Biocompounds

www.bic.reapress.com

Biocompd. Vol. 2, No. 1 (2025) 53–62.

Paper Type: Original Article

Development and in Vitro Characterization of Niosomal Carriers Niosomal Carriers for Sustained Drug Delivery

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Citation:

Received: 26 October 2024 *Revised: 16 January 2025* *Accepted: 01 March 2025*	Hosseinzadeh Gavon, A., Khanpour, M., & Farrokh Pour, M. (2025). Development and in vitro characterization of niosomal carriers niosomal carriers for sustained drug delivery. *Biocompounds, 2*(1), 53-62.

Abstract

Drug delivery systems are designed to enhance bioavailability while overcoming the limitations of conventional dosing strategies. Vesicular carriers, such as niosomes, have attracted attention for their excellent stability, cost-effectiveness, and sustained-release properties. Niosomes can encapsulate both lipophilic and hydrophilic drugs, making them versatile carriers for a wide range of therapeutics. Recent studies have focused on optimizing niosomes for targeted drug delivery, exemplified by the encapsulation of 4-Hydroxyisoleucine (4-HIL) for diabetes management. In this study, 4-HIL isolated from fenugreek seeds was incorporated into niosomes and PEGylated niosomes using the thin-film hydration method. The resulting 4-HIL-loaded niosomes exhibited semi-spherical, smooth morphologies with a particle size of ~200 nm, a Zeta Potential (ZP) of -22 mV, and entrapment efficiencies ranging from 55.1% to 87.1%. Fourier-Transform Infrared (FTIR) spectroscopy confirmed hydrogen bonding between Span 60 and cholesterol within the niosomal structure. PEGylation increased vesicle sizes to 460–580 nm and improved entrapment efficiencies to 75.43–90.1%, highlighting the potential of this formulation as a promising carrier for antidiabetic therapy.

Keywords: Niosomes, 4-Hydroxyisoleucine, Drug delivery, Polyethylene glycol, Encapsulation.

1 | Introduction

Conventional drug dosing regimens often suffer from limitations such as frequent administration, short half-life, and low bioavailability due to their non-targeted nature [1]. In recent years, significant efforts have focused on developing advanced drug delivery systems capable of overcoming biological barriers, enhancing bioavailability, minimizing the shortcomings of standard formulations [2], and creating intelligent systems that mimic physiological drug release, reducing the risk of hypoglycemia [3].

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https://doi.org/10.48313/bic.v2i1.35

Vesicular systems, including liposomes, have demonstrated considerable technological advantages in clinical practice [4]. Recently, diverse vesicular carriers, such as niosomes, transfersomes, ethosomes, and pharmacosomes, have gained attention. While these carriers share a lamellar structure, they differ in their constituent components [5]. Over the past three decades, novel strategies for developing vesicular carriers have emerged, leading to a variety of drug delivery platforms, including liposomes [4], niosomes [5], nanoparticles [6], microemulsions [7], microspheres [8], and magnetic microcapsules [9].

Niosomes are innovative self-assembling vesicles formed from non-ionic surfactants in aqueous solution, facilitated by physical stirring or elevated temperature. They have gained prominence due to their versatility as drug delivery platforms [10], [11]. Due to their biocompatibility and biodegradability, niosomes have been used in cosmetics, biotechnology, and pharmaceuticals, particularly for targeted drug delivery. Their advantages include high stability, cost-effectiveness, improved bioavailability, biodegradability, non-carcinogenicity, longer shelf life, controlled release, and pH stability [12–14]. Additionally, niosomes enhance drug stability and solubility, prolong therapeutic effects, reduce side effects, and enable co-loading of incompatible drugs for combination therapies [15]–[19].

The unique structure of niosomes, including an internal aqueous compartment surrounded by a hydrophobic membrane and tunable surfactant/lipid chain lengths, allows them to encapsulate both hydrophilic and hydrophobic drugs, enhancing therapeutic efficacy while minimizing side effects [20]. Niosomes are non-toxic, non-immunogenic, osmotically active, biocompatible, and biodegradable. They have been extensively investigated as carriers for drugs, hormones, antigens, and immune modulators [21].

Despite their advantages, conventional niosomal formulations face challenges such as drug leakage, limited control over release rates, and difficulty achieving high local drug concentrations. Functional modifications, such as PEGylation or incorporation of stimuli-responsive elements, can overcome these limitations, enabling targeted and controlled drug release [22], [23].

Among natural compounds, 4-Hydroxyisoleucine (4-HIL), a rare amino acid found in fenugreek, shows significant antidiabetic potential. 4-HIL reduces hepatic glucose production, lowers blood glucose levels, improves the glucose-to-insulin ratio, and mitigates liver indices [24]–[27]. Encapsulation of 4-HIL into niosomes has been explored to enhance its bioavailability, prolong release, and achieve sustained therapeutic effects, highlighting its promise as a novel treatment for diabetes [28].

In this study, we developed nano-niosomes for 4-HIL delivery, evaluating Encapsulation Efficiency (EE), controlled drug release, and potential for targeted therapy. Niosomes were synthesized using Span 60 and cholesterol at varying molar ratios in a buffered medium, and PEGylation was applied to enhance stability and performance. The physicochemical properties of the niosomes were characterized, along with their ability to encapsulate and release 4-HIL effectively, demonstrating their potential as a resilient, targeted drug-delivery system.

2 | Materials and Methods

2.1 | Preparation and Characterization of 4-Hydroxyisoleucine-Loaded Niosomes

4-HIL-loaded niosomes were prepared using the widely adopted thin-film hydration method, with minor modifications to ensure precise encapsulation and optimal drug-loading efficiency [29], [30]. As shown in *Table 1*, six formulations were prepared using Span 60 and cholesterol at molar ratios of 2:1 (without Polyethylene Glycol (PEG)), 1:1 (without PEG), 1:2 (without PEG), 2:1 (with 2.5 mg PEG), 1:1 (with 2.5 mg PEG), and 1:2 (with 2.5 mg PEG). The components were placed in six 100 mL containers, followed by the addition of 10 mL ethanol and 2.5 mg 4-HIL. The mixtures were stirred at 6 rpm for 24 hours at room temperature to achieve complete homogenization.

Ethanol was subsequently removed using a rotary evaporator at 70°C, yielding a white, powder-like residue. The residues were then hydrated with 12 mL phosphate buffer (pH 7.4) and stirred for 30 minutes at 25°C to ensure thorough mixing. Finally, the suspensions (milky white solutions) were sonicated for 10 minutes to reduce particle size to the nanoscale. The amounts of drug, Span 60, and ethanol were maintained at 2.5 mg, 5 mg, and 10 mL, respectively [31].

2.2 | Morphological Characterization by Scanning Electron Microscopy

The surface morphology and structural characteristics of the 4-HIL-loaded niosomes were examined using Scanning Electron Microscopy (SEM) (JEOL JSM-IT100, Japan). A small amount of lyophilized niosomal powder was mounted on an aluminum stub using double-sided conductive carbon tape and coated with a thin layer of gold under vacuum using a sputter coater (Quorum SC7620, UK) to ensure conductivity. The samples were then imaged at different magnifications under an accelerating voltage of 10–15 kV. SEM micrographs revealed the vesicles' surface texture, uniformity, and approximate shape. The 4-HIL-loaded niosomes displayed a spherical-to-semi-spherical morphology with smooth, homogeneous surfaces, indicating successful vesicle formation without aggregation [32].

2.3 | Zeta Potential

The Zeta Potential (ZP) of the niosomes was measured to evaluate surface charge and predict colloidal stability. Samples were diluted with deionized water and analyzed using a Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C. Each measurement was performed in triplicate, and the average value was reported. Higher absolute values of ZP indicate stronger electrostatic repulsion and improved stability against aggregation.

2.4 | Fourier-Transform Infrared Spectroscopy

Fourier-Transform Infrared (FTIR) spectroscopy was performed to investigate potential chemical interactions between Span 60, cholesterol, and 4-HIL in the niosomal formulation. Samples were analyzed using an FTIR spectrometer (PerkinElmer, USA) over the spectral range of 4000–400 cm^{-1}. Characteristic peaks were identified to confirm the presence of hydrogen bonding and other interactions that contribute to the structural stability of the niosomes [32].

2.5 | Encapsulation Efficiency

EE was assessed by centrifuging the niosomal suspensions at 15,000 rpm for 30 minutes at 4°C to separate the unencapsulated drug. The supernatant was analyzed spectrophotometrically at 210 nm. EE was calculated using [31]:

$$\mathrm{EE}(\%) = \left(\frac{\text{total amount of drug added} - \text{amount of free drug}}{\text{total amount of drug added}}\right) \times 100.$$

3 | Results and Discussion

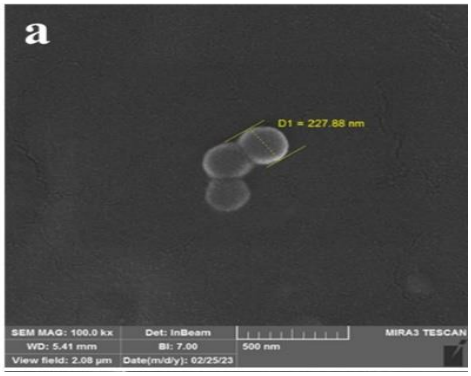
3.1 | In Vitro Characterization of 4-HIL-Loaded Niosomes

The 4-HIL-loaded niosomes were successfully prepared using the thin-film hydration method [30], which enabled efficient encapsulation of 4-HIL and ensured optimal drug loading. Various physicochemical analyses were performed to characterize the prepared niosomal formulations.

SEM was employed to analyze the morphology and surface characteristics of the synthesized niosomes. As observed in *Fig. 1a*, the non-PEGylated niosomes (Group B, *Table 1*) exhibited a uniform particle distribution (~200 nm) with smooth, quasi-spherical surfaces, consistent with previous reports describing niosomal vesicles. Furthermore, the PEGylated niosomes (Group E, *Table 1*) demonstrated a distinct increase in particle

size, ranging from 460–580 nm (*Fig. 2*), due to the formation of a PEG coating layer surrounding the vesicles. The SEM micrographs clearly revealed this outer PEG layer, confirming the successful surface modification of niosomes.

These findings are consistent with those of Baranei et al. [33], who reported that PEG coating of Cholesterol–Hemisuccinate (CHEMS)-based niosomes loaded with green tea extract resulted in increased vesicle diameter and improved surface smoothness. Collectively, the morphological observations validate the effective PEGylation of niosomes, which likely enhances colloidal stability and prevents aggregation.



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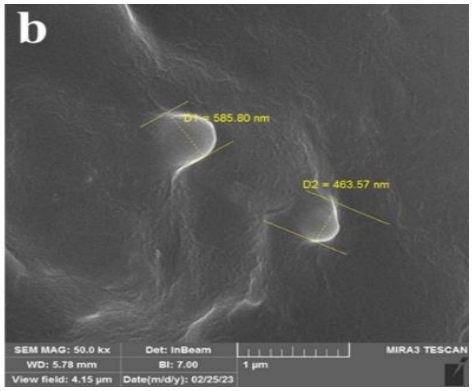
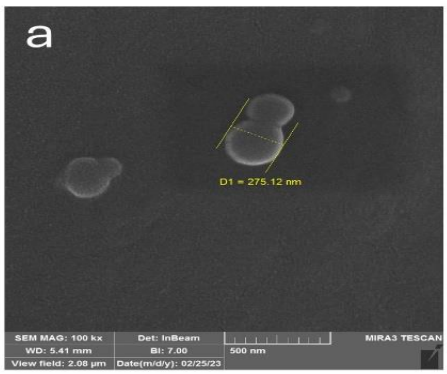


b.

Fig. 1. SEM image of optimized noisome: a. without PEG, and b. with added PEG.



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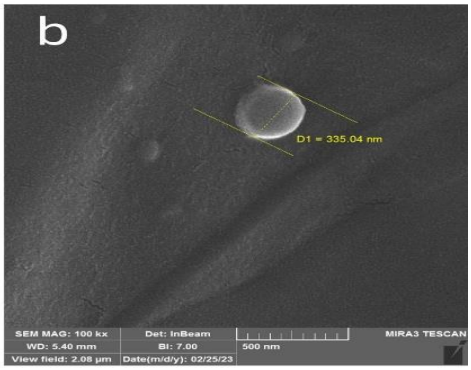

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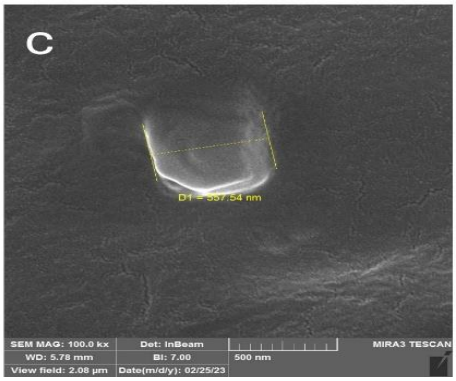

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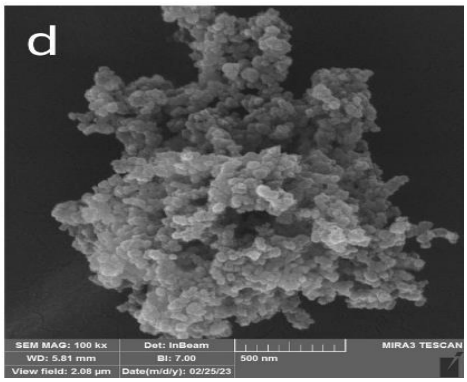

d.

Fig. 1. SEM image of optimized niosome, showing a-b. Without PEG, and c-d. with added PEG to the noisome.

The stability of niosomal formulations (Groups A–F) was evaluated by optical imaging at days 1, 4, and 7. Samples were stored at two distinct temperatures: refrigeration (4°C) and room temperature (25°C) to assess the effect of temperature on niosome integrity.

Under refrigeration (4°C), all niosomal formulations (A–F) exhibited stable and uniform dispersions with no evidence of sedimentation or aggregation throughout the observation period. Notably, formulations containing equal molar ratios of Span 60 and cholesterol (with or without PEG) showed superior stability, suggesting that this balance between surfactant and cholesterol helps maintain vesicle integrity by preventing fusion and particle aggregation.

Conversely, samples stored at room temperature (25°C) exhibited visible aggregation and phase separation after the 4th day, indicating a significant temperature-dependent destabilization effect. These observations emphasize that storage temperature and lipid composition are critical for preserving the structural and

functional integrity of niosomal vesicles. The stability behavior observed here supports the robustness and suitability of the optimized formulation for pharmaceutical applications requiring prolonged shelf life and consistent dispersion stability [34].

The ZP serves as an essential physicochemical parameter that reflects the surface charge of nanoparticles and their colloidal stability. A higher absolute value of ZP (positive or negative) corresponds to greater electrostatic repulsion among vesicles, thereby reducing aggregation tendencies.

As summarized in *Table 2*, the maximum ZP value (−22 mV) was obtained for the formulation containing the highest proportion of Span 60 (2:1 ratio), indicating enhanced surface charge and stability. This suggests that increasing the Span 60 concentration strengthens the vesicular bilayer structure and enhances resistance to aggregation. These findings are consistent with recent studies by [35], who similarly reported that higher surfactant content yields niosomes with greater colloidal stability.

Table 1. Composition of 4-HIL-loaded Niosomes.

Group (Formulation)	Cholesterol	Group (Formulation)	Cholesterol
A	2.5	0	1:2
B	5	0	1:1
C	10	0	2:1
D	2.5	2.5	1:2
E	5	2.5	1:1
F	10	2.5	2:1

Table 2. Effect of cholesterol on the span 60 ratio, with or without PEG, on EE (%EE) and ZP.

Group (Formulation)	Ratio (Cholesterol Ch): Span (S)	ZP (mV)	EE (EE%)
A	Ch:S 1:2	-22 ± 1.13	71.76
B	Ch:S 1:1	0.5 ± 0.14	87.10
C	Ch:S 2:1	2.5 ± 1.70	55.10
D	Ch:S 1:2 with PEG	-1.65 ± 1.68	75.43
E	(Ch:S 1:1 with PEG)	-3.56 ± 0.87	90.10
F	(Ch:S 2:1 with PEG)	2.16 ± 0.83	60.43

The nearly identical ZP values obtained across all niosomal formulations indicate a uniform structural arrangement and confirm the absence of free drug molecules on the vesicle surfaces. After PEGylation, a reduction in the absolute ZP values was observed, attributed to the hydration layer formed by PEG, which partially neutralizes the surface charge. This phenomenon occurs because the PEG corona shields the negative surface charge of the uncoated niosomes, leading to a shift toward neutrality. The resulting near-neutral surface potential serves as evidence of the successful PEGylation of the vesicles. Similar findings were previously reported by [36], who observed surface charge neutralization in PEG-modified niosomes encapsulating doxorubicin.

Further investigation using FTIR spectroscopy (FTIR) provided valuable insights into the molecular interactions within the niosomal formulation (*Fig. 3*). The FTIR spectra exhibited prominent absorption peaks at 720, 1393, 1504, 2850, 2918, and 2800–3000 cm^{-1}, characteristic of the functional groups present in Span 60 and cholesterol (*Fig. 2*). Additionally, a broad band between 3000–3700 cm^{-1} indicates hydrogen bonding interactions among these components, confirming the formation of stable bilayer structures.

The incorporation of PEG further influenced the spectral characteristics. A broad stretching vibration around 3417 cm^{-1}, along with minor shifts in the C–O–C stretching band from 1054 cm^{-1} to 1057–1058 cm^{-1}, was observed in PEGylated niosomes with varying cholesterol-to-Span 60 ratios. These spectral changes confirm the successful integration of PEG chains into the niosomal matrix and suggest enhanced structural organization and hydrogen bond formation in the modified systems.

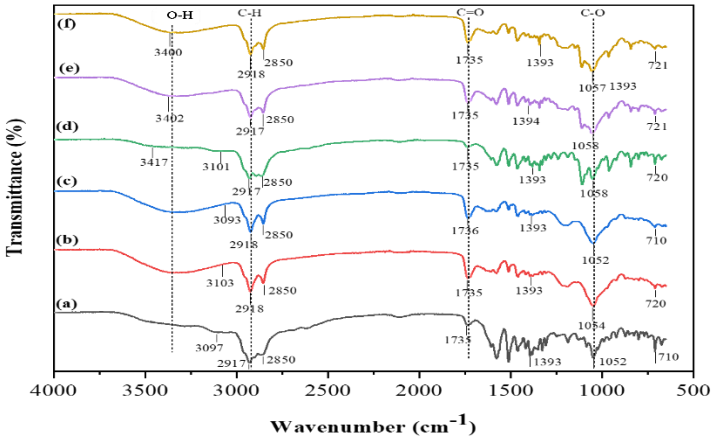

Fig. 2. FTIR spectrum of prepared nano-niosomes with various ratios of cholesterol (Ch) to Span 60 (S) and PEG.

The FTIR spectroscopy data confirmed the formation of hydrogen bonds between Span 60 and cholesterol within the niosomal bilayer, which is crucial for maintaining vesicular stability and structural integrity. Characteristic absorption peaks observed at 1735 cm^{-1} (C=O ester bond), 2850 and 2918 cm^{-1} (aliphatic C–H stretching), and 720 cm^{-1} (–CH_2– stretching) correspond to Span 60, whereas the peaks at 1054 cm^{-1} (C–O stretching), 2800–3000 cm^{-1} (C–H stretching), and 1393 cm^{-1} (C–H bending) are typical for cholesterol.

The incorporation of PEG into the niosomal formulation was evident from the broad O–H stretching band at 3417 cm^{-1} and the shift of the C–O–C stretching peak from 1054 cm^{-1} to 1057–1058 cm^{-1}, confirming the successful PEGylation of the vesicles. The absence of major spectral shifts across formulations with different cholesterol-to-Span 60 ratios suggests that the niosomal bilayer structure remains stable, even upon compositional variation.

The EE (%EE), a key metric for evaluating drug loading performance, was found to be strongly influenced by the cholesterol-to-Span 60 ratio. As summarized in *Tables 1* and *2* and illustrated in *Figs. 1* and *2*, the %EE of 4-HIL ranged from 55.1% to 87.1% across formulations with ratios of 1:1, 2:1, and 1:2. These values lie within the acceptable range for colloidal drug delivery systems, confirming the effective encapsulation of 4-HIL within the niosomal vesicles.

The observed variations in %EE indicate that lipid composition plays a crucial role in modulating drug entrapment and release kinetics. A balanced proportion of cholesterol and Span 60 enhances membrane packing density, minimizes drug leakage, and improves overall niosome stability and drug retention efficiency.

3.2 | Impact of PEGylation on Encapsulation Efficiency

The incorporation of PEG into the niosomal formulation markedly enhanced the EE (%EE) of 4-HIL, as summarized in *Tables 1* and *2* and illustrated in *Figs. 1* and *2*. The %EE values for PEGylated nanoniosomes prepared with varying cholesterol-to-Span 60 ratios ranged from 75.43% to 90.1%, representing a significant improvement over non-PEGylated formulations.

This enhancement in EE can be attributed to the steric stabilization and hydration layer provided by PEG molecules, which reduces vesicle aggregation and leakage during formulation. The hydrophilic PEG chains create a hydration barrier around the vesicles, minimizing drug diffusion out of the bilayer and thereby increasing the drug loading capacity and overall stability of the niosomal system.

These findings are consistent with previous reports demonstrating that PEGylation can improve the physicochemical properties of nanocarriers by enhancing membrane integrity, reducing inter-vesicular fusion, and prolonging drug retention time. Consequently, PEGylated niosomes present an efficient and stable platform for the controlled and sustained delivery of hydrophilic and amphiphilic therapeutics.

3.1 | Role of Stabilizing Agents in Enhancing Encapsulation Efficiency

Several studies have emphasized the critical role of stabilizing agents, such as PEG, in enhancing the EE (EE%) of nanoparticulate systems [36], [37]. The ability of PEG chains to entrap or interact with drug molecules contributes significantly to the increased EE observed in the present study. PEG and other stabilizers improve vesicle performance by modulating key parameters such as Hydrophilic–Lipophilic Balance (HLB), chain rigidity, lipid membrane packing order, and intermolecular spacing within the bilayer. These modifications enhance vesicle compactness, reduce drug leakage, and facilitate more potent drug–lipid interactions, ultimately leading to improved drug loading capacity and controlled release behavior.

Overall, the inclusion of PEG as a stabilizing agent not only reinforces the structural stability of the niosomal membrane but also provides a hydrophilic protective shell, minimizing aggregation and promoting long-term dispersion stability, making PEGylated niosomes a superior platform for drug delivery applications.

5 | Conclusion

In this study, 4-HIL-loaded niosomes and their PEGylated analogs were successfully synthesized, optimized, and comprehensively characterized. The results demonstrated satisfactory EE, uniform morphology, and favorable physicochemical stability of the nano-niosomal systems. PEGylation notably enhanced EE, particle stability, and sustained drug release, confirming its role as an effective surface modifier.

This approach offers a promising strategy to overcome limitations associated with conventional drug delivery, such as rapid clearance and poor bioavailability. Furthermore, the superior performance of PEGylated niosomes compared with their non-PEGylated counterparts highlights their potential as a robust and efficient platform for the controlled delivery of antidiabetic agents such as 4-HIL.

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