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Formulation and Evaluation of Nanostructured Lipid Carriers (NLCS) Containing Thymoquinone

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KEYWORDS

Thymoquinone, nanostructured lipid carrier, Hot Homogenization Method, Bioavailability, and Controlled release

ABSTRACT:

Nanotherapeutics is increasingly dominating worldwide research and is regarded as a new therapy paradigm in the medical industry. This study outlines the creation of a stable nanostructured lipid carrier (NLC) technology for thymoquinone delivery. This work seeks to construct gelatin-coated nanostructured lipid carriers (NLC) for encapsulating sage extract, with the objective of enhancing the quality characteristics of beef burger samples. Nanostructured lipid carriers (NLC) were synthesized using the hot homogenization ultrafiltration method and then assessed for particle size, zeta potential, polydispersity index (PDI), encapsulation efficiency (EE), and in vitro drug release.

Introduction:

Targeted drug administration is often used to provide pharmaceuticals selectively and in elevated doses to neoplastic tissue [1]. Lipid-based nanoparticles serve as a flexible instrument with significant application potential; they can solubilize various compounds with diverse physicochemical characteristics inside a biocompatible and biodegradable matrix that has well-established safety profiles. Herbal remedies from traditional medicine have been used to address health issues since antiquity, although their efficacy is contingent upon prolonged bioavailability. The administration of herbal medications to patients by conventional means is not necessarily the most effective. Many phytocompounds have various limitations, including issues with bioavailability (poor solubility), the barrier function of the gastrointestinal system, and extensive primary metabolism. Nanotechnology is a unique and effective method to address the physicochemical limits of phytocompounds [4]. Consequently, nanostructured delivery methods for herbal medicine may augment biological activity. Nanostructured lipid systems provide a viable approach among colloidal delivery technologies to address the primary limitations of bioactive chemicals derived from diverse herbal sources that hinder their utilization [5]. The goals include the discovery of formulation factors that influence NLC formulation and its features, such as size and zeta potential (ZP). The additional purpose was to conduct in vitro cytotoxicity and brain distribution investigations of the formulated products. Nanoencapsulation is a unique technique for encapsulating bioactive substances inside a protective shell. This approach is particularly successful in stabilizing, transferring, solubilizing, conserving, and enhancing the activity of active substances. Recently, the food industry has focused significantly on the nanoencapsulation of bioactive substances via lipid-based systems and the development of nanocarriers for diverse purposes [6]. Lipid-based carriers are distinctive delivery methods that can encapsulate both hydrophilic and lipophilic substances, owing to their low toxicity, cost-effectiveness, and simplicity of manufacture, contingent upon the kind of lipids and microencapsulation techniques used [7]. Nanostructured lipid carriers (NLCs) are systems composed of solid and liquid lipids, surfactants, and water, whereby a liquid lipid core is encased by a matrix of solid lipids. NLCs may enhance the stability of physicochemical qualities, solubility, bioavailability, and controlled release of functional substances, including nutraceuticals and natural preservatives in food items [8]. Consequently, NLC, as an innovative carrier system, may be very useful in the production of enhanced or functional foods and in extending the shelf life of foods by mitigating

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deterioration (food preservation). Recent studies have focused on the encapsulation of phytochemical substances, extracts, and essential oils using NLC systems, including Mentha pulegium essential oil. Utilizing polymer coatings for NLC may enhance its effectiveness by augmenting absorption rates and distribution efficiency, boosting the chemical and enzymatic stability of the encapsulated substance, and improving colloidal stability.

Materials and methods

Hot Homogenization Method

Thymoquinone was solubilized in ethanol and combined with an acetone solution including a mixture of the lipid phases stearic acid and oleic acid. The mixture was then added dropwise to a combination of Tween 80 and sodium lauryl sulfate, which were used to stabilize the NLC. The mixture was sonicated at 85 ºC for 30 minutes at 1200 rpm with magnetic stirrer 8. The basic emulsion was transformed into the NLC system using a hot homogenizer at 15,000 PSI. The emulsion was then cooled to room temperature while being continuously stirred, resulting in the recrystallization of the lipid to create a nanostructured lipid carrier (NLC). The acquired NLC dispersions were subjected to lyophilization for further analysis [9]. The compositions of several substances were shown in Table 1.

Table 1: Formulation of thymoquinone containing Nanostructured Lipid Carriers by Hot Homogenization Method

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

Characterization of NLCs

The mean particle size (MPS) and polydispersity index (PDI): Both were assessed using photon correlation spectroscopy (PCS) utilizing a Malvern Zetasizer (Nano ZS 90, Malvern Ltd., Malvern, UK). The measurement using PCS relies on the light-scattering phenomenon, whereby the statistical intensity variations of scattered light from particles inside the measuring cells are quantified. Before the measurements, all samples were diluted with double-distilled water to achieve an appropriate scattering intensity. The z-average and PDI values were measured at a 90° angle using disposable polystyrene cells with a diameter of 10 mm at 25 °C, equilibrating for 120 seconds. The refractive index (RI) for measuring the size of lipid nanoparticle dispersion was established at RI = 1.330 (abs = 0.01). All measurements were conducted in triplicate at 25 °C. [10]

Zeta potential: The ZP, which denotes the electric charge on the particle surface and signifies the physical stability of colloidal systems, was assessed by measuring the electrophoretic mobility using the Malvern Zetasizer (Nano ZS 90, Malvern Ltd., Malvern, UK). The measurements were conducted

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subsequent to dilution in double-distilled water.

The measurement was conducted using the Dip cell with a field strength of 20 V/cm, and the average of the ZP was derived from 30 trials.

Efficiency of entrapment and drug loading: Entrapment efficiency (EE): These parameters are defined as the percentage of drug integrated into the lipid nanoparticles in relation to the amount drug added. It delineates the percentage of drug contained inside the particles and the percentage of free drug remaining in the dispersion medium. The TQ-NLCs dispersion was centrifuged at 45,000 rpm for 35 minutes; 1.0 ml of the resultant supernatant was diluted with 3.0 ml of DMSO, and the absorbance was measured spectrophotometrically at 423 nm using a UV-Visible spectrophotometer (UV 1700, Shimadzu, Kyoto, Japan) (Liu & Wu, 2010). Loading capacity or drug loading (DL) is the percentage of drug integrated into the lipid nanoparticles in relation to the overall weight of the lipoidal phase (i.e., lipid plus drug). CRM was extracted from lyophilized powder using DMSO and methanol. The CRM concentration was spectrophotometrically measured (UV 1700, Shimadzu, Kyoto, Japan) at 423 nm, using a DMSO and methanol combination as a blank [11].

Morphological analysis: The NLCs were assessed morphologically using a scanning electron microscope at an accelerating voltage of 20 kV. Samples were made by depositing NLCs onto an aluminum specimen stub, let them to dry overnight, and then sputter coating with gold before imaging.

In-vitro permeation study: Fresh nasal tissues were meticulously excised from the nasal cavity of sheep sourced from the local abattoir. Tissue samples were placed in Franz diffusion cells with a permeation area of 0.785 cm^2. At 37 °C, 25 ml of SNF with a pH of 6.4 containing 1% SLS was introduced into the acceptor compartment. The temperature in the chambers was maintained at 37 ◦C. Following a pre-incubation period of 20 minutes, a lyophilized powder corresponding to 50 mg of TQ was dispersed in 3 ml of SNF at pH 6.4 and positioned in the donor chamber. At specified intervals, 3 ml of samples were extracted from the acceptor compartment, with the sampled volume being replenished with SNF at pH 6.4 after each extraction, during a duration of 11 hours. The extracted materials were filtered and used for analysis. Control samples (devoid of drug) were concurrently analyzed throughout the experiment to detect any potential interference. The quantity of penetrated medication was quantified with a UV-Visible spectrophotometer at 254 nm [12].

Findings and discourse: The TQ-loaded NLCs were synthesized using the hot high-pressure homogenization method. Lecithin and almond oil were chosen as the solid matrix for TQ-NLCs, while poloxamer served as the liquid lipid and Tween 80 as the stabilizer. The equilibrium of emulsifiers is necessary at the oil-water interface for the stability of dispersions. NLCs may be synthesized by many techniques, including hot and cold homogenization, solvent diffusion, and microemulsion. The hot high-pressure homogenization technique was straightforward and rapid at the laboratory scale, and it did not involve the use of organic solvents in formulation development. Consequently, the hot homogenization process was used for the formulation of TQ-NLCs, using lecithin and almond oil poloxamer, which yielded the maximum drug payload without any issues of drug leakage. The homogenizer pressure was set to 600 bar for four cycles at 85 ± 0.5 °C. Figure 1 illustrates a minor peak succeeded by a more extensive peak, signifying two distinct populations of particles: the initial population exhibits a negligible presence with a diameter of 35.95 nm, while the subsequent broader peak corresponds to a population with a larger diameter of 146.8 nm, accompanied by polydispersity indexes (PDI) of 0.18. The particle size of the NLCs' dispersion is a critical determinant of both the pace and degree of drug release and absorption. A reduced droplet size increases the interfacial surface area for medication absorption. Particles with an average diameter of up to 200 nm may be readily transferred transcellularly by the intranasal pathway. Furthermore, it was proposed that the reduced size facilitates an accelerated release rate. Furthermore, it has been shown that a reduced particle size may facilitate accelerated absorption and enhance bioavailability. PDI also quantifies the breadth of particle size dispersion. A PDI below 0.5 may indicate strong homogeneity among the particle population, whereas higher PDI values imply a wide size dispersion. The ZP denotes the electrical charge on the surface of the NLCs. A higher ZP value indicates a better likelihood of suspension stability, since the repulsion between charged particles counteracts their inherent inclination to assemble. It is presently acknowledged that elevated ZP values, whether positively or negatively charged, indicate that dispersion will exhibit enhanced long-term stability. The ZP value was determined to be -31.4 ± 1.87 (Figure 2). Lipid nanoparticles serve as significant drug carriers

due to their potential for drug loading. In CRM-NLCs, a rise in EE was seen with the augmentation of precirol concentration. However, when the concentration of capmul MCM above 35%, a drop in the encapsulation efficiency (EE) was detected. This may result from lipid precipitation occurring during particle formation. Following the formation of NLCs, cooling induces the recrystallization of lipids, leading to a drug-free core or a core with diminished drug content. Consequently, a rise in lipid over a particular threshold results in decreased encapsulation efficiency. A notable impact was noticed with Tween-80. With the elevation of Tween 80 concentration, the encapsulation efficiency of the formulation improved. The EE of the NLC dispersion was determined to be 90.86%. The morphological analysis of the NLC formulation was conducted using scanning electron microscopy (SEM) images of freeze-dried NLCs. It was shown that they have an oval morphology (Figure 3). The size analysis results corroborate the findings of the photon correlation spectroscopy study. Figure 3-4 illustrates the release profile of TQ from drug-loaded NLC and plain drug suspension (PDS) over the dialysis membrane in SNF (pH 6.4). NLC formulations exhibited a biphasic release pattern, characterized by an early burst release followed by a persistent release at a consistent rate. A potential reason for this phenomenon is the disparity in melting points between solid lipid and liquid lipid. A solid lipid with a higher melting point may crystallize first, resulting in a liquid lipid core that is either absent of or contains little lipid. Ultimately, the majority of the liquid lipids situated at the nanoparticle's outer shell result in a drug-enriched shell, precipitating a burst release during the early phase (Figure 4-5).

Conclusion: This research distinctly delineates a novel formulation of TQ-NLCs exhibiting antihypertensive effect characteristics. A high-pressure homogenization technique was used to fabricate the TQNLC, enhancing drug incorporation and release characteristics. Phytochemicals from medicinal plants contained in nanostructured carriers will increasingly influence therapies in the future. Comprehensive and distinctive study was conducted to elucidate the function and primary contribution of phytochemicals in the development of herbal-enriched nanostructured carriers with enhanced therapeutic effectiveness. The integration of specific phytochemicals from diverse herbal sources with lipid nanocarriers has several options, primarily to formulate enhanced products, augment the efficacy of herbal delivery, and enhance the preferred attributes of herbal therapy.

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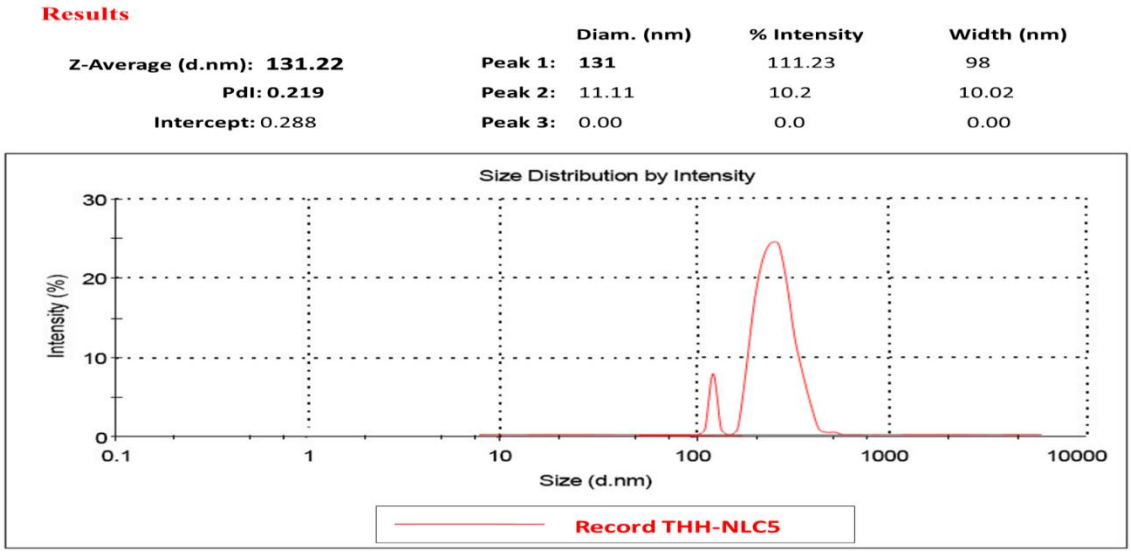

Figure 1: Particle size distribution & Polydispersity Index (PDI) of prepared thymoquinone containing Nanostructured Lipid Carriers by Hot Homogenization Method (THH-NLC5)

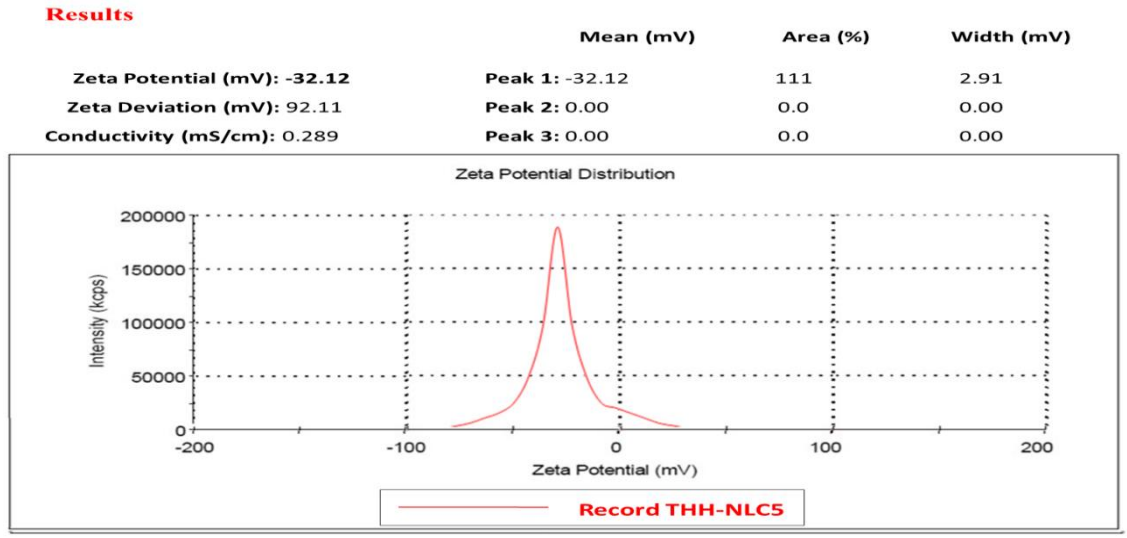

Figure 2: Zeta potential (mV) of prepared thymoquinone containing Nanostructured Lipid Carriers by Hot Homogenization Method (THH-NLC5)

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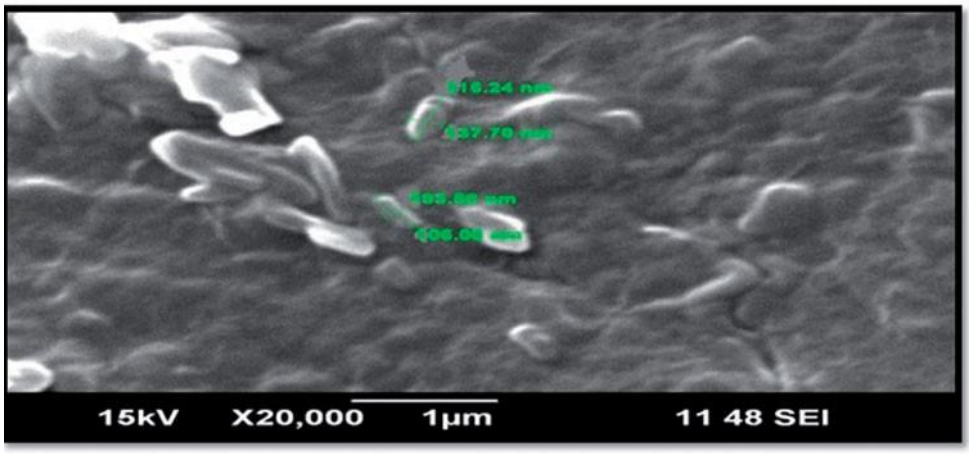

Figure 3. SEM image of THH-NLC5

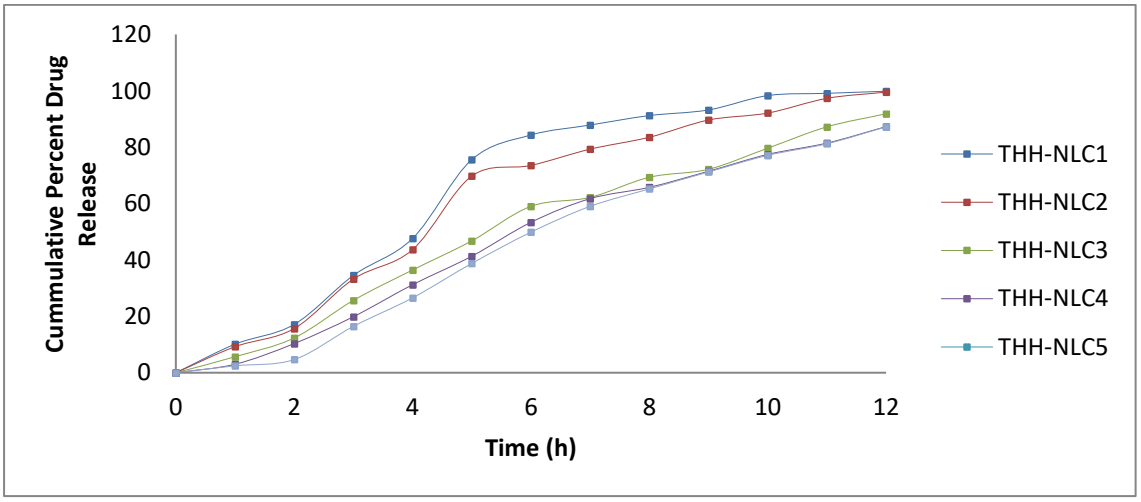

Figure 4: in-vitro drug release study (zero-order kinetics) of prepared thymoquinone containing Nanostructured Lipid Carriers by Hot Homogenization Method (THH-NLC1 - THH-NLC5)

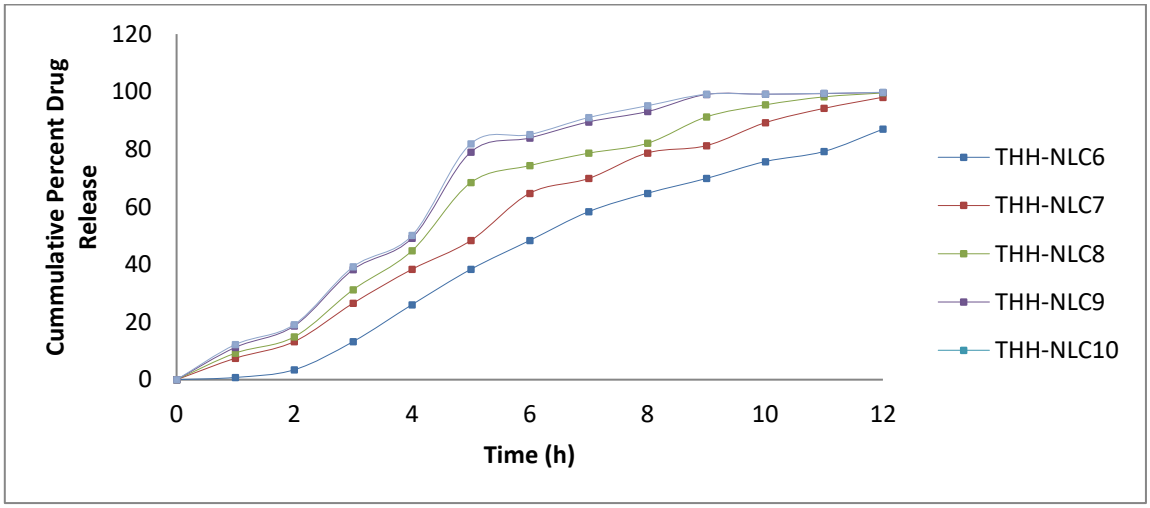

Figure 5: in-vitro drug release study (zero-order kinetics) of prepared thymoquinone containing Nanostructured Lipid Carriers by Hot Homogenization Method (THH-NLC6 - THH-NLC10)