Niosomal Encapsulation of Curcumin: Formulation and Characterization

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: Because of its many actions, curcumin, a plant-derived polyphenolic substance found naturally in turmeric (Curcuma longa), has been the focus of a significant investigation. The utilization of safe, useful, and highly functional chemicals obtained from natural sources in human nutrition/prevention/therapy needs some modifications to achieve multifunctionality, enhance the bioavailability, and delivery methods, all to enhance their efficacy. Curcumin's limited water solubility, fast metabolism and removal from the body, and hence low bioavailability, are significant obstacles to its use. To address these issues, a variety of new drug delivery systems with multiple routes of administration have evolved. Encapsulating the medication in vesicular structures is one such technique that, if successful in enabling selective absorption, can be predicted to extend the drug's life in systemic circulation and reduce toxicity. As a result, several vesicular drug delivery methods, including liposomes, niosomes, transfersomes, and pharmacosomes, have been developed. Since then, developments in vesicular drug delivery have resulted in the creation of systems that enable drug targeting as well as the prolonged or controlled release of traditional medications. The present study aimed to develop and characterize curcumin-loaded niosomes.

Design of Study: In present study 3² factorial method was used to formulate different formulations of niosomes containing curcumin.

Place and Duration of Study: Department of Pharmacy, IFTM University Moradabad, From December to May 2021.
Methodology: Various niosomal formulations of curcumin were developed by using surfactant and cholesterol by thin-film hydration technique. Total 9 formulations were developed and characterized ($3^2$ factorial designs).

Results: Formulation N7 was considered as an optimized formulation since formulation F7 has maximum drug entrapment and a prolonged drug release rate. The present study suggests that the concentration of surfactant and cholesterol affects % drug loading efficiency of niosomes. The percentage entrapment efficiency of niosomes increases on the increasing concentration of surfactant (Span 60) and cholesterol but above a certain concentration of cholesterol further increment in cholesterol concentration reduces drug entrapment efficiency of niosomes.

Keywords: Niosomes; curcumin; surfactant; cholesterol.

1. INTRODUCTION

Curcumin is a yellow substance found in the plant *Curcuma longa*. Curcumin, among other things, has anticancer, anti-inflammatory, antibacterial, hepatoprotective, and antirheumatic effects. Curcumin appears to have anti-inflammatory properties via inhibiting several inflammatory chemicals. Curcumin can help reduce post-operative inflammation. Turmeric's main active component, curcumin, is a powerful antioxidant as vitamins C, E, and Beta-Carotene, making it a popular consumer choice for cancer prevention, liver protection, and premature aging. Several researchers have researched curcumin because of its therapeutic advantages. Although curcumin is well tolerated by humans and animals, its poor bioavailability limits its therapeutic use. Recent studies have discussed the poor bioavailability of curcumin due to its poor absorption, rapid metabolism, and systemic elimination [1,2].

Vesicles have emerged as the preferred drug delivery method in recent years. Immunology, membrane biology, diagnostic techniques, and, most recently, genetic engineering have all highlighted the relevance of lipid vesicles. Vesicles can be used to mimic biological membranes as well as for active drug administration and targeting. Vesicular systems such as niosomes, phytosomes, liposomes, and others are crucial in overcoming these barriers [3]. Niosomes are non-ionic surfactant vesicles formed by combining nonionic surfactant with cholesterol and then hydrating in an aqueous medium [4, 5].

Niosomes protect the medication molecule from the external environment, improving its therapeutic efficacy. To produce niosomes, various surfactants such as alkyl ethers, alkyl glyceryl ethers, and sorbitan fatty acid esters are employed in various combinations and molar proportions. The addition of cholesterol increases niosome stiffness, resulting in fewer leaky niosomes [6]. Niosomes produced with nonionic surfactants in conjunction with cholesterol inhibit curcumin degradation better than micelles [3]. The primary components of niosomes include nonionic surfactants, a hydration medium, and lipids such as cholesterol. Nonionic surfactants self-assemble in aqueous conditions, resulting in bilayered structures called niosomes. The construction of bilayers often requires some sort of energy input, such as thermal or mechanical. Based on their size and bilayer structure, niosomes are divided into three kinds. Small unilamellar vesicles ($10–100$ nm), large unilamellar vesicles ($100–3000$ nm), and multilamellar vesicles with numerous bilayers are all examples of vesicles. In the present study, curcumin niosomes were synthesized by nonionic surfactant span 40 and cholesterol according to $3^2$ factorial design. The physiochemical properties like size, curcumin entrapment efficiency, and stability during storage were investigated [7].

1.1 Niosomes Versus Liposomes

Liposomes and niosomes are structurally comparable and equiactive; both have similar physicochemical features; both may be utilized as targeted drug delivery systems; both liposomal and niosomal vesicles can accomplish sustained release action. The structural units of liposomal and niosomal vesicular systems differ; liposomes are formed of phospholipids, whereas niosomes are made of surfactants. Niosomes have better durability and eliminate numerous drawbacks associated with liposomes, such as the fact that they do not require specific conditions such as low temperature and an inert atmosphere during production [8,9,10].

1.2 Niosomes as Drug Carriers

Niosomes are used as carriers for a variety of drug molecules since ancient times. Because of
their nonionic nature, they have high biocompatibility and minimal toxicity. Niosome formulations have several uses and may be given via a variety of methods including intramuscular, intravenous, peroral, and transdermal. Furthermore, niosomes have been proven to enhance medication absorption through cell membranes and localization in specific organs as drug delivery vesicles. Because of niosomes’ distinct structure, a delivery system capable of loading both hydrophilic and lipophilic medicines can be created. Pharmaceuticals that are hydrophilic or lipophilic are stored in the aqueous core and membrane bilayer of the niosome, respectively [11]. Ying-Qi Xu et al. developed curcumin-loaded niosomes to solve many curcumin-related issues, including low stability, restricted solubility, and rapid degradation in vivo. This technique enabled curcumin to be released in a controlled manner, enhancing its medicinal availability.

1.3 Anticancer Therapy

Chemotherapy is the most often utilized cancer treatment. Many anticancer drugs’ therapeutic efficacy is hindered by their poor penetration into tumor tissue and substantial unfavorable effects on healthy cells. Several attempts have been made to overcome these restrictions, including the usage of niosomes as a novel medication delivery mechanism.

1.4 Melanoma

Artimisone is an antimalarial medication with anticancer effects. Artimisone-encapsulated niosomes cytotoxicity against tumor cells was very selective. Gude et al. synthesized niosomal cisplatin from Span 60 and cholesterol and tested its antimetastatic effectiveness in a B16F10 melanoma metastatic model. When compared to free cisplatin, their findings demonstrate that cisplatin encapsulated in niosomes has significant antimetastatic effectiveness and lower toxicity.

1.5 Anti-inflammatory Drugs

Niosomes have also been demonstrated to have the ability to transport antiviral medicines. Ruckmani et al. created zidovudine-loaded niosomes and studied their entrapment efficiency and release sustainability. Tween, Span, and cholesterol were combined in various amounts to form the niosomes. Large quantities of zidovudine were captured by tween 80-based niosomes, and the addition of diacetyl phosphate delayed drug release. Tween 80 formulations containing diacetyl phosphate were removed from the blood within five hours, according to a pharmacokinetic investigation in rabbits. Ghadi et al. formulated curcumin-loaded hyaluronan containing niosomes, the study suggests that hyaluronan containing niosomes were spherical with entrapment efficiency of 98.27% and possess higher anti-inflammatory efficiency than simple suspension of curcumin.

1.6 Anti Viral Drugs

Niosomes have also been demonstrated to have the ability to transport antiviral medicines. Ruckmani et al. created zidovudine-loaded niosomes and studied their entrapment efficiency and release sustainability. Tween, Span, and cholesterol were combined in various amounts to form the niosomes. Large quantities of zidovudine were captured by tween 80-based niosomes, and the addition of diacetyl phosphate delayed drug release. Tween 80 formulations containing diacetyl phosphate were removed from the blood within five hours, according to a pharmacokinetic investigation in rabbits.

1.7 Advantages of Niosomes [10,12,13]

1) They increase drug molecules’ therapeutic efficacy by enhancing the oral bioavailability of poorly absorbed medicines, delaying clearance from circulation, and shielding the drug from the biological environment.
2) They are osmotically active, stable, and increase the entrapped drug’s stability. Their administration might take place orally, parenterally, or topically.
3) Because niosomes include both hydrophilic and hydrophobic components, they can serve as a carrier for medicines with a wide range of solubility.
4) Niosomes are proposed to pass through the cornea, allowing them to be used for ocular drug delivery.

2. MATERIALS AND METHODS

2.1 Materials

Curcumin was purchased from SD fine chem limited, Mumbai. Span 40 was purchased from world chem industries, Gujrat. All other chemicals and solvents were of analytical grade.
2.2 Method of Preparation of Curcumin Niosomes

Thin-film hydration was used to create niosomes. The thin-film hydration method is a well-known and straightforward method for producing niosomes. Niosomes containing curcumin were created utilizing a nonionic surfactant and cholesterol in this technique. Nonionic surfactant and cholesterol were combined with 20 mL chloroform in a rotatory evaporator immersed in a water bath at 60°C. Then accurately weighed drug was added to the solvent. At low pressure, the rotatory evaporator was allowed to evaporate until a thin layer developed. The thin film was dried and dissolved in 20 mL of phosphate buffer, which was then placed in a water bath at 60°C and rotated at 120 rpm to hydrate the layers [14,15,16,17].

2.3 Characterization of Niosomes

2.3.1 Standard graph of curcumin

A standard graph of curcumin was prepared by dissolving different concentrations of drugs in methanol. The absorbance was measured at 423 nm and a linear relationship was observed with absorption to the concentration of the drug. The standard curcumin graph was shown in the result and discussion section.

2.3.2 Surface morphology

The surface morphology of niosomes was investigated using a scanning electron microscope (SEM) and transmission electron microscope (TEM) and results were shown in Fig. 5 and Fig. 6 [18].

2.3.3 Particle size distribution and zeta potential

The particle size and zeta potential of the produced liposomes were measured. The optical microscope was used to measure the vesicle size. Individual sizes of 200 niosomes were measured, and an average value was determined [19,20,21]. Particle movement velocity in electric field and particle charge were determined to measure zeta potential. The prepared niosomes were diluted 10 times with distilled water and analyzed with a zeta sizer (Malvern, Ver. 6.01) [22].

2.3.4 Drug content

The calculated drug content in produced niosomal vesicles was determined by placing 100 mg of niosomal suspension in a 100 mL volumetric flask and filling the flask with phosphate buffer (PH 7.4). Take 1ml of this solution and made up the volume of 10 ml with phosphate buffer of PH 7.4. The Drug content was calculated using a UV spectrophotometer [21].

2.3.5 Drug entrapment efficiency

The niosomal suspension was sonicated along with phosphate buffer and centrifuged. (Nair et al., 2017) Separation of unentrapped drugs was carried out and analyzed using UV Visible spectrophotometer. The formula for calculating encapsulation efficiency is shown below [23].

Encapsulation efficiency = Entrapped drug/total amount of drug (mg) * 100

Table 1. Formulation table

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Weight Taken (mg)</th>
<th>Drug</th>
<th>Surfactant (Span 60)</th>
<th>Cholesterol</th>
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<td>N9</td>
<td>200</td>
<td>400</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
2.3.6 In vitro drug release study

The niosomal preparation was placed in a dialysis bag with an effective length of 8 cm. The dialysis bag act as a donor compartment. The dialysis bag was placed in phosphate buffer saline (PH 7.4) which act as a receptor compartment. The medium was agitated using a magnetic stirrer at 37ºC. 1 ml of volume was withdrawn at a specific time interval from the receiver compartment through the side tube and after each withdrawal, the same volume of medium was uploaded. The sample was analyzed by UV spectrophotometer at nm [24].

2.3.7 Kinetics of drug release

In vitro dissolution data was used to analyze the Kinetics of drug release from the Niosomal gel. The in vitro dissolution data was fitted to various models like zero order, first order, Higuchi release model, Korsmeyer, and Peppas model [25].

2.3.8 Stability study

Stability studies were carried out to study the leaching of drugs from niosomes. The niosomal preparation was stored at two different conditions at room temperature (25ºC±2ºC) and freeze condition (4ºC±2ºC) for 10 weeks, drug content of niosomes was checked periodically [26].

2.3.9 FTIR

The sample for IR spectroscopy was prepared by mixing the samples with spectroscopic grade potassium bromide and compressed into transparent pellets, then scanned in the IR range from 500 to 4000 cm⁻¹. Infrared spectra of curcumin and the physical mixture of curcumin and excipients were taken to study the interaction between curcumin and excipients [22].

3. RESULTS AND DISCUSSION

Curcumin-loaded niosomes were prepared using 3² factorial designs by thin-film hydration technique. The thin-film hydration method was selected because curcumin is hydrophobic, hence the capability for loading the higher mass of hydrophobic drug is more to multilamellar vesicles than unilamellar vesicles. The entrapment of the drug depends upon Pka value to a considerable extent.

3.1 UV Standard Curve of Curcumin

A double beam Ultraviolet-visible spectrophotometer (Shimadzu) was used to determine the maximal drug concentration. Curcumin 4 g/ml methanol solution was scanned in the 400-800 nm range.

3.1.1 Estimation of curcumin by ultraviolet-visible spectrophotometer

Methanol was used to make a standard stock solution of curcumin (1mg/ml). Dilutions ranging from 1 to 7 g/ml were prepared by using methanol. The absorbance of these solutions at 423 nm was measured using a UV-visible spectrophotometer against methanol as a blank, and a standard curve was generated versus concentration.

3.2 FTIR

Drug and excipients stability study was performed using FTIR. Results showed no physical and chemical reaction between drug and excipients.

Table 2. Result of analysis of Ultraviolet spectroscopy method for estimation of Curcumin

<table>
<thead>
<tr>
<th>Statistical parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max</td>
<td>423 nm</td>
</tr>
<tr>
<td>Equation of regression</td>
<td>0.129x-0.009</td>
</tr>
<tr>
<td>Slope</td>
<td>0.129</td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.009</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.999</td>
</tr>
</tbody>
</table>

3.3 SEM

SEM was used to examine the morphology and surface appearance of an optimized batch of produced niosomes. Scanning electron microscopy revealed that all of the produced niosomes appeared to have a similar spherical shape. All of the formulations were measured in nanometers.

3.4 TEM

TEM photomicrograph revealed spherical shape and morphology of the niosomes at a magnification of 100xs. The drug-loaded niosomes of spherical shape are represented by TEM imaging for optimized formulation. The vesicle’s inner dark spherical core was surrounded by a faint background.
Fig. 1. UV spectrum of Curcumin in methanol

\[ y = 0.1296x - 0.0098 \]
\[ R^2 = 0.9998 \]

Fig. 2. Standard calibration curve of Curcumin in methanol

Fig. 3. FTIR Spectrum of curcumin
3.5 Entrapment Efficiency

From the obtained result it was concluded that surfactant concentration has a significant impact on drug entrapment efficiency. Formulation N7 has maximum drug entrapment efficiency which was considered an optimized batch. Cholesterol can seal the leaking space in the bilayer membrane but increasing cholesterol concentration beyond a certain level starts disrupting the natural bilayer structure, hence decreases the drug entrapment. (Nagalakshmi S. et al., 2016).
3.6 Stability Study

The stability study of the optimized batch was carried out at 4ºC±2ºC (refrigeration temperature) and 25ºC±C(room temperature) for 10 weeks. The results showed that drug retention capacity was more at 4ºC±2ºC 80.2% and 67.5% at 25ºC±C. Results showed that an increase in temperature decreases drug retention capacity due to the degradation of the polymer.

3.7 Particle Size and Zeta Potential

From each batch (N1, N2, N3, N4, N5, N6, N7, N8, N9) 200 niosomes were measured for diameter. The average vesicular size was calculated which was found in a range of 4.22 µm to 4.83 µm. The results show that niosomes formed were of uniform size.

3.8 In vitro Drug Release

From the in vitro drug release study it has been observed that the rate of drug release depends on percentage drug entrapment efficiency. Optimized formulation N7 shows a prolonged release than other formulations. At a predetermined time intervals the sample was withdrawn and the amount of drug was determined by measuring the absorbance at 425 nm using an ultraviolet-spectrophotometer.

![Fig. 7. Stability study](image)

Table 4. Particle Size analysis and zeta potential

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>The average diameter of niosomes (µm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>4.72±0.54(µm)</td>
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</tr>
<tr>
<td>N2</td>
<td>4.65±0.45(µm)</td>
<td>-17.5</td>
</tr>
<tr>
<td>N3</td>
<td>4.43±0.28(µm)</td>
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<tr>
<td>N4</td>
<td>4.73±0.35(µm)</td>
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<td>N5</td>
<td>4.50±0.46(µm)</td>
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<td>N6</td>
<td>4.32±0.52(µm)</td>
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</tr>
<tr>
<td>N7</td>
<td>4.75±0.58(µm)</td>
<td>-16.6</td>
</tr>
<tr>
<td>N8</td>
<td>4.31±0.71(µm)</td>
<td>-15.5</td>
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<tr>
<td>N9</td>
<td>4.22±0.47(µm)</td>
<td>-14.6</td>
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</table>
Fig. 8. Size distribution of formulation N1

Fig. 9. Size distribution of formulation N2

Table 5. *In vitro* drug release study

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
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<td>84.25</td>
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</table>
Fig. 10. Size distribution of formulation N3

Fig. 11. Size distribution of formulation N4

Fig. 12. Size distribution of formulation N5
Fig. 13. Size distribution of formulation N6

Fig. 14. Size distribution of formulation N7

Fig. 15. Size distribution of formulation F8
Table 6. Kinetics of drug release of optimized formulation N7

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero-order R²</th>
<th>Zero-order AIC</th>
<th>First-order R²</th>
<th>First-order AIC</th>
<th>Second-order R²</th>
<th>Second-order AIC</th>
<th>Higuchi R²</th>
<th>Higuchi AIC</th>
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<td>0.999</td>
<td>72.16</td>
<td>0.711</td>
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</table>
3.9 Release Kinetics

The drug release kinetics of the optimized formulation was best fitted in the Korsmeyer-Peppas model. The result of drug release kinetics of optimized formulation N7 was shown in Table 6.

CONCLUSION

In the present study, Various formulation of curcumin was prepared using a varying concentration of surfactant and cholesterol. The present study suggests that these niosomal formulations provide sustained release of drugs with enhanced bioavailability. Optimized formulation N7 shows maximum drug entrapment efficiency and prolonged drug release. Thus niosomes represent a promising drug delivery approach for herbal extracts. Bioavailability problems of herbal drugs can be overcome by this vesicular structure.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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