Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 9s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Formulation And Evaluation of Thymoquinone Loaded NLC by Emulsion Evaporation Method

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Cite this paper as: Akash Dewangan, Dr. Lavakesh Kumar Omray, Dr. Naveen Gupta, Dr. Dharmendra Singh Rajput, Dr. Dusmanta Kumar Pradhan, (2025) Formulation And Evaluation Of Thymoquinone Loaded Nlc By Emulsion Evaporation Method. *Journal of Neonatal Surgery*, 14 (9s), 772-780.

ABSTRACT

Nanoparticles based controlled drug release can also reduce the side effects of drugs. Benefits of nanoparticle drug delivery systems include minimised irritant reactions and improved penetration within the body due to their small size, allowing for intravenous and other delivery routes. Oral administration is the most convenient route among various routes of drug delivery as it offers high patient compliance. However, the poor aqueous solubility and poor enzymatic/metabolic stability of drugs are major limitations in successful oral drug delivery. There are several approaches to improve problems related to hydrophobic drugs. Among various approaches, nanotechnology-based drug delivery system has potential to overcome the challenges associated with the oral route of administration. Novel drug delivery systems are available in many areas of medicine. Nanoparticles (NPs) and other colloidal drug-delivery systems modify the kinetics, drug distribution in the body and release profile of an associated drug. Nanostructured lipid carriers (NLCs) have been promising formulation for effective oral drug delivery and opening new possibilities for NLCs in future pharmaceutical applications. Nanostructure lipid carriers (NLCs) are nanosized-based carrier systems which comprise solid lipid matrix combined with liquid lipids and surfactants. The aim of the paper is to explore the various advantages of formulation technology along with the characterization parameter of the NLCs.

Keywords: *Thymoquinone, NLC, Emulsion Evaporation Method*

1. INTRODUCTION

Natural products have been effectively treating numerous diseases with multifaceted therapeutic effects and a higher safety profile. Today, natural products play a vital role in global rehabilitation and healthcare, serving as a valuable source of new drugs. Novel Nano-Drug Delivery System (NDDS) refers to a novel approach in the pharmaceutical field, harnessing the potential of nanotechnology for drug delivery. Depending on the carrier materials and structures used, NDDS fall into distinct categories, including nanosuspensions, nanoliposomes, micelle, microemulsions/self-microemulsions, nanocapsules, and solid lipid nanoparticles [1]. Natural products are delivered with diverse nano-novel drug delivery systems, including nanosuspensions, microemulsions, nanoliposomes, micelles, nanocapsules, and solid lipid nanoparticles via optimal administration routes, such as oral, intravenous, nasal, inhalational, transdermal, and ocular routes [2]. NLCs present several advantages such as easy manufacturing, low toxicity, physical stability, custom-tailored release, high drug entrapment, no drug leaching during storage, and improvement of drug's solubility and stability, which are some excellent features that grant them an upper hand over other drug delivery systems. NLCs, by their biocompatible nature, can be administered via the oral, parenteral, topical, rectal, and pulmonary routes [3]. Owing to the several advantages of NLCs, this review focuses on the events that occurred late and recently in the successful oral delivery of poorly soluble medications using NLCs. Lipid-based nanoparticles have been utilized in the delivery of poorly soluble drugs of late with solid lipid nanoparticles being the earlier drug delivery system that has shown great potential in the delivery of medications across the

GIT. The use of biodegradable natural lipids and surfactants and toxic solvent-free methodologies in their preparation enable lipid-based nanoparticles to be the foremost selection for the delivery of poorly soluble drugs [4]. NLCs have provoked the incessant impulsion for the development of safe and valuable drug delivery systems owing to their exceptional physicochemical and then biocompatible characteristics. Throughout the earlier period, a lot of studies recounting NLCs based formulations have been noticeably increased. They are binary system which contains both solid and liquid lipids aiming to produce less ordered lipidic core. Their constituents particularly influence the physicochemical properties and effectiveness of the final product. NLCs can be fabricated by different techniques which are classified according to consumed energy. More utilization NLCs is essential due to overcome barriers surrounded by the technological procedure of lipid-based nanocarriers' formulation and increased information of the core mechanisms of their transport via various routes of administration. They can be used in different applications and by different routes such as oral, cutaneous, ocular and pulmonary [5].

2. MATERIAL AND METHODS

Determination of wavelength of maximum absorbance (λmax value): For the standardization of the drug using UV spectroscopy, the drug is firstly subjected to wavelength scan for the determination of absorbance maxima (λmax). The prepared sample was scanned in UV spectrophotometer in the wavelength ranging from 200-400 nm. To determine the wavelength at which maximum absorbance was observed and selected as the λmax of the drug in that particular media. UV spectrum of thymoquinone was taken and the absorption maxima were noted. Absorption maximum (λmax) determination by ultraviolet spectroscopy of thymoquinone 20 mg of drug was dissolved in 50 ml of phosphate buffer saline pH 6.8 solution. It was further diluted to 100 ml with the similar solvent i.e. ethanol. 10 ml thymoquinone solution was further diluted to 100 ml with phosphate buffer saline pH 6.8. After, the dilution and volume make-up it was scanned or examined in the range between 200 to 400 nm [6].

Standard plot of thymoquinone in phosphate buffer saline pH 6.8: The standard calibration plot of drug was prepared in phosphate buffer saline pH 6.8. Last, the calibration curve was prepared in phosphate buffer saline pH 6.8. It was prepared to carry out the drug release medium in the dissolution study as it mimics the intestinal conditions of the body. For the preparation of standard plot of drug in pH 6.8. In this, the drug was firstly solubilized in 30% phosphate buffer saline pH 6.8 and after complete solubilized the volume was made up to 100 ml with phosphate buffer saline pH 6.8 in 100 ml volumetric flask to yield the solution of concentration 100 µg/ml. From the above standard stock solution, further aliquots were diluted to get the working standard solutions i.e. 0, 2, 4, 6, 8, 10 µg/ml were made in triplicate to prepare a calibration plot of the drug in phosphate buffer saline pH 6.8 for drug release study. The absorbance of further aliquots was taken at 254 nm λmax of drug by using phosphate buffer saline pH 6.8 as blank.

Linearity: CL (50.0 mg) was weighed accurately and dissolved in 10 mL 0.1 M sodium acetate buffer (pH 5) (5000 µg mL-1 stock solution). Six samples of 5-400 µL were taken from this stock solution and diluted to 1.0 mL with the mobile phase (25-800 µg mL-1 solutions). drug peak responses of these samples were determined. Regression equation and regression coefficients were calculated (n=3).

Accuracy: It was calculated as the percentage of recovery by the assay of the known amount of analyte which contained 50, 100 and 150 % active agent in the sample, using the regression equation (n=6).

Precision: Three concentrations of drug solutions (25, 400, and 800 µg mL-1) were prepared using stock solution of drug. The peak responses of these samples were measured. The relative standard deviation (coefficient of variation) of a series of measurements was calculated. The same procedure was performed on consecutive days (n=3).

Specificity: Method selectivity was assessed by the analysis of eight placebo formulations at the same assay conditions. Sensitivity (detection and quantification limits) the limit of detection is the lowest concentration of the analyte which can be detected in a sample. Limit of quantification is the parameter of quantitative assays for the low-level compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals.

Physical appearance: Powder samples of thymoquinone were inspected visually to check colour, appearance and other physical characteristics.

Solubility: Solubility of thymoquinone were studied in water and other organic solvents.

Melting point: Capillary tube method was used for the determination of melting point of the drug i.e. cetirizine hydrochloride. It was determined by using digital melting point apparatus. To carry out thymoquinone was filled in the capillary tube. It was kept in melting point apparatus. It was uninterruptedly observed to determine the temperature at which the drug was almost melted. The temperature at which drug was melted; it was noted as the melting point of the pure drug. Drug-excipients compatibility studies: The drug-excipients interaction was carried out by using Fourier transform infrared (FTIR) spectroscopy analysis. To carry out the study the physical mixtures of drug and polymers in the ratio of 1:1 were mixed uniformly with IR grade KBR to make pellets by compressing in a hydraulic pressure. The prepared pellets were then scanned over a group frequency range of 4000-5000 cm-1 to observe the peaks corresponding to different functional groups using PerkinElmer spectrum 400 USA, FTIR instrument. The drug-excipient compatibility studies were performed by visual and non-thermal (FTIR) techniques [7].

Preparation of NLC by Emulsion Evaporation Method: In emulsion evaporation method, drug, solid lipid, liquid lipid and lipophilic surfactant were dissolved in the organic solvent in a water bath at 75 ◦C. The resultant organic solution was added drop wise in the aqueous phase containing amount given Poloxamer 188 with surfactant Tween 80. The resultant material was subjected to mechanical agitation at1000 rpm and cooled for obtaining NLCs [8]. The formulations are depicted in Table 6.

Table 6: Formulation of thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method

F. Code	Drug (mg)	Lipid		Poloxamer 188 (%)	Tween 80 (%)
		Lecithin	Linseed oil		
TQ-NLC1	10	100		10	5
TQ-NLC2	10		100	10	5
TQ-NLC3	10	50	50	10	5
TQ-NLC4	10	25	75	10	5
TQ-NLC5	10	75	25	10	5
TQ-NLC6	10	100		5	10
TQ-NLC7	10		100	5	10
TQ-NLC8	10	50	50	5	10
TQ-NLC9	10	25	75	5	10
TQ-NLC10	10	75	25	5	10

Measurement of the Particle Size and Polydispersity Index (PDI): The average diameter and polydispersity index (PDI) of TQ-NLC were analyzed at a fixed angle of 173° and at 25°C with the Malvern software using photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern, UK). TQ-NLC was diluted with deionized water (1 : 9) prior to analysis to prevent back-scattering effect. The analysis was performed in triplicate.

Determination of TQ-NLC Encapsulation Efficiency and Drug Loading Capacity: Encapsulation efficiency (EE) and drug loading capacity of TQ-NLC were calculated by determining the amount of free drug using an ultrafiltration technique. Briefly, 5 mL of TQ-NLC solution was placed in the upper chamber of a centrifuge tube matched with an ultrafilter (Amicon Ultra, Millipore Co., USA, MWCO 10 kDa) and centrifuged for 10 minutes at 2000 ×g. The ultrafiltrate containing the unencapsulated drug was determined by UV spectrophotometry analysis at 254 nm. The drug loading content was the ratio of incorporated drug to lipid (w/w). The TQ encapsulation efficiency and drug loading capacity were calculated by the following equation:

$$\text{TQ encapsulation efficiency} = \frac{W_{\text{total drug}} - W_{\text{free drug}}}{W_{\text{total drug}}} \times 100,$$

$$\text{Drug loading capacity} = \frac{W_{\text{total drug}} - W_{\text{free drug}}}{W_{\text{lipid}}} \times 100,$$

where “W total drug” is the mass of the total TQ used, “W free drug” is the mass of the free drug detected in the filtrate of lower chamber of post centrifugation of the aqueous dispersion, and “W lipid” is the mass of lipid added into the aqueous matrix.

In *vitro* drug release studies: In vitro drug release studies from NLC were performed in sink conditions according to the dialysis bag method and compared to a simple drug suspension, prepared by suspending 0.2% p/v of TQ in water under stirring. Cellulose acetate dialysis bags (Sigma-Aldrich, St. Louis, MO, USA, 12,500 cut-off) were soaked overnight in pH 6.8 gastric buffer, filled with 1 mL of NLC dispersion or suspension containing 2 mg of drug and immersed for 2 h in 100 mL of pH 6.8 phosphate buffer simulating the infant gastric pH (drug solubility in the medium about 0.7 mg/mL) and then for 4 h in 100 mL of pH 6.8 phosphate buffer (simulating the intestinal pH), thermostated at 37 °C and stirred at 50 rpm. The concentration of released drug was spectrometrically assayed at 254 nm at given time intervals. Each withdrawn sample from the receiver solution was replaced with an equal volume of fresh solvent. A correction for the cumulative dilution was calculated [9]. Experiments were performed in triplicates and results expressed as mean values ± S.D.

3. RESULTS AND DISCUSSION

The maxima wavelength of thymoquinone was determined in Phosphate Buffer Solution (pH 6.8) was determined 254 nm (Figure 1). The standard curves of thymoquinone were prepared in phosphate buffer solution (pH 6.8) at λmax 254. The data were regressed to obtain the straight line. The correlation coefficient greater than 0.99 was observed in all the cases, which indicated that, the drug follows Beer-Lambert's law in the concentration range of 2-10 µg/ml (Figure 2). Specificity is defined as the ability to detect the analyte of interest in the presence of interfering substances. Specificity can be shown by spiking

known levels of impurities or degrading agents in to a sample with a known amount of the analyte of interest. The ICH guidelines classified precision in to two parts; repeatability and intermediate precision. Accuracy is the difference between the measured value and the taken value (Table 1-5).

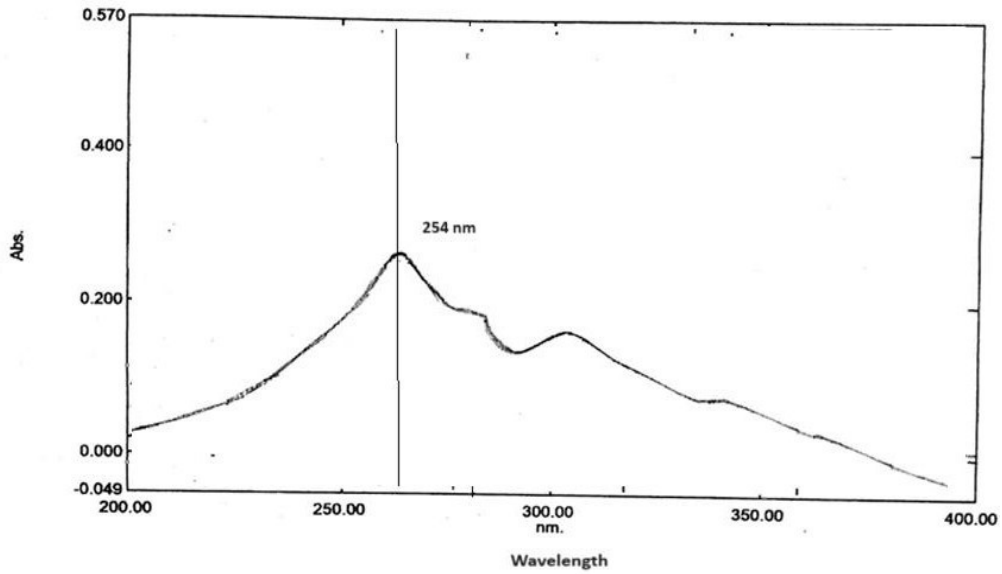

Figure 1: UV absorption maxima of thymoquinone in Phosphate Buffer Solution (pH 6.8) at λmax 254 nm

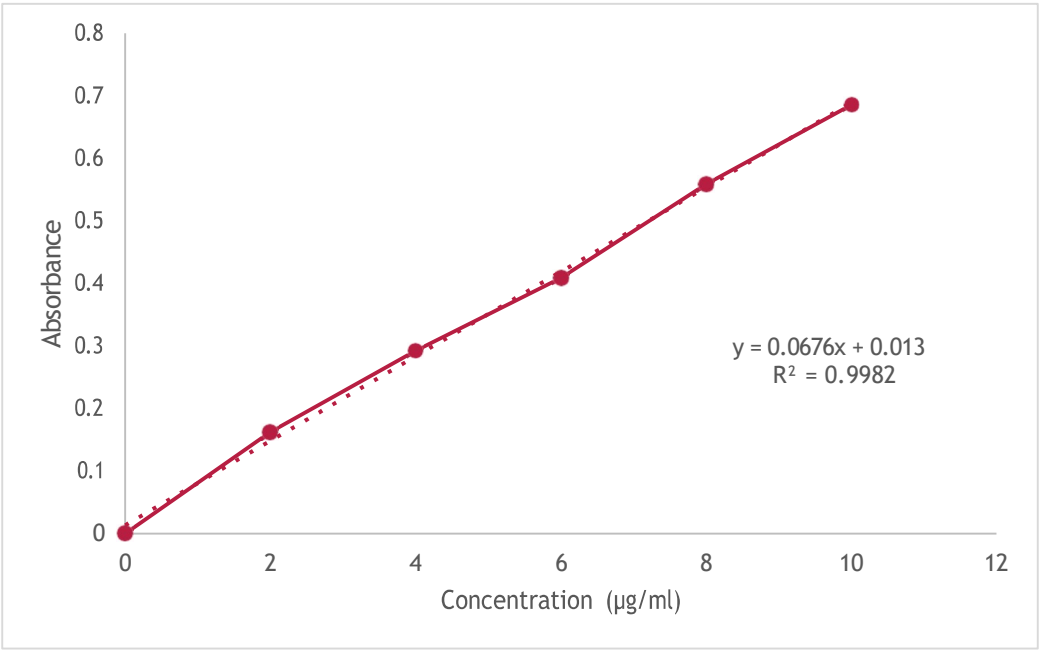

Figure 2: Standard Curve of thymoquinone in Phosphate Buffer Solution (pH 6.8) at λmax 254 nm

Table 1: Results of specificity test

Test solution	Thymoquinone (Drug content)
Standard drug solution	25.01
Placebo solution	0
Drug solution containing Excipients	24.98

Table 2: Results of repeatability test of 10 µg / ml drug solution

S. No.	Thymoquinone (Drug content)
1	0.561
2	0.558
3	0.557
4	0.557
5	0.562
6	0.561
7	0.563
8	0.562
9	0.561
10	0.564
Mean	0.56

Table 3: Results of intra-day precision of 10 µg / ml drug solution

Time	Thymoquinone (Drug content)
0 hr	0.561
1 hr	0.558
2 hr	0.557
3 hr	0.557
4 hr	0.562
5 hr	0.561
6 hr	0.564

Table 4: Results of inter-day precision of 10 µg / ml drug solution

Time	Thymoquinone (Drug content)
Day 1	0.558
Day 2	0.557
Day 3	0.557
Mean	0.188

The physical characteristics of drug powder thymoquinone such as Pale-yellow color, odorless and tasteless were noted visually. Melting point of drug was determined by melting point apparatus and practically observed melting point was 46˚C. The sample was qualitatively tested for its solubility in various solvents. It was determined by dissolving 10 mg of drug sample in conical flask and solvent as water, methanol, ethanol, ether and chloroform etc., was contain in burette. Now use incremental method for identify the fix amount of drug soluble by quantity of solvent and get the concentration of solution for calculation of solubility in mg/ml. S. The solubility in Water 1.013 mg/ml, pH 1.2 0.1N HCl 0.0411 mg/ml, pH 6.8 phosphate buffer 1.781 mg/ml and pH 7.4 phosphate buffer0.214 mg/ml. The partition coefficient of the drug in was – 0.1034. The FTIR spectra observed that the characteristic absorption peaks of pure Thymoquinone were obtained at 2984, 1643, 1249 cm-1 corresponding to C-H, C=C, C=O (Figure 3).

Table 5: Results of accuracy test for thymoquinone

Recovery level	Amount of drug recovered	Amount of drug added (mg)	% recovery	Mean	Standard Deviation	%RSD
25%	25.01	25	100.01	99.96	0.2213	0.0965
	24.95	25	99.98			
	24.78	25	99.16			
50%	49.54	50	99.92	99.97	0.2129	0.0964
	49.91	50	99.96			
	49.92	50	99.98			
100%	99.97	100	99.97	99.96	0.1111	0.0935
	99.92	100	99.92			
	99.98	100	99.98			

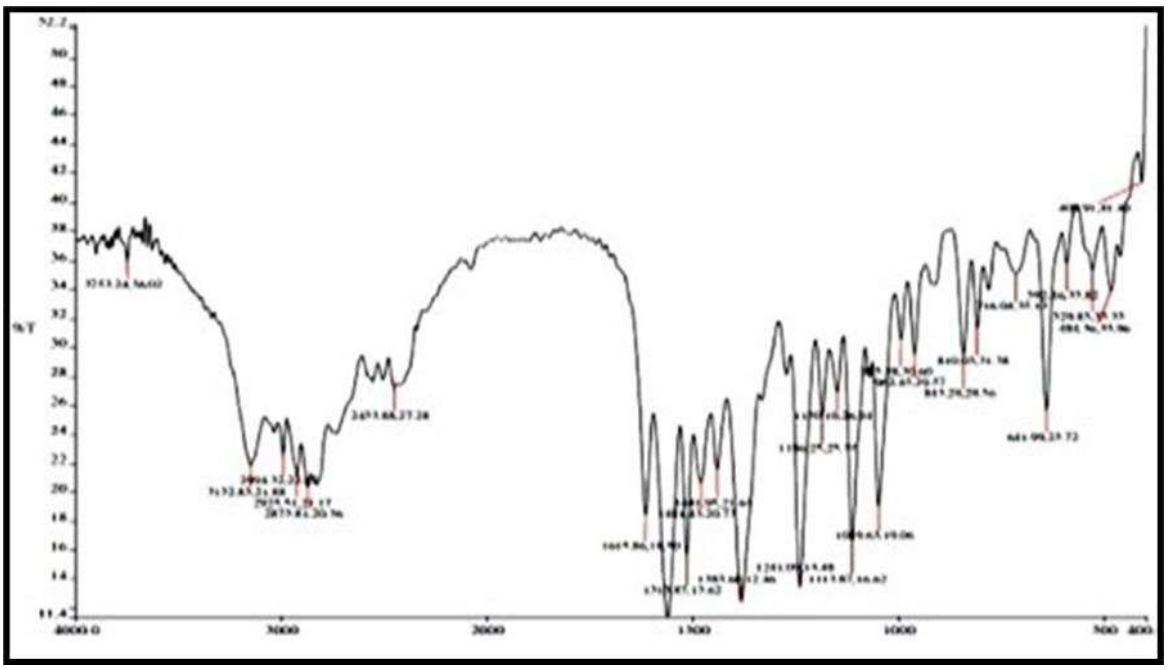

Figure 3: FTIR spectra of drug thymoquinone

Particle size measurements are a good indicator of instability and are used to characterize the product. Physical properties of prepared thymoquinone containing Nanostructured Lipid Carriers by solvent emulsion method (TQ-NLC1 to TQ-NLC10) were performed using microscope. Some formulation showed double layer as some formulation showed single layer. Physical properties of prepared thymoquinone containing nanostructured lipid carriers by using microscope. In evaluating the particle size distribution within all formulations, dynamic light scattering was employed to calculate the polydispersity index (PDI) (Table 7 & Figure 4). To maintain the stability of a nanostructured lipid carriers' formulation, it is imperative that the polydispersity index remains within an acceptable range. High values indicate a broad range of particle sizes and a greater variability, which can lead to issues such as aggregation or sedimentation over time. In contrast, low polydispersity indices signify a more uniform and narrow size distribution, which is desirable for achieving formulation stability and ensuring consistent performance. Measurement of zeta potential (ZP) allows predictions to be made about the storage stability of colloidal dispersions. In general, particle aggregation is less likely to occur with charged particles (high zeta potential) due to electrical repulsion (Table 7 & Figure 5). In this study, an essential aspect was to assess the content of drug within the NLC dispersions. This evaluation served to gauge any potential loss of drugs that might have transpired during the formulation process of the optimized NLC dispersions. The drug content percentages obtained are consistent with data in the literature showing that high drug content percentages are obtained when NLC is used as a carrier system. The in-vitro release of drug from thymoquinone loaded NLC was performed using treated dialysis membrane. The NLC suspension in 1 mL quantity was poured to dialysis tube and sealed. The release of drug from NLC was compared with the release of drug from pure drug suspension (Figure 6-7).

The data obtained were subjected to one-way analysis of variance (ANOVA) using XL STAT followed by Newman- keuls multiple comparison value obtained (P< 0.05). The results were considered to be statistically significant. The current research proved that Nanostructured Lipid Carriers is a adaptable technology that have a potential ability to enhance the solubility of thymoquinone NLC are the nano lipid carriers which encapsulate the lipophilic drug in the lipid matrix. The NLC approach was utilized to increase the solubility and oral bioavailability of thymoquinone.

The NLC containing thymoquinone were prepared by solvent evaporation technique with lecithine and linseed oil were used as a lipid, tween 80 as a surfactant and polaxomer as a stabilizer in the formulation. The optimized batch of TQ-NLC5 was found to be stable in accelerated stability studies at room temperature. The in-vitro release studies showed the extended release of drug from drug loaded NLC in 24 hrs of studies. The optimized formulation of drug loaded NLC were further subjected to in-vivo studies.

Table 7: Evaluation parameter of thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method

Formulation Code	Layers	Particle size (nm)	PDI	Zeta potential (mV)	Drug content (%)
TQ-NLC1	Double	138.11±1.12	0.223±0.18	-32.01±1.11	96.71%±1.11%
TQ-NLC2	Double	141.23±1.11	0.224±0.12	-32.08±1.11	96.21%±1.21%
TQ-NLC3	Double	136.12±1.01	0.226±0.15	-35.11±1.14	97.21%±1.52%
TQ-NLC4	Single	141.14±1.24	0.225±0.23	-33.11±1.11	94.14%±1.21%
TQ-NLC5	Single	138.14±1.31	0.223±0.15	-34.12±1.01	96.23%±1.31%
TQ-NLC6	Double	137.61±1.12	0.231±0.14	-33.17±1.04	95.21%±1.25%
TQ-NLC7	Double	139.01±1.14	0.234±0.17	-32.14±1.12	95.23%±1.22%
TQ-NLC8	Double	141.15±1.18	0.234±0.17	-33.14±1.11	96.25%±1.21%
TQ-NLC9	Single	141.12±1.25	0.234±0.21	-32.16±1.12	95.12%±1.21%
TQ-NLC10	Single	139.15±1.31	0.236±0.24	-33.14±1.11	96.32%±1.21%

Summary and conclusion: Since the beginning of time, formulators have been faced with the task of designing and delivering medicines and biologicals via the oral route. There are many different kinds of therapeutic compounds that have problematic pharmacological characteristics, and lipid formulations offer a viable strategy for such molecules. It has been shown that the formulation of NLCs has a significant potential for administration via the oral route. This is due to the fact that it enhances the oral bioavailability by avoiding the first-pass effect and also solves the drawbacks of the SLN formulation. The formulations of a variety of lipophilic and hydrophilic medicines that are created using NLCs are discussed in the previous paragraph. Biocompatible, biodegradable, non-irritating, and non-sensitizing are all characteristics of NLCs. Despite this, there have been very few studies conducted on the process of improving the solubility, bioavailability, stability, and permeability of medicines. The Nanostructured Lipid Carrier is one of the many lipid formulations that provides additional benefits, including the increase of solubility, bioavailability, permeability, and stability, as well as the simplicity of manufacturing and the flexibility to scale up production. The nanostructured lipid carrier was developed with the intention of resolving challenges such as low solubility, bioavailability, stability, and permeability of hydrophilic and lipophilic drugs.

Results

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 138.14	Peak 1:	129	112.24	105
PdI: 0.223	Peak 2:	0.00	0.0	0.00
Intercept: 0.306	Peak 3:	0.00	0.0	0.00

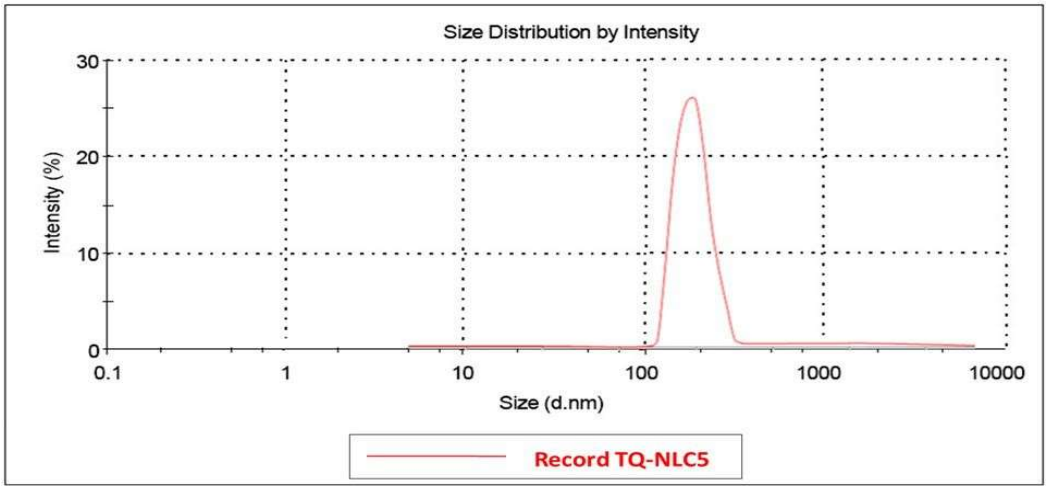

Figure 4: Particle size distribution & Polydispersity Index (PDI) of prepared thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method (TQ-NLC5)

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -34.12	Peak 1:	-34.12	99	3.96
Zeta Deviation (mV): 59.99	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.309	Peak 3:	0.00	0.0	0.00

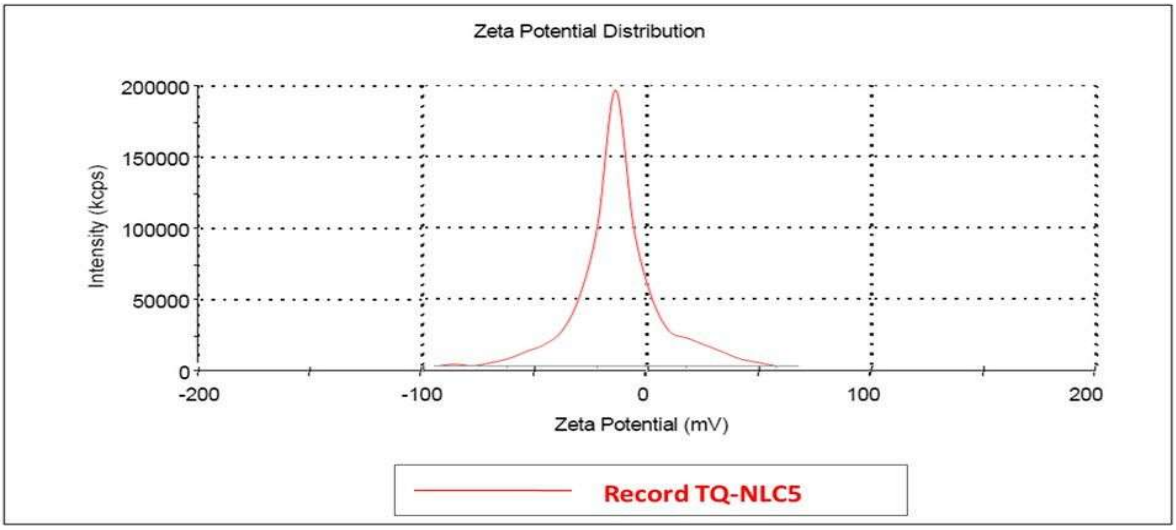

Figure 5: Zeta potential (mV) of prepared thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method (TQ-NLC5)

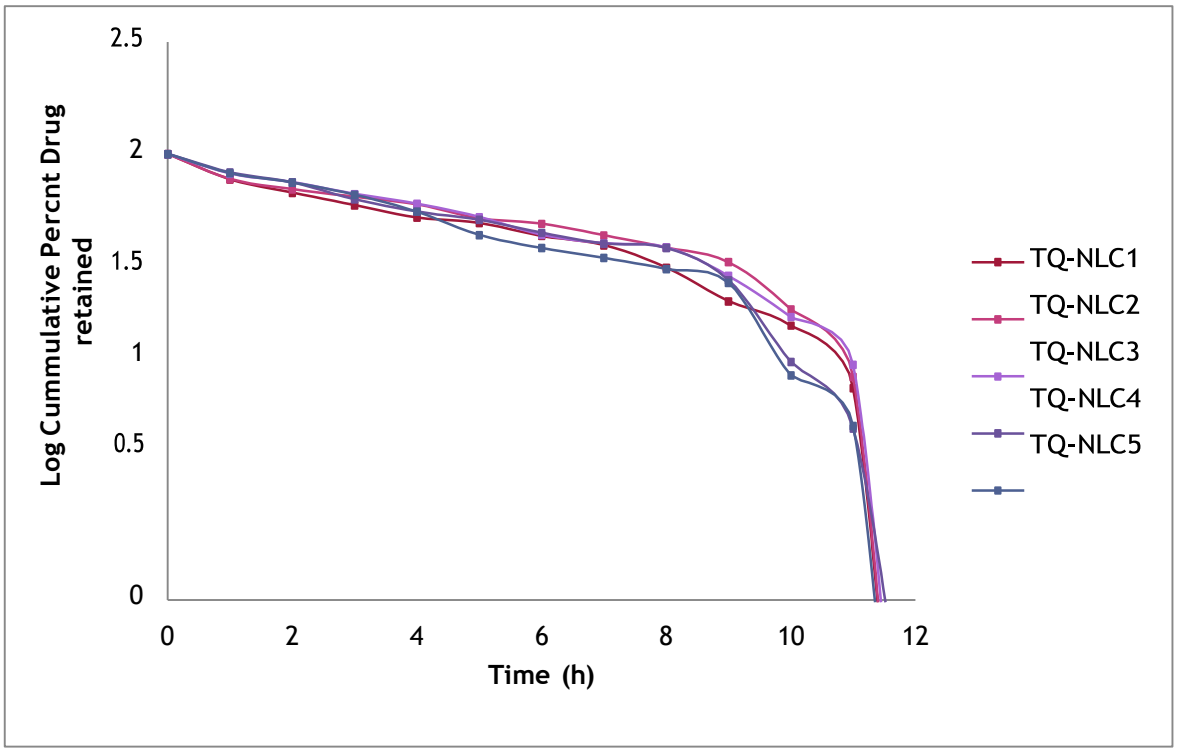

Figure 6: in-vitro drug release study (zero-order kinetics) of prepared thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method (TQ-NLC1 - TQ-NLC5)

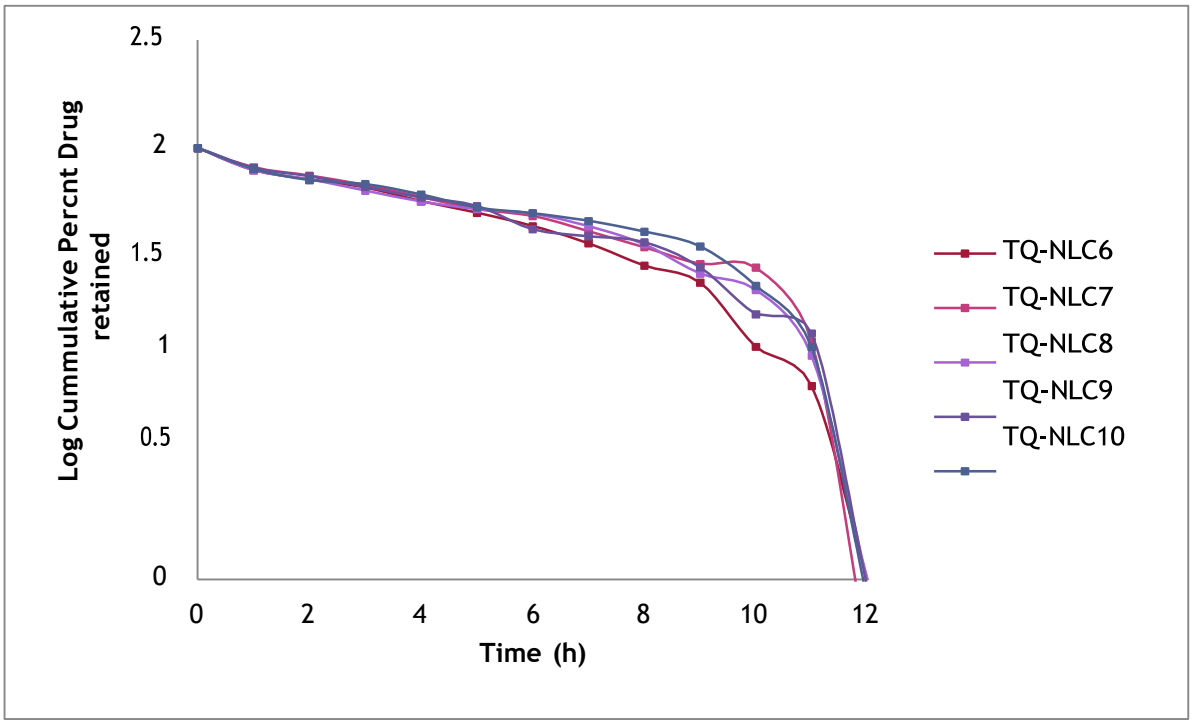

Figure 7: in-vitro drug release study (zero-order kinetics) of prepared thymoquinone containing Nanostructured Lipid Carriers by Emulsion Evaporation Method (TQ-NLC6 - TQ-NLC10)

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