RESEARCH PAPER

Formulation And Optimization Of Curcumin And α-Tocopherol Loaded Nanostructured Lipid Carrier's System

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ABSTRACT

Curcumin and α-tocopherol are natural antioxidants with significant anti-inflammatory, antimicrobial, and wound-healing properties. However, their therapeutic effectiveness is limited by poor aqueous solubility, low stability, rapid degradation, and poor bioavailability. The present study aimed to develop and optimize Curcumin and α-Tocopherol-loaded Nanostructured Lipid Carriers (NLCs) to improve their stability, encapsulation efficiency, and drug release characteristics. The NLCs were prepared using suitable solid and liquid lipids along with surfactants and optimized through a factorial design approach. The formulations were characterized for particle size, polydispersity index (PDI), zeta potential, morphology, Fourier Transform Infrared Spectroscopy (FTIR), encapsulation efficiency, drug loading, and in vitro drug release behavior.

FTIR analysis confirmed the compatibility of curcumin and α-tocopherol with the selected excipients, indicating the absence of significant drug–excipient interactions. The optimized formulation exhibited nanosized particles with good uniformity and high encapsulation efficiency. In vitro drug release studies demonstrated a sustained release profile, achieving approximately 85% cumulative drug release over the study period. Furthermore, antibacterial studies revealed enhanced inhibitory activity of drug-loaded NLCs against Escherichia coli and Staphylococcus aureus compared with blank formulations. Overall, the developed Curcumin–α-Tocopherol-loaded NLCs showed improved physicochemical properties and biological performance, indicating their potential as an effective lipid-based delivery system for topical and pharmaceutical applications.

Keywords: Curcumin; α-Tocopherol; Nanostructured Lipid Carriers; Encapsulation; Efficiency; Antibacterial Activity.

How to cite this article: Sharma R, Gupta A K, Formulation And Optimization Of Curcumin And α-Tocopherol Loaded Nanostructured Lipid Carrier's System. Int J Drug Deliv Technol. 2026;16(67s):1991-2007. DOI: 10.25258/ijddt.16.67s.178

Source of support: Nil

Conflict of interest: None

1. INTRODUCTION

Nanotechnology is used in many areas including environmental science, health, cosmetics and nutritional research . Recently nanotechnology has appeared as an alternative to the existing medicine delivery techniques . Nanocarriers are colloidal systems with a mean diameter of less than one micron . The distinctive properties of nanoparticles, such as a high surface to volume ratio and nanoscale size, render them appropriate for medical applications . Due to their smaller size than biological macromolecular medicines or traditional chemotherapeutic drugs, they can be combined with a broad range of support components and pharmaceutically active compounds . These components provide stimulus-based activation, imaging, targeting and resistance to degradation. However, the body responds differently to nanoparticles than to traditional drugs . Nanoparticles have unique biodistribution patterns and hydrodynamic characteristics. It is interesting to note that interactions on nanobiological level can improve medicine delivery . Nanocarriers can be modified by changing the encapsulating moieties to optimize pharmacokinetic and biodistribution properties, reduce toxicity, control release, enhance solubility and stability and transport their payload to targeted sites. Physiochemical features of nanocarriers like surface, composition and shape can be modified to maximize their activities and minimize negative impacts. Nanostructured lipid carriers (NLCs) are a major advancement in drug delivery systems, but they suffer from many problems that hinder their widespread use. These challenges include formulation complexity, stability problems and biological barriers .

Recent studies have been oriented to antioxidant molecules due to their broad dermatological, pharmacological and biological effects. These chemicals have properties like free radical scavenging, melanin reduction, wound healing, and photoprotection . Curcumin (cur) is a lipophilic phenol isolated from the rhizomes of Curcuma longa . Several research have established cur's biological activity including antioxidant, anti-inflammatory, antibacterial, anticancer and wound healing properties . α-tocopherol (α-toc), the most active and effective form of vitamin E, is found naturally and used as an antioxidant in the food, cosmetics and pharmaceutical industries . Topical α-toc has been shown to protect skin and promote wound healing . α-toc can scavenge peroxyl radicals and protect phospholipids and fatty acids in the skin membrane . Dermatology is an area in which both cur and a-toc are very effective but their therapeutic usefulness is limited by many circumstances. The low water solubility (<0.1 mg/mL), low physicochemical stability, unsustainable bioavailability, low skin permeability, and quick metabolism of Cur results in low cur levels in tissues . α-Toc is, however, rapidly oxidized by ambient oxygen, has limited solubility in water (~30 mg/L) and is sensitive to light. Moreover, it has been associated with skin irritation, limiting its use in products . These restrictions prevent good topical and systemic efficacy of cur and α-toc. To overcome these disadvantages, the development of co-encapsulation delivery systems offers several advantages, such as protection of the therapeutic molecule and its integrity, ease of incorporation, formulation flexibility with respect to the ratio and dose, controlled release, and the possibility of synergistic effects when several therapeutic molecules are incorporated. The development of specific co-encapsulation delivery systems is critical for the development of new dermatological products. Nanostructured Lipid Carriers (NLCs) are second generation lipid based nanocarriers consisting of mixture of solid and liquid lipids which show superior performance than the conventional lipid delivery systems in terms of drug loading capacity, stability, controlled release and bioavailability. NLCs are emerging as potential carriers for topical and transdermal drug delivery due to their nanoscale size, biocompatibility, and the ability to encapsulate lipophilic bioactives. Their small particle size and lipidic nature allows them to closely interact with stratum corneum, enhancing skin penetration and retention of medicinal substances.

This leads to a less organized lipid matrix in NLCs compared to classical liposomes and other vesicular systems because the liquid lipids are incorporated into solid structures. This faulty crystalline structure offers more room to host the drug with higher encapsulation efficiency and lower drug ejection during storage. Also, NLCs have good physicochemical stability making them suitable as carriers for the delivery of bioactive chemicals vulnerable to degradation.

Curcumin (Cur) and α-tocopherol (α-Toc) are potent natural antioxidants with anti-inflammatory, antioxidant, antibacterial, wound healing and skin protective effects. However, their medicinal applications are restricted because of their poor water solubility, low bioavailability and susceptibility to degradation under physiological and environmental conditions. Encapsulation in NLCs is a potential strategy to overcome these limitations, protecting active compounds from degradation, increasing solubility and allowing sustained release at the target site.

Previous studies indicate that the entrapment of curcumin and α-tocopherol in lipid-based nanocarriers enhances their stability and therapeutic activity. Some research groups found that co-delivery systems with antioxidants successfully prevented active chemicals from oxidative breakdown and enhanced their biological activity. Moreover, co-encapsulation methods offered superior antioxidant activity and stability compared to single-drug delivery systems.

We propose the co-encapsulation of curcumin and α-tocopherol in Nanostructured Lipid Carriers (Cur/α-Toc-NLCs) because of their potent antioxidant properties and the benefits of NLCs as delivery vehicles. Curcumin-loaded NLCs and α-tocopherol-containing lipid nanoparticles have been well-studied but data on the co-encapsulation of the two antioxidants in one NLC system aiming at improved stability and topical delivery are scarce.

The present study is focused on the production and characterization of Cur/α-Toc-NLCs and evaluation of their physicochemical properties and biological activity. The effect of formulation variables like lipid content and surfactant concentration will be studied. Cur/α-Toc-NLCs will be characterized for particle size, polydispersity index (PDI), zeta potential, morphology, FTIR, encapsulation efficiency (EE), drug loading capacity (DL) and stability. In addition, the improved formulation will be tested for antioxidant activity, cytocompatibility, skin permeation behavior and stability. The present study presents a promising approach to improve solubility, stability and antioxidant efficacy of curcumin and α-tocopherol using a novel co-loaded NLC delivery system for topical and cutaneous medicinal applications.

2. MATERIALS AND METHODS

2.1 Materials

The α-Tocopherol was procured from authentic sources which include Merk Limited India and the *Curcuma longa* (turmeric) plant was collected from its natural habitat in Sirmaur District of Himachal Pradesh, India. The drug and excipient constituents used for the

preparation of Nano lipid carrier system of different formulations were obtained from reliable and approved suppliers. The formulations were prepared using analytical grade compounds without further purification.

2.2. Preparation of Drug-Loaded Nanostructured Lipid Carriers

The warmed aqueous phase was poured into the lipid phase and stirred continuously at 800 rpm for 2-4 min for preparation of coarse emulsion. The pre-emulsion was ultrasonicated at 20% amplitude for 20 minutes to reduce particle size and achieve uniform dispersion. The NLC formulation was allowed to cool down to room temperature (25 °C) by itself and stored at 4 °C for further use.

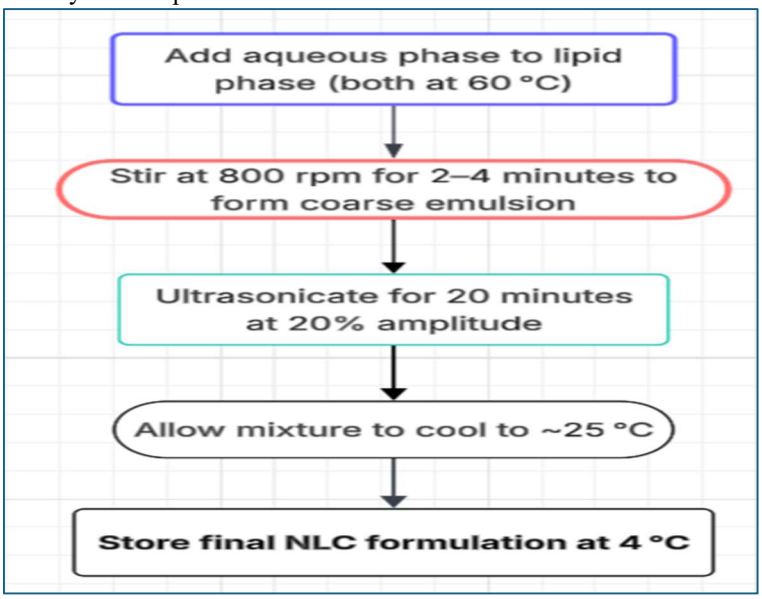


Table 1 Composition of NLCs Formulations

Formulation Code	Curcumin (mg)	α-Tocopherol (g)	Compritol 888 ATO (g)	Poloxamer 188 (%)	Tween 80 (%)	Water (q.s.)
F1	0	0	3.0	1	0.5	100ml
F2	50	1.0	3.0	1	0.5	100ml

Figure 1 Image showing the formulation of curcumin and alpha -tocopherol loaded Nano Lipid Carriers System

2.3 Preformulation Parameter of NLCs

2.3.1 Melting Point Determination

A pre-sealed capillary tube was used and the drug was injected until it reached a height of 3 mm. The closed end was tapped on a hard surface to push the medicine to the bottom of the tube. Then the capillary tube was placed into the melting point apparatus. The melting point of the drug was determined and compared with the standard value.

2.3.2 Appearance

Visual examination was carried out to investigate the appearance of the drugs and the respective standards of the drugs were compared to the same.

2.3.3 Solubility studies

Solubility was studied in a number of solvents. Briefly, 10 mg of drug sample was dissolved in 10 ml of solvents (water, methanol, ethanol, 0.1 N HCl, pH buffer 6.8) in small test tubes and shaken for 5 min. The mixed vials were kept in an orbital shaker at 25±1.0°C for an equilibration period. After equilibrium was achieved, samples were removed and centrifuged at 3000 rpm for 15 min. The supernatant was decanted and filtered using Whatman filter paper. The filtrate was diluted with pH 6.8 buffer and the concentration of drug extracts was measured by UV-Visible spectrophotometer at respective wavelength.

2.3.4 Determination of wavelength (λmax)

2.3.4.1 Development of calibration curve

A standard stock solution was prepared by dissolving 100 mg of drug in 100 mL of phosphate buffer with pH 7.4. The stock solution of curcumin and phytosterol (1000 µg/ml) was prepared in a pH buffer solution of 6.8. The solution was diluted using the same solvent to a concentration of 100µg/ml. The solution was then scanned in twin beam UV spectrophotometer in the range 200-500nm.

2.4 Evaluation of prepared aprepitant loaded NLCs

2.4.1 Fourier transform infrared spectroscopy (FTIR)

Disperse NLC samples and relevant controls (blank solid-lipid nanoparticles, NLC with liquid lipid only, drug-loaded NLC and physical components like pure drug/essential oil) and convert them into dry powders prior to FTIR. To prepare pellets, dispersions were snap frozen, stored at -80 °C and lyophilized without cryoprotectant (48 h; chamber pressure ~3.0 mbar; shelf ~-18 °C). Pellet formation for transmittance measurements, the dry sample should be thoroughly mixed with spectroscopic grade KBr and formed into translucent pellets by compression in a hydraulic press . FTIR analysis was performed to investigate the possible drug-excipient interactions. Samples were prepared by KBr disc method and then placed in the sample holder of the FTIR instrument. The spectra covered the range from 4000 to 400 cm^{-1}.

2.4.2 *Scanning electron microscope (SEM)*

Surface form affects drug release kinetics and interaction with biological membranes. Scanning electron microscopy (SEM), which visualizes nanoparticle form and topography, shows spherical particles with flat surfaces after dilution, drying, and gold/palladium coating. This confirms the integrity of the nanostructured core and ensures that no aggregation has taken place. Moreover, it's even dispersion prevents any sensory changes in applications such as food fortification.

2.4.3 Particle Size, Zeta Potential, and Polydispersity Index (PDI)

The size of the particles is important for the bioavailability, penetration, and cellular uptake of NLC. The hydrodynamic diameter (Z-average) can be determined by the dynamic light scattering (DLS), which is based on the light intensity fluctuations caused by the Brownian motion. The polydispersity index (PDI) is a measure of size uniformity, with a value of 0 indicating a monodisperse system and a value of 1 indicating heterogeneity. A value < 0.3 indicates a homogenous dispersion, which is desirable for uniform drug release. The surface charge and colloidal stability were expressed by the zeta potential, which was estimated from the electrophoretic mobility using the Helmholtz-Smoluchowski equation. Values <= -30 mV are sufficient to avoid aggregation due to electrostatic repulsion. Dilute to reduce various scattering effects prior to measurement with deionized water. The size of NLC particles (50-500nm) plays a significant role in their bioavailability, penetration and absorption. The hydrodynamic diameter is often determined by dynamic light scattering (DLS). The size is determined by the lipid content, the concentrations of surfactant, and processing variables such as homogenization and sonication. The addition of liquid lipids reduces the particle size compared to SLNs, but drug loading can increase the particle size due to higher viscosity. Particles smaller than 200 nm are preferred to improve absorption, stability and solubility of poorly soluble drugs.

2.4.4 Entrapment efficiency

These important criteria for evaluating nanostructured lipid carriers (NLCs). EE is the percentage of drug successfully loaded into nanoparticles and DL is the amount of drug in the total weight of lipids and entrapped drug. Higher EE reduces the waste of medication and higher DL improves the carrier design and therapeutic efficacy.

The parameters are calculated using the following equations:

$$EE\ (\%) = Toal\ Drug\ Total\ Drug - Free\ Drug \times 100$$

$$DL\ (\%) = Total\ Lipid\ Initial\ Drug - Free\ Drug \times 100$$

HPLC quantification of the free drug after isolation from the nanoparticles by centrifugation or ultrafiltration is used to experimentally determine the EE and DL. Formulation properties such as lipid composition, drug to lipid ratio and surfactant concentration have significant effect on EE and DL, which are important for the development of efficient NLC-based drug delivery systems.

2.4.5 *In-vitro Drug release studies*

The dialysis bag diffusion method is commonly used to study the in vitro drug release from nanostructured lipid carriers (NLCs). A pre-soaked dialysis membrane (molecular weight cut-off 12-14 kDa) is loaded with a pre-defined volume of NLC dispersion containing an accurately determined dose of medication. The sealed bag is placed in a release medium (usually phosphate buffer saline (PBS, pH 7.4) or simulated physiological fluids) and is maintained at 37 ± 0.5 °C with continuous stirring to maintain sink conditions. Aliquots were taken at regular intervals from the receptor chamber and replaced with fresh medium to maintain a constant volume. The collected samples are filtered and the discharged drug is quantified by spectrophotometry or HPLC. This method enables the determination of the kinetics and processes of release that are often fitted to mathematical models, such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations to describe the release behavior. The dialysis method is a simple and effective method for mimicking drug diffusion across biological membranes. Other systems such as Franz diffusion cells or USP dissolving apparatus can be used depending on formulation characteristics.

2.4.6 Antibacterial Assay

The antibacterial efficacy of drug loaded NLCs against Escherichia coli and Streptococcus aureus was studied by using liquid and solid media methods .

2.4.6.1 Quantitative Antibacterial Analysis in Liquid Medium

Activated bacterial cultures were incubated with various formulations of NLC mixed with LB broth medium. The cultures were shaken periodically and maintained at 37°C. The percent of bacterial inhibition was calculated using the following formula .

$$Bacterial\ Inhibition\ (\%) = I_c - I_s / Ic \times 100$$

2.4.6.2 Qualitative Antibacterial Assessment in Solid Medium

Antibacterial activity was also estimated by agar well diffusion method. We cut wells in the LB agar plates with a sterile borer and spread the bacterial suspension evenly on the plates. The formulations were filled in the wells and the plates were kept at 37 °C for a complete day. The inhibition zone was assessed and compared to other formulations .

3. RESULT AND DISCUSSION

3.1 RESULTS

3.1.1 Preformulation studies of drugs

Pre-formulation studies of curcumin were conducted and compared with standard. It was observed that the melting point of the sample was almost same as that of the standard. Sample and standard were similar. The solubility of the different samples of medication was also found to be the same as the standard. No change in the wavelengths of the associated samples was observed with respect to individual standards.

Table 2 Preformulation studies of Curcumin

Sr. No.	Parameters	Standard	Sample
1	Melting Point	1830C	1800C
2	Appearance	Yellowish-orange powder	Yellowish-orange powder
3	Solubility	Ethanol	Ethanol
4	Wavelength	420nm	423nm

3.1.2 Solubility studies

It was clear from the solubility studies that Curcumin showed high solubility in Ethanol in contrast to other solvents

Table 3 Solubility study of drug in different solvents

Sr. No	Solvents used	Curcumin
1	Water	++
2	Methanol	++

3	Buffer	+
4	Ethanol (95%)	+++

Highly soluble +++, Soluble ++, poorly soluble +

3.1.3 Preformulation studies of drugs

The pre-formulation studies of Alpha Tocopherol were carried out and compared with standards. It was observed that the melting point of the sample was approximately equal to that of the standard. The appearances of the standard and the sample were also identical. The solubility of the different drug samples was also found to be similar to that of standard. Contrary to individual standards no change in the wavelengths of the matching samples was observed.

Table 4 Preformulation studies of α-Tocopherol

Sr. No	Parameters	Standard	Sample
1	Melting Point	2.5-3.50C	3.10C
2	Appearance	Pale yellow to amber, viscous oily liquid	Pale yellow viscous liquid
3	Solubility	Insoluble in water, freely soluble in ethanol, ether, acetone, chloroform and oils	Complies
4	Wavelength	292nm	291nm

3.1.4 Solubility studies of Alpha Tocopherol

It was clear from the solubility studies that α-tocopherol showed high solubility in ethanol in contrast to other solvents.

Table 5 Solubility study of drug in different solvents

Sr. No	Solvents	Solubility of Alpha Tocopherol
1	Water	+
2	Ethanol	+++
3	Methanol	++
4	Buffer	+

Highly soluble +++, Soluble ++, poorly soluble +

3.1.5 Determination of maximum wavelength (λmax)

The λmax of Curcumin was found to be 420nm which is in agreement with the standard drug. The maximum absorbance is shown in the figure below and the graph.

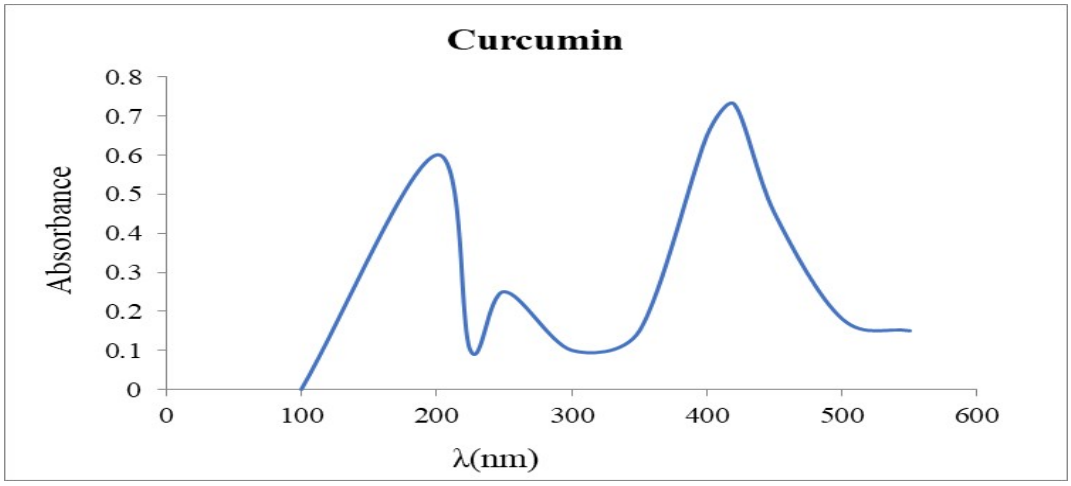


Figure 2 Absorption maxima(λmax) of curcumin at 420nm

3.1.6 Standard calibration curve

Curcumin standard curves were prepared in a buffer solution at pH 6.8 with different concentrations. Absorption was seen at 2, 4, 6, 8 and 10 ug/ml. Also, the regression coefficient was calculated and found to be near to unity, which indicates that the standard graphs can be used for further study.

Table 6 Absorbance of curcumin at different Concentration

Sr. No	Concentration (µg/ml)	Absorbance@290 nm
1.	2µg/ml	0.154
2.	4µg/ml	0.318
3.	6µg/ml	0.427
4.	8µg/ml	0.585
5.	10µg/ml	0.781

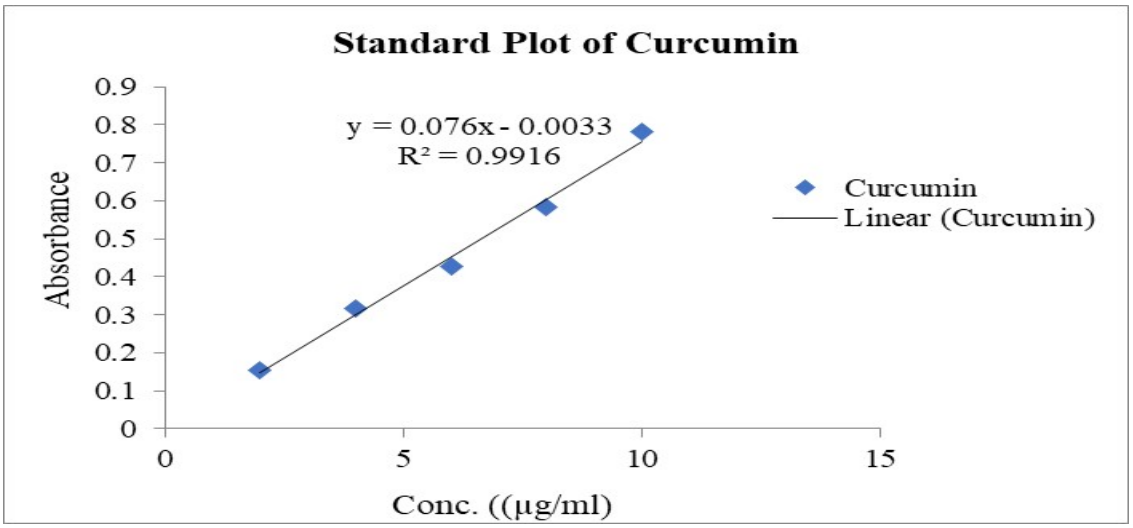


Figure 3 Standard calibration curve of Curcumin

3.1.7 Determination of maximum wavelength (λmax)

The maximum wavelength (λmax) for curcumin was 290nm in accordance with the standard drug. The figure below shows the maximum absorbance and the graph.

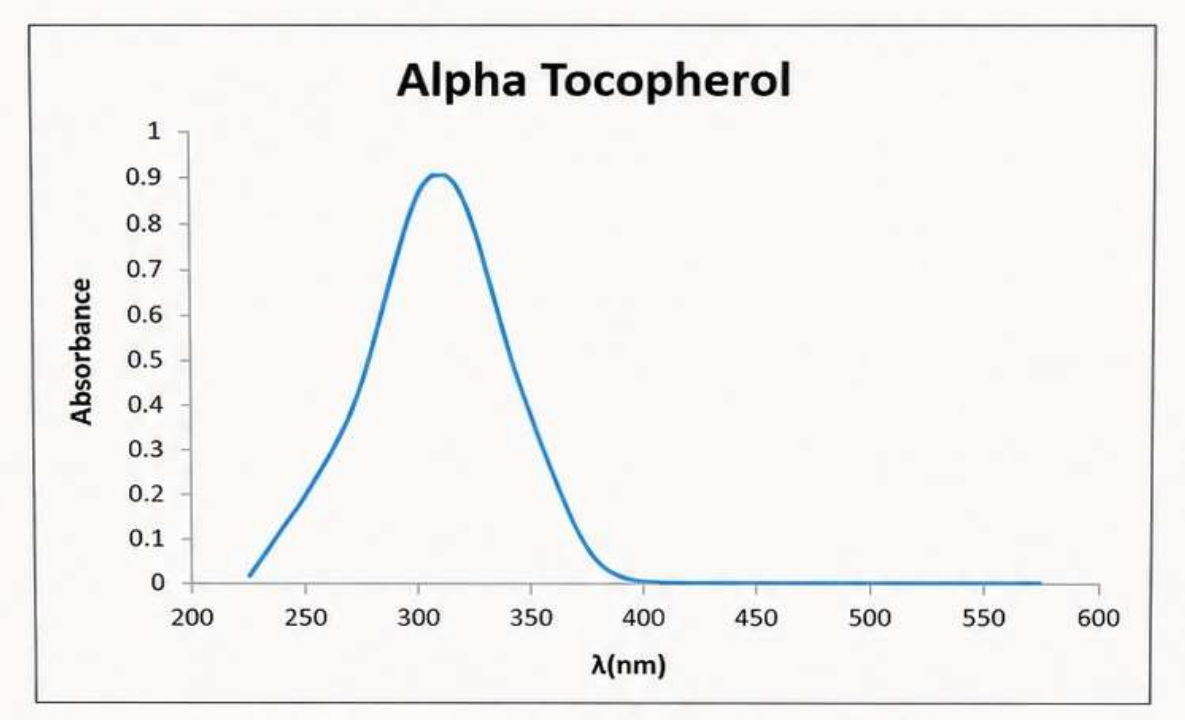


Figure 4 Absorption maxima (λmax) of α-tocopherol (292 nm)

3.1.8 Standard calibration curve

The standard curve of Alpha Tocopherol was developed using different concentrations of ethanol. Absorption was observed at concentrations of 2, 4, 6, 8 and 10 µg/ml. Also, the regression coefficient was calculated and found to be near to unity, which indicates that the standard graphs can be used for further study.

Table 7 Absorbance of α-tocopherol at different Concentration

Sr. No	Concentration (µg/ml)	Absorbance @292 nm
1.	2µg/ml	0.142
2.	4µg/ml	0.279
3.	6µg/ml	0.399
4.	8µg/ml	0.579
5.	10µg/ml	0.711

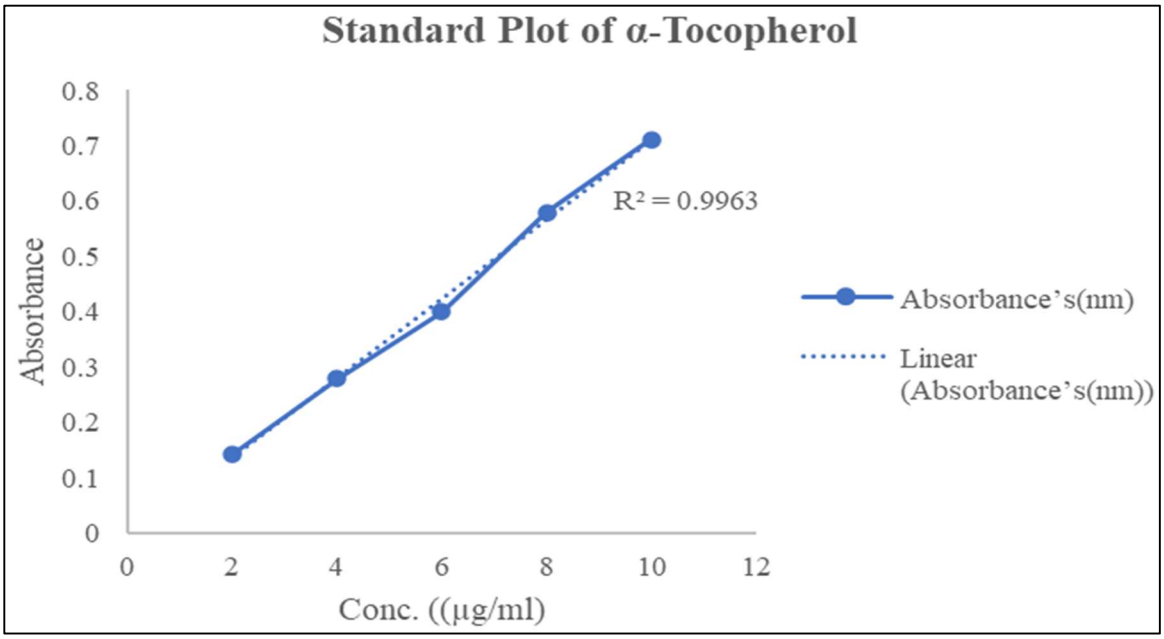


Figure 5 Standard calibration curve of α-tocopherol

3.2 Evaluation of NLCs

3.2.1 Fourier Transfer Infrared (FTIR) Spectroscopic

The FTIR spectroscopic analysis was performed to evaluate possible chemical interaction of drug under study along with all excipients.

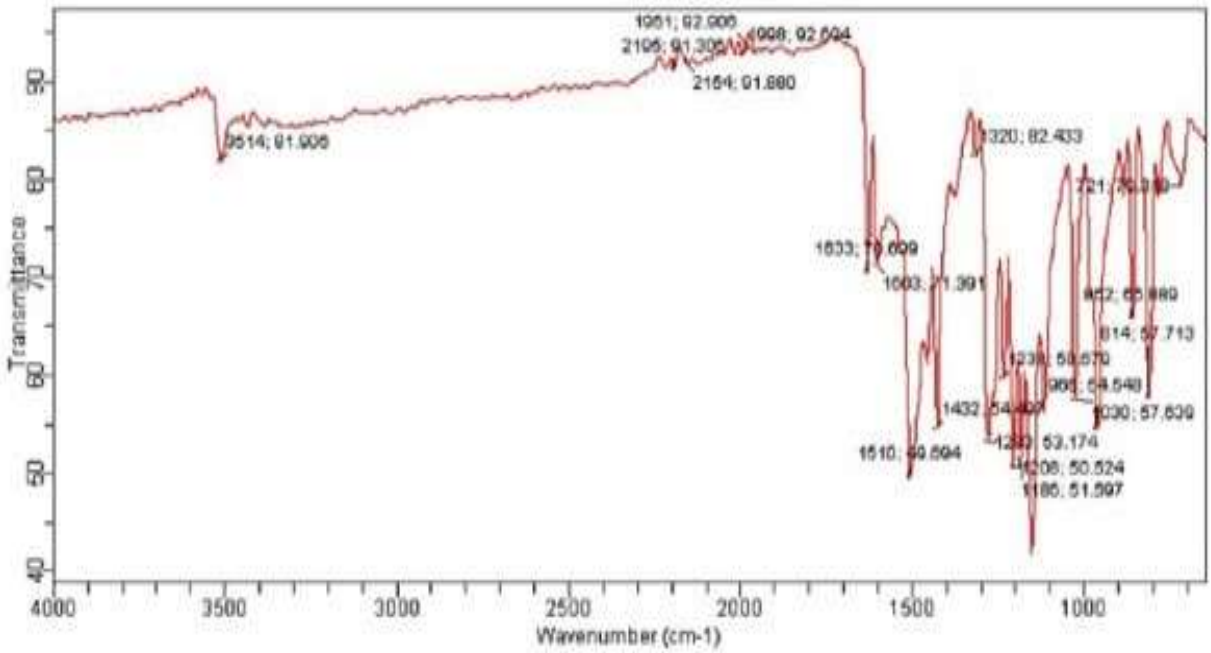


Figure 6 FTIR spectrum of Curcumin

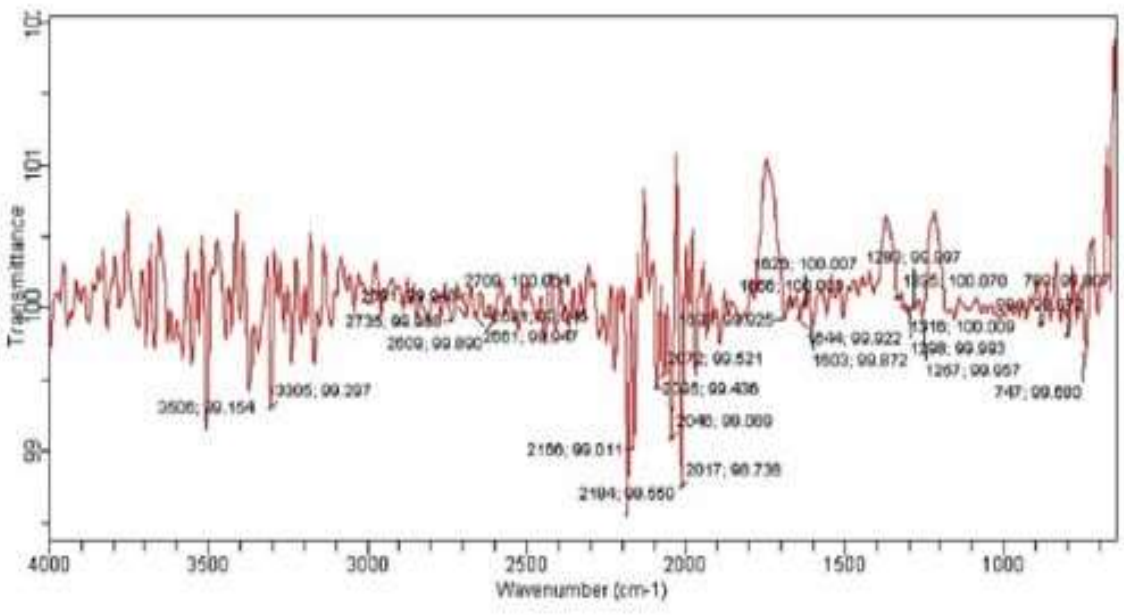


Figure 7 FTIR Spectrum of α-Tocopherol

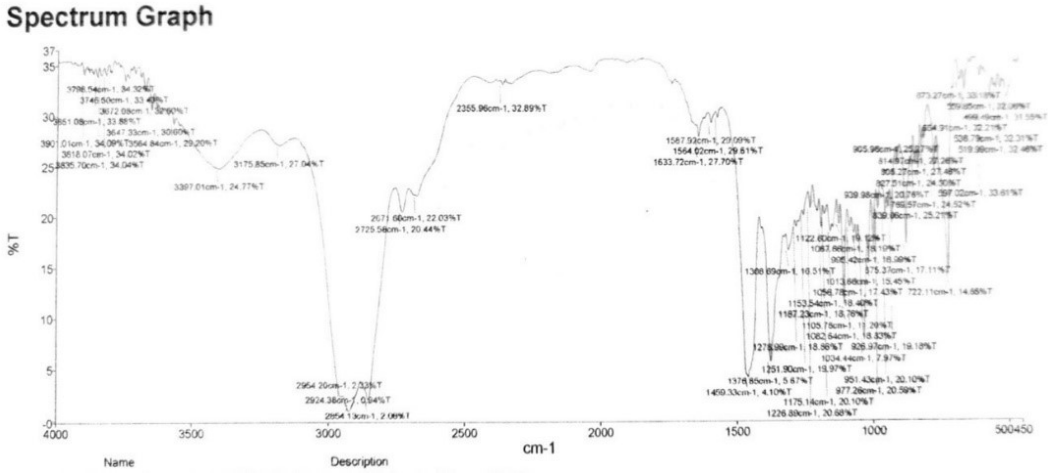


Figure 8 FTIR Spectrum of Drug and Excipients

FTIR spectroscopy was performed to analyze the sample of curcumin and the spectrum showed the peaks at 3514 cm-1, 1633 cm-1, 1510 cm-1, 1432 cm-1, 1273 cm-1 and 1033 cm-1 which are characteristic of the functional groups such as O-H stretching, C=O stretching, aromatic C=C stretching, CH bending and C-O stretching vibrations respectively.

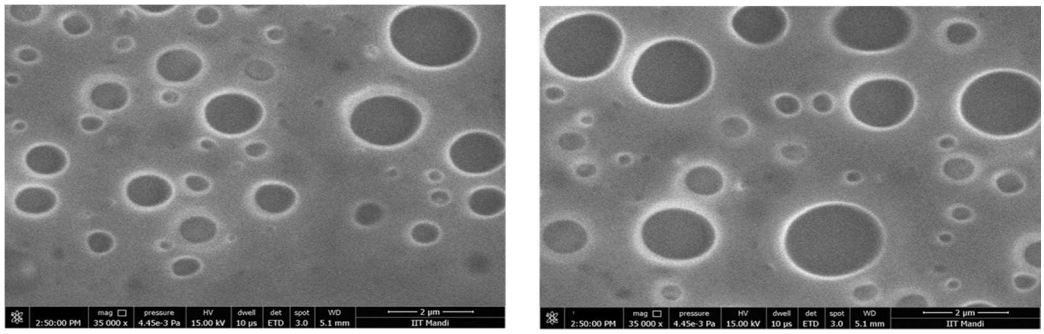
The FTIR spectrum of α-Tocopherol exhibited peaks at 3506 cm^{-1}, 3305 cm^{-1}, 1627 cm^{-1}, 1455 cm^{-1}, 1269 cm^{-1}, and 1043 cm^{-1} corresponding to phenolic O-H stretching, aliphatic C-H stretching, aromatic vibrations, CH_3 bending, and C-O stretching respectively which confirmed the presence of its functional groups.

The compatibility of curcumin and α-tocopherol in NLC formulation was assessed by FTIR spectroscopy. The optimized formulation showed characteristic peaks of both drugs with little change in the position and intensity of the peaks. The absence of new peaks or disappearance of major peaks suggested that there was no chemical interaction between the drug and the excipients.

The FTIR analysis confirmed a successful incorporation of curcumin and α-tocopherol into the NLC system, indicating the acceptable formulation compatibility and stability.

3.2.2 Surface morphology

The morphological qualities of various formulations were characterized by surface analysis. SEM analysis showed smooth surface morphology and spherical shape of the generated NLCs.



(Blank NLCs) (Drug-Loaded-NLCs)

Figure 9 Scanning Electron Microscope (SEM) of nanoparticles (a) Blank NLCs (b) Drug-Loaded-NLCs (Best Formulation)

3.2.3 Particle size distribution

The particle size investigation revealed that the average particle size of the generated drug-loaded nanostructured lipid carriers was 501.6 nm.

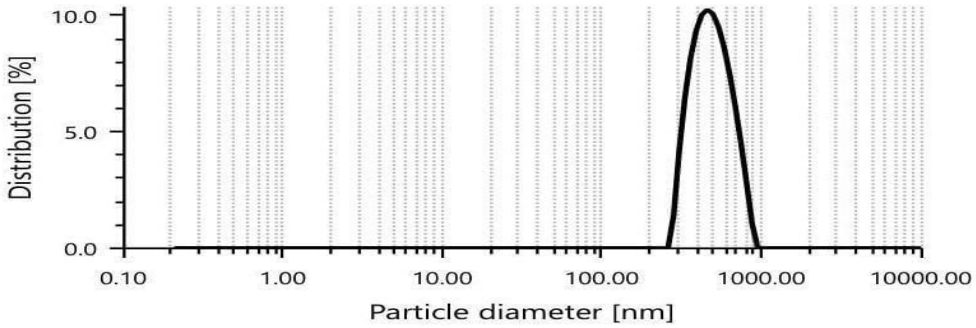


Figure 10 Particle size distribution graph of Drug-Loaded-NLCs

3.2.4 Zeta potential

Colloidal stability of the system was measured by zeta potential of the curcumin nanostructured lipid carrier (CNL) formulation. The sharp narrow peak in the zeta potential distribution graph indicated a uniform surface charge distribution across the particles. The most of particles had a positive zeta potential value in range of 0 to +40 mV and the peak value was in range of +30 to +40 mV.

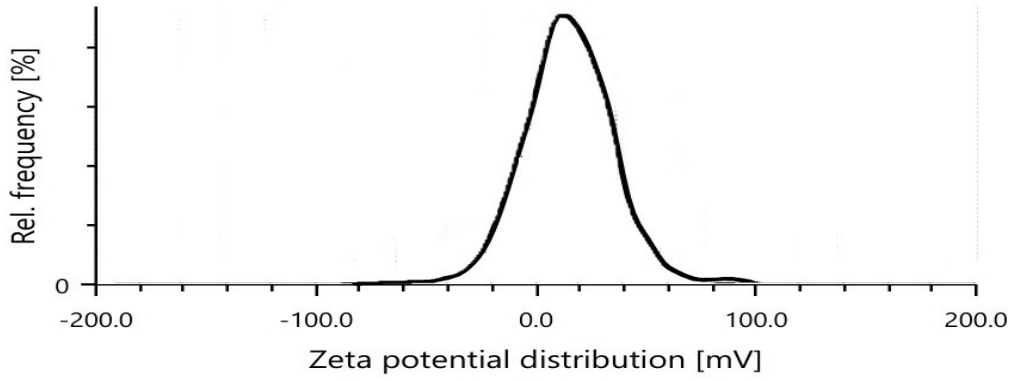


Figure 11 Zeta potential distribution of Drug Loaded NLCs

The positive zeta potential of this formulation indicates the particles have sufficient electrostatic repulsion to inhibit aggregation and promote stability. The lipid-based nanoparticles are generally considered to be stable with

zeta potential values greater than +30 mV due to the high repulsive interactions among the particles. The limited distribution also confirms the uniform distribution of surface charge in the CNL formulation.

3.2.5 Polydispersity Index

The poly-dispersity index of each formulation was found to be in the range of 0.28 to 0.49, which indicated that the developed NLCs had a uniform size distribution.

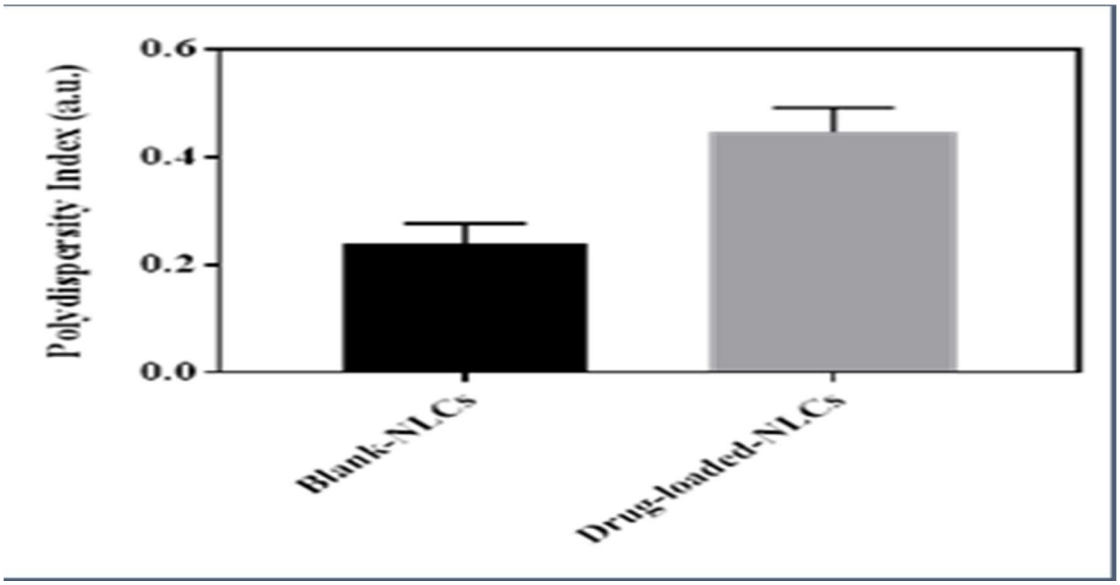


Figure 12 Polydispersity index of Drug Loaded NLCs

3.2.6 Drug entrapment efficiency and drug loading capacity

The drug entrapment efficiency and drug loading capacity of D-NLCs were found to be 88.24% and 7.14%, respectively, as indicated in the study.

Table 8 Particle size, Zeta potential, PDI, percent EE and DL of the formulations

Formulation Code	Particle Size(nm)	Zeta Potential(mV)	PDI	%EE	%DL
F1	307.4	±18	±0.28	0%	0%
F2	501.6	±30	±0.49	88.24%	7.14%

3.2.7 In-vitro drug release studies

As shown in the figure, the developed D-NLCs exhibited controlled drug release profile with an initial burst release of drug followed by a steady rate release for 72 hours. About 54.1% of the medicine was released in the first 24 hours. Most of the remaining medication was discharged within 72 hours. The drug particles loosely attached to the surface of D-NLCs could be the origin of the burst release pattern of the drug observed in the release experiments. Moreover, the free drug showed drug release within 30 min whereas no drug release was observed from blank formulation.

Table 9 Percent CDR at different time interval

Sr.No.	Time (min)	%CDR
1.	0	0%
2.	15	22.96%
3.	30	27.41%
4.	60	30.37%
5.	120	35.92%

6.	180	40%
7.	240	44.44%
8.	360	47.41%
9.	720	51.85%
10.	1440	54.1%
11.	2880	66.9%
12	4320	85.3%

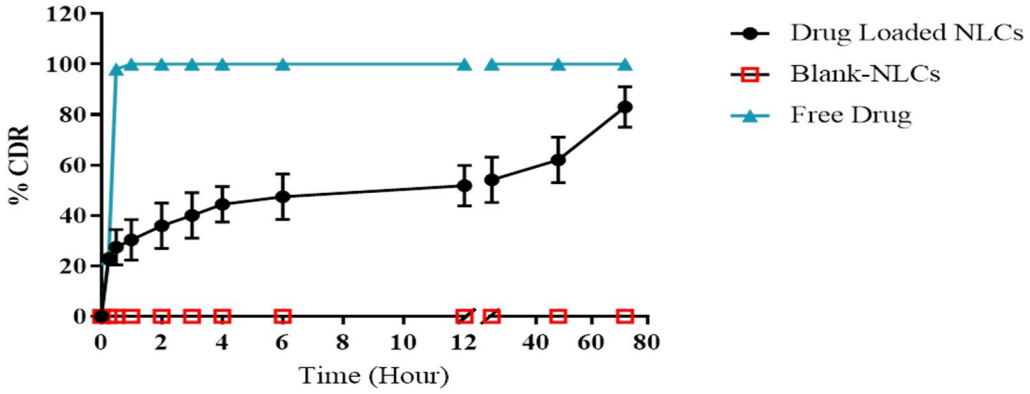


Figure 13 In vitro drug release profile for Drug loaded nanostructured lipid

3.2.5 In-vitro Antibacterial assays

The of D-NLCs and NLCs were evaluated for antibacterial activity and results were compared. Quantitative studies in liquid medium have shown that D-NLCs have higher antibacterial activity than NLCs. This is due to the immediate release of the drug from the formation on the surface of D-NLCs responsible for the mechanistically controlled release of the drug.

The D-NLCs exhibit antibacterial activity after 72 hours of incubation, whereas the NLCs do not. Similarly, after incubation with S. aureus for 72 h, the NLCs showed no effect on bacterial growth compared to D-NLCS.

The bacterial inhibitory efficacy of the D-NLCs and NLCs was further studied by the cup plate method. It is clear that NLCs in saline do not have bacterial inhibition rings in any of the tested locations. A bacterial inhibitory ring can be seen at all time points on the positive control in the form of D-NLCs.

After 72 h of incubation, the inhibition ring of bacteria in D-NLCs is larger than the positive control and almost disappears in NLCs. Similarly, for S. aureus, after 72 hours of incubation, the bacterial inhibition ring D-NLC is larger than the positive control and absent in the NLCs, unlike the others.

The effect of D-NLCs on microbial strain. NLCs show no bacterial inhibition ring in contrast to positive control.

Table 10 Antimicrobial Susceptibility Testing [Zone of Inhibition]

Species	Concentration of the drug (µg/ml)	Zone of Inhibition (mm)
Gram (-ve) *Escheric hia coli*	1000	55±5
	2000	75±5
	3000	105±5

	4000	115±5
	5000	145±5
Gram (+ve) ***Staphylococcus aureus***	1000	30±5
	2000	55±5
	3000	85±5
	4000	95±5
	5000	135±5

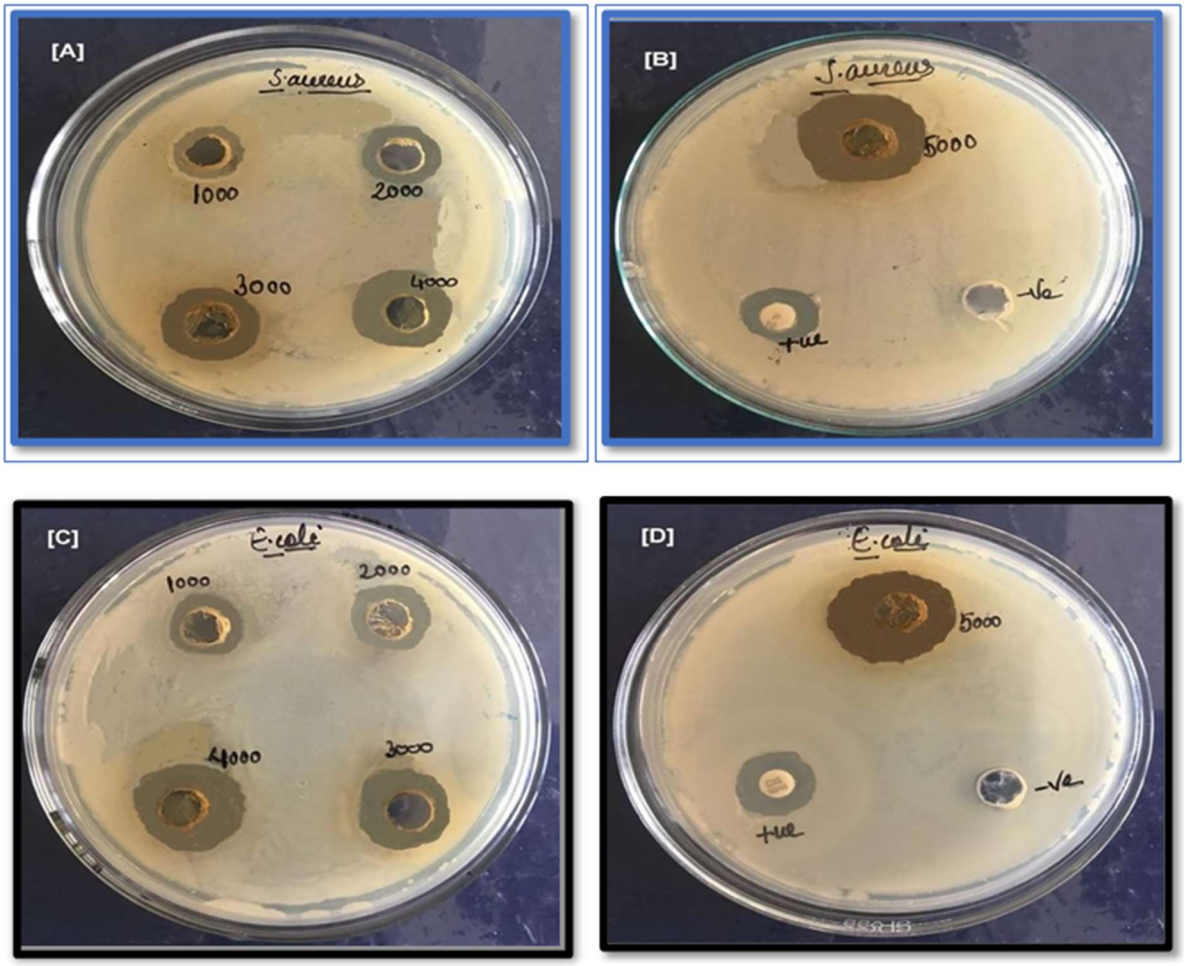


Figure 2.Qualitative growth inhibition assay of bacteria on agar plates with effect on S.aureus&E.coli

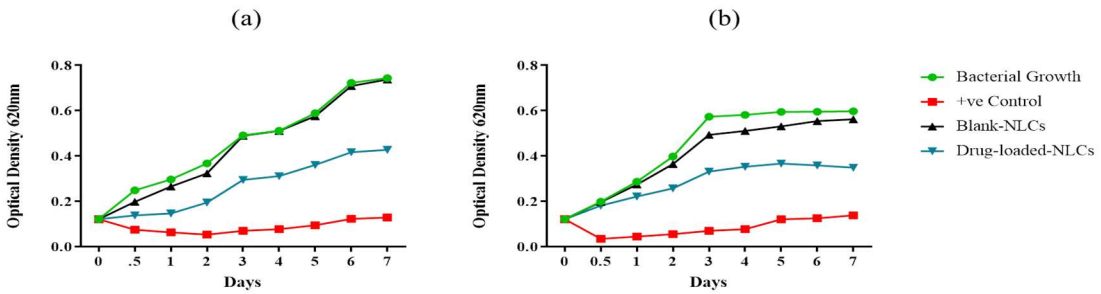


Figure 15 Quantitative in vitro antibacterial activity assay showing inhibition of bacteria E. coli growth in L.B. liquid medium and S. aureus.

CONCLUSION

The current research aimed to optimize Curcumin and α-Tocopherol loaded Nanostructured Lipid Carriers (NLCs) as an effective lipid-based delivery system. The improved formulation showed desirable physicochemical properties such as nanoscale particle size, good stability and high encapsulation efficiency. The FTIR analysis confirmed the compatibility of the active compounds with the excipients of the formulation, demonstrating that there were no major chemical interactions. The NLC formulation exhibited sustained drug release with approximately 85% total drug release over the period of evaluation, possibly contributing to longer therapeutic activity. The drug-loaded NLCs exhibited superior antibacterial activity against *E. coli* and *S. aureus* than the blank formulations indicating a possible synergistic effect of curcumin and α-tocopherol in the lipid carrier system. The NLC formulation resulted in improved stability, release profile, and biological activity of antioxidants. Curcumin-α-tocopherol-loaded NLCs show promise for topical drug delivery, wound healing, antioxidant therapy, and treatment of oxidative stress-related skin diseases. Further in vivo investigations are required to determine clinical efficacy and safety.

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