encapsulation of food ingredients. *Journal of Agriculture and Food Research*. 2020 Dec 1;2:100084.
- [6]. Posso MC, Domingues FC, Ferreira S, Silvestre S. Development of phenothiazine hybrids with potential medicinal interest: a review. *Molecules*. 2022 Jan 3;27(1):276.
- [7]. Singh S, Sharma H, Gohri S. A comprehensive review of the synthesis, characterization, and antioxidant potential of phenothiazine derivatives. *Current Bioactive Compounds*. 2025 Oct;21(8):E15734072334547.
- [8]. Danaei MR, Dehghankhold M, Ataei S, Hasanazadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari YM. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 2018 May 18;10(2):57.
- [9]. Veseli A, Žakelj S, Kristl A. A review of methods for solubility determination in biopharmaceutical drug characterization. *Drug development and industrial pharmacy*. 2019 Nov 2;45(11):1717-24.
- [10]. Samuelsen L, Holm R, Schönbeck C. Simultaneous determination of cyclodextrin stability constants as a function of pH and temperature—A tool for drug formulation and process design. *Journal of Drug Delivery Science and Technology*. 2021 Oct 1;65:102675.
- [11]. Mao F, Kong Q, Ni W, Xu X, Ling D, Lu Z, Li J. Melting point distribution analysis of globally approved and discontinued drugs: A research for improving the chance of success of drug design and discovery. *ChemistryOpen*. 2016 Aug;5(4):357-68.
- [12]. De Luca M, Ioele G, Spatari C, Ragno G. Optimization of wavelength range and data interval in chemometric analysis of complex pharmaceutical mixtures. *Journal of Pharmaceutical Analysis*. 2016 Feb 1;6(1):64-9.
- [13]. Enders AA, North NM, Fensore CM, Velez-Alvarez J, Allen HC. Functional group identification for FTIR spectra using image-based machine learning models. *Analytical Chemistry*. 2021 Jun 30;93(28):9711-8.
- [14]. Bang KH, Na YG, Huh HW, Hwang SJ, Kim MS, Kim M, Lee HK, Cho CW. The delivery strategy of paclitaxel nanostructured lipid carrier coated with platelet membrane. *Cancers*. 2019 Jun 11;11(6):807.
- [15]. Mishra A, Imam SS, Aqil M, Ahad A, Sultana Y, Ameenuzzafar, Ali A. Carvedilol nano lipid carriers: formulation, characterization and *in-vivo* evaluation. *Drug delivery*. 2016 May 3;23(4):1486-94.
- [16]. Shekunov, AH C. Particle size analysis in pharmaceuticals: principles, methods and applications. *Pharm Res*. 2007;24:411-37.
- [17]. Mohammed, A., & Abdullah, A. (2018, November). Scanning electron microscopy (SEM): A review. In Proceedings of the 2018 international conference on hydraulics and pneumatics—HERVEX, BăileGovora, Romania (Vol. 2018, pp. 7-9).
- [18]. Sadeghi S, Bakhshandeh H, Cohan RA, Ehsani P, Norouzian D. Physical-chemical characterizations of synthetic dual niosomes for antibacterial delivery of lysostaphin and LL-37. *Pharmaceutical Chemistry Journal*. 2023 Dec;57(9):1418-27.



- 
- [19]. Paswan SK, Saini T. Comparative evaluation of *in vitro* drug release methods employed for nanoparticle drug release studies. Clin. Trials. 2021 Nov;14(17):10-4227.
- [20]. González-Barcia M, Lema MI, Otero-Espinar FJ. Lactoferrin-loaded nanostructured lipid carriers (NLCs) as a new formulation for optimized ocular drug delivery. European journal of pharmaceuticals and biopharmaceutics. 2022 Mar 1;172:144-56.
- [21]. D'Souza S. A review of *in vitro* drug release test methods for nano-sized dosage forms. Advances in pharmaceuticals. 2014;2014(1):304757.
- [22]. Cha J, Gilmor T, Lane P, Ranweiler JS. Stability studies. In Separation science and technology 2011 Jan 1 (Vol. 10, pp. 459-505). Academic Press.