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RESEARCH ARTICLE

Hypericum perfolatum Oil-Loaded Niosomal Gel for Vaginal Delivery

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SUMMARY

The objective of this study is to formulate and assess a niosomal gel formulation with Hypericum perforatum oil for potential vaginal administration. Different Span®60:Tween®80 ratios (1:1, 1:2, and 2:1) were used to prepare the niosomes using the thin-film hydration method, and the physicochemical properties of the prepared niosomes were assessed by particle size, polydispersity index (PDI), and zeta potential. The F2 formulation was selected based on optimal particle size and stability conditions. Morphological analysis with SEM confirmed spherical and homogeneously dispersed vesicles. The selected niosomal formulation was incorporated into a 2% HPMC gel base to prepare a vaginal gel product. The developed gel had an adequate viscosity (17,000 ± 0.520 cP), a physiologically relevant pH value (5.5 ± 0.2), and an effective entrapment of Hypericum oil (71.6 ± 0.4%). In vitro drug release studies across a cellulose membrane showed sustained release of the niosomal gel compared to the non-niosomal product. Stability studies conducted over 3 months under accelerated stability conditions revealed negligible changes in physicochemical properties. Overall, the niosomal gel system efficiently promoted the retention and controlled release of Hypericum perforatum oil, justifying its potential as a promising carrier for vaginal drug delivery purposes.

Keywords: *Hypericum perforatum oil, niosomes, HPMC vaginal gel, stability.*

Vajinal İçin Hypericum perfolatum Yağ Yüklü Niosomal Jel

ÖZ

Bu çalışmanın amacı, potansiyel vajinal uygulama için Hypericum perforatum yağı içeren bir niozomal jel formülasyonu formüle etmek ve değerlendirmektir. İnce film hidrasyon yöntemi kullanılarak niozomlar hazırlamak için farklı Span®60:Tween®80 oranları (1:1, 1:2 ve 2:1) kullanılmış ve hazırlanan niozomların fizikokimyasal özellikleri partikül boyutu, polidispersite indeksi (PDI) ve zeta potansiyeli ile değerlendirilmiştir. F2 formülasyonu, optimum partikül boyutu ve stabilite koşulları temelinde seçilmiştir. SEM ile yapılan morfolojik analiz, küresel ve homojen olarak dağılmış vezikülleri doğrulamıştır. Seçilen niozomal formülasyon, vajinal jel hazırlamak için %2'lik bir HPMC jel bazına eklenmiştir. Geliştirilen jel, yeterli bir viskoziteye (17.000±0,520 cP), fizyolojik olarak anlamlı bir pH değerine (5,5±0,2) ve Hypericum yağının etkili bir şekilde tutulmasına (%71,6±0,4) sahipti. Selüloz membran üzerinden yapılan in vitro etkin madde salım çalışmaları, niozomal olmayan ürüne kıyasla niozomal jelin sürekli salınımını göstermiştir. Hızlandırılmış stabilite koşulları altında 3 ay boyunca yürütülen stabilite çalışmaları, fizikokimyasal özelliklerde ihmal edilebilir düzeyde değişiklikler olduğunu ortaya koymuştur. Genel olarak, niozomal jel sistemi, Hypericum perforatum yağının tutulmasını ve kontrollü salımını etkili bir şekilde desteklemiş ve vajinal ilaç iletimi için umut verici bir taşıyıcı olma potansiyelini kanıtlamıştır.

Anahtar Kelimeler: *Hypericum perforatum yağı, niozomlar, HPMC vajinal jel, stabilite.*

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INTRODUCTION

The vaginal route presents a promising option for delivering drugs locally, thanks to its expansive surface area, abundant blood supply, and the ability to avoid the liver's first-pass metabolism. However, the unique characteristics of the vaginal environment, such as its fluctuating pH, various enzymes, and natural self-cleaning process can often limit how long drugs stay effective and how well they work. This is why creating mucoadhesive, biocompatible, and stable delivery systems is crucial to tackle these challenges and improve treatment outcomes (Karmazyn & Zulfakar, 2021; Osmałek et al., 2021).

Hypericum perforatum oil, better known as St. John's Wort oil, is a well-known herbal remedy celebrated for its anti-inflammatory, antioxidant, and wound-healing benefits. Its traditional applications for skin and mucosal issues are increasingly backed by scientific research. However, the tendency to be oily and sensitive to oxidation poses significant hurdles in formulation, especially when aiming for mucosal tissues like the vaginal lining. These challenges call for a delivery method that not only stabilizes the oil but also ensures it stays in place longer and releases its benefits in a controlled manner (Ishak & Handy, 2023; Pereira et al., 2024).

Niosomes vesicles made from non-ionic surfactants have come to the forefront as effective carriers for both water-soluble and oil-soluble compounds. Their resemblance to biological membranes, straightforward preparation, and solid physical stability make them appealing for delivering drugs through mucosal routes. When designed correctly, niosomes can encapsulate lipophilic substances like Hypericum oil, shielding them from breakdown and allowing for controlled release in the mucosal setting. Adding these vesicles into a gel base boosts their ability to stick, extends the time they stay in contact, and enhances how well patients accept the treatment (Abdelkader et al., 2020; Vashist et al., 2020).

This study is all about creating and characterizing a new niosomal gel formulation that includes *Hypericum perforatum* oil, specifically designed for vaginal use. The formulation process utilizes the thin-film hydration method, incorporating a Span/Tween-based surfactant system and sodium citrate buffer as the hydration medium to enhance electrostatic stabilization. Comprehensive physicochemical evaluations such as particle size, zeta potential, pH, and viscosity control were performed to determine how suitable this system is for vaginal delivery. The aim of the study is to develop a stable, biocompatible, and effective topical formulation that can deliver Hypericum oil right where it's needed while tackling the physiological challenges associated with the vaginal route.

MATERIALS AND METHODS

Hypericum perforatum extract was purchased from Merck KGaA (Darmstadt, Germany). Span®60, Tween® 80, cholesterol, ethanol, sodium sitrate, glycerin, and hydroxypropyl methylcellulose (HPMC) were obtained from Interlab (Sigma-Aldrich, Türkiye). All solvents were of analytical grade and used without further purification. Throughout the study, freshly distilled water was utilized in all preparations.

Preparation of niosome formulations containing *Hypericum perforatum* oil

Formulations were prepared using different ratios of Span®60 and Tween®80 (1:1, 1:2, and 2:1), and their physicochemical properties were subsequently evaluated, with particular emphasis on particle size and zeta potential to determine stability and dispersion characteristics (Shehata et al., 2021). Table 1 presents the components and their respective amounts used in the preparation of the niosomal formulations.

Table 1. Formulations of niosomes

Ingredients	Quantity		
	F_1	F_2	F_3
Hypericum perforatum extract	50 mg	50 mg	50 mg
Span®60	200 mg	150 mg	100 mg
Tween®80	100 mg	150 mg	200 mg
Cholesterol	100 mg	100 mg	100 mg
Ethanol	2 mL	2 mL	2 mL
0.1 M sodium citrate solution	100 mL	100 mL	100 mL

The predetermined amounts of surfactants (Span®60 and Tween®80), cholesterol, and *Hypericum perforatum* oil were dissolved in ethanol. The solvent was then evaporated using a rotary evaporator at 60 °C under reduced pressure, forming a thin lipid film on the inner surface of the flask. The resulting dry film was hydrated with 10 mL of 0.1 M sodium citrate buffer by magnetic stirring for 30 minutes. To reduce the particle size of the obtained niosomal formulation, the dispersion was sonicated at 40°C for 10 min, applying approximately 25 kHz power, and then subjected to magnetic stirring for another 5 min. This process was repeated twice to ensure homogeneity and optimal size reduction (Chasanah et al., 2021; Rezaei et al., 2024; Yeo, 2022).

Characterization studies on the niosomal formulations

A comprehensive characterization was carried out on niosomal formulations prepared with varying Span®60 to Tween®80 ratios, including assessments of particle size, surface charge (zeta potential), encapsulation efficiency (EE%), pH, and morphological characteristics. The most suitable Span®60 to Tween®80 ratio was determined according to the characterization results, and the corresponding niosomal formulation was optimized accordingly (Obeid et al., 2022; Temprom et al., 2022; Witika et al., 2022).

Determination of particle size, polydispersity index (PDI), and zeta potential

Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano ZS (Malvern Instruments, UK) to determine the mean particle diameter, polydispersity index (PDI), and zeta potential of the developed niosomal formulations. Each formulation was analyzed three times at 25 °C to obtain accurate and consistent data. PDI values reflected the size distribution profile, whereas zeta potential was indicative of the electrostatic stability of the vesicles.

Evaluation of encapsulation efficiency (EE%)

The encapsulation efficiency of the niosomal formulations was determined by centrifuging 1 mL of the niosomal formulation at 15,000 rpm for 45 minutes. The supernatant containing free drug was analyzed using the validated UV spectrophotometric method at 268 nm (Alahmad et al., 2022; Błońska-Sikora et al., 2025). The EE% was calculated using the formula:

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$$EE\% = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100$$

Equation 1

Evaluation of pH

The pH of niosome formulations was determined using a calibrated digital pH meter (Ohaus, USA) at ambient temperature (25±1°C). Each formulation was tested in triplicate, and the average value was recorded. pH measurements were evaluated for compatibility with physiological vaginal pH (~4.5), as maintaining this range is important to minimize mucosal irritation and ensure patient compliance during intravaginal administration (Gvozdevan & Staynova, 2025).

Morphological characteristics

The structural features of the niosomal systems were characterized by Scanning Electron Microscopy (SEM) (Thermo Fisher Scientific, USA). A dilut-

ed aliquot of the niosomal dispersion was deposited onto an aluminum stub that had been pre-prepared with double-sided conductive carbon tape. The film was allowed to air-dry under vacuum at ambient temperature to minimize any alteration of the vesicular architecture. After drying, the specimens received a sputtered gold overlayer generated by a vacuum coater to improve electrical conductivity during imaging. Imaging was performed at an accelerating voltage of 15 kV, and high-magnification micrographs were recorded to evaluate the morphology, dimension distribution, and surface topology of the niosomes.

Preparation of non-niosomal *Hypericum perforatum* oil - HPMC gel

HPMC (2% w/v) was gradually dispersed in 30 mL of warm water with gentle stirring and allowed to swell for 10–15 min. After cooling to room temperature, *Hypericum perforatum* oil was added and gently mixed using a magnetic stirrer.

Preparation of niosomal vaginal gel

The required amount of HPMC (2% w/v) was gradually dispersed in 30 mL of warm water with gentle stirring and allowed to swell for 10–15 minutes. After cooling to room temperature, the optimized niosome formulation was added and mixed slowly using a magnetic stirrer (Limpongsa et al., 2023).

Evaluation of the structural and functional properties of HPMC vaginal gel

The HPMC vaginal gel products were characterized in comprehensive characterization studies to evaluate their physicochemical and functional attributes. Visual inspection was done to inspect color, transparency, and homogeneity (Bondre et al., 2023; Zhao et al., 2022). Room temperature pH was determined to ensure compatibility with the natural vaginal pH. Viscosity with a viscometer (Brookfield, USA) was determined to evaluate flow behavior and the convenience of application. Uniformity of drug content was determined spectrophotometrically following appropriate extraction procedures.

To determine the content of *Hypericum perforatum* oil in the gel formulation, precisely 1 g of the gel was accurately weighed and dissolved in 10 mL of ethanol by vortexing for 5 minutes, followed by sonication in an ultrasonic bath for 10 minutes. The resulting mixture was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected, and the absorbance was measured at 268 nm, the previously identified maximum absorbance wavelength of the oil, using a UV spectrophotometer (Sotirova et al., 2025).

In vitro drug release was investigated by using a dialysis bag to investigate release kinetics. The *in vitro* release of *Hypericum perforatum* oil from the prepared HPMC-based vaginal gel was evaluated using a dialysis membrane (molecular weight limit 12,000-14,000 Da). Before use, the membrane was soaked in release medium overnight to ensure proper hydration and permeability. The receptor compartment was filled with phosphate-buffered saline (PBS, pH 4.5) containing 20% (v/v) ethanol, selected to enhance the solubility of the lipophilic oil while mimicking the vaginal pH environment. The temperature was maintained at 37 ± 0.5 °C using a water-jacketed system, and the receptor medium was continuously stirred at 100 rpm to ensure homogeneous mixing. Approximately 1g of the gel formulation was placed in the donor compartment directly on the membrane. At predetermined time intervals (2, 6, 10, 15, 20, and 24 h), 1-mL aliquots of receptor medium were withdrawn and replaced with an equal volume of fresh, prewarmed release medium to maintain sedimentation conditions. The amount of *Hypericum perforatum* oil released at each time point was determined by UV-visible spectrophotometry at 268 nm, based on a previously established standard calibration curve. Cumulative percent release was calculated relative to the amount of oil initially loaded into the gel formulation (Thapa et al., 2022).

The formulations were investigated further under accelerated conditions for the initial stability by investigating changes in appearance, pH, and viscosity with time (Ali et al., 2020a).

RESULTS

Results of the physicochemical and morphological characterization of niosomal formulations

The physicochemical characterization results of the niosomal formulations prepared with three different Span®60 and Tween®80 ratios (1:1, 1:2, and 2:1) are presented in Table 2.

Table 2. Physicochemical properties of niosomal formulations with varying Span®60:Tween®80 ratios (n=3)

Niosomal formulations	Particle size (nm) ± SD	Zeta potential (mV) ± SD	EE(%)±SD	PDI±SD	pH±SD
F1	302.9±0.1	-42.40±0.3	60.5±0.6	0.457±0.1	5.6±0.1
F2	190.8±0.1	-27.30±0.1	72.4±0.5	0.205±0.1	5.3±0.3
F3	327.1±0.3	-33.80±0.5	51.7±0.8	0.244±0.3	5.5±0.2

EE (%): percentage of encapsulation efficiency

PDI: Polydispersity index

SD: Standard deviation

In this study, niosomal formulations were developed using varying molar ratios of Span®60 and Tween®80 (1:1, 1:2, and 2:1) to evaluate the effect of surfactant composition on particle characteristics. Among the tested ratios, the 1:1 Span®60:Tween®80 formulation exhibited the smallest average particle size and the lowest polydispersity index (PDI), indicating a more uniform vesicle population. This result can be attributed to the optimal balance between the hydrophilic (Tween®80) and lipophilic (Span®60) surfactants, which likely promoted the formation of stable and homogeneous bilayer structures. In contrast, increasing the concentration of either surfactant alone disrupted this balance, leading to larger and more polydisperse vesicles. The 1:1 ratio may thus represent a critical point where the surfactant mixture provides the most favorable interfacial tension and membrane curvature for the self-assembly of stable, nano-sized niosomes.

The zeta potential values of the niosomal formulations varied according to the ratio of Span®60 to Tween®80, reflecting differences in surface charge distribution and colloidal stability. The formulation with a 1:1 Span®60:Tween®80 ratio exhibited a moderately negative zeta potential, indicative of a balanced bilayer organization that favors electrostatic repulsion between vesicles and supports dispersion stability. When the proportion of Tween®80 was increased (1:2 ratio), the zeta potential tended to decrease in magnitude, possibly due to the enhanced hydrophilicity of the surface, leading to a reduction in surface charge density and, consequently, colloidal repulsion. Conversely, increasing the Span®60 content (2:1 ratio) resulted in a relatively more negative zeta potential, which may be attributed to the predominance of lipophilic moieties contributing to a denser bilayer structure and stronger surface charge. However, excessive Span®60 can also disrupt vesicle integrity and lead to aggregation. Therefore, the 1:1 ratio appeared to offer the most favorable surface charge profile for maintaining niosome stability.

The 1:1 Span®60:Tween®80 ratio provided the highest encapsulation efficiency among the tested formulations. This is likely due to the optimal amphiphilic balance, which facilitated the formation of well-organized bilayers capable of efficiently entrapping the lipophilic Hypericum oil. Formulations with higher Tween®80 content showed lower EE% values, presumably due to increased membrane fluidity and potential drug leakage. Conversely, while higher Span®60 ratios enhanced bilayer rigidity, excessive concentrations may have led to partial exclusion of the active compound, slightly reducing EE%.

The hydration medium consisted of 0.1 M sodium citrate buffer adjusted to pH 5.0 ± 0.3, ensuring both the physicochemical stability of the vesicles and compatibility with the intended vaginal application site.

Morphological results of niosome formulation

Based on the evaluation of the physicochemical properties of the niosome, including particle size, PDI, and zeta potential, formulation F2 was identified as the most optimal. The surface morphology of this formulation was further examined by SEM, and the resulting image is presented in Figure 1.

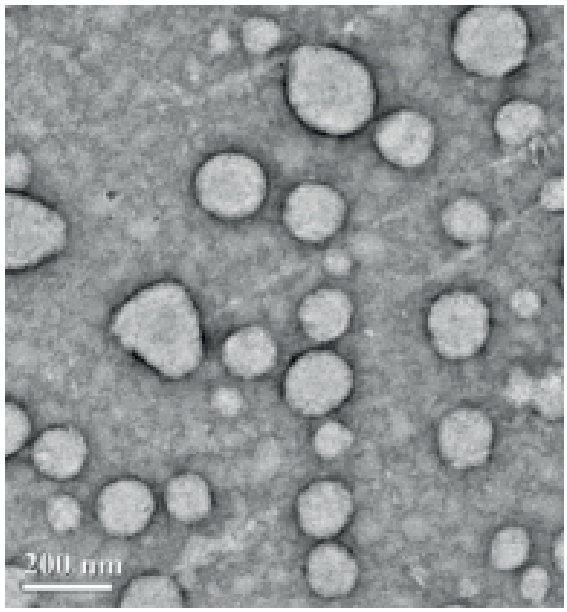

Figure 1. SEM image of the optimized niosomal formulation (F_2)

As shown in Figure 1, the particles exhibit a spherical shape and are uniformly distributed, indicating successful formation of stable vesicles with the desired niosomal structure. Although particles of varying sizes are present in the image, the majority appear to fall within the 100–200 nm range.

Results of physicochemical properties of niosome-loaded HPMC vaginal gel

The HPMC gel prepared with the optimized F_2 niosomal formulation was evaluated in terms of its visual appearance, viscosity, and pH. Additionally, the content of *Hypericum perforatum* oil and its release rate from the gel matrix were determined.

The prepared gel exhibited an opaque white color, consistent with the visual characteristics of the incorporated niosomal formulation. The formulation displayed a homogeneous appearance and a viscosity of 17,000 ± 52 cP, as determined from three parallel measurements. The pH of the gel was measured at 5.5 ± 0.2 using a calibrated pH meter, aligning well with the physiological range for vaginal applications. The content of *Hypericum perforatum* oil, quantified via UV spectrophotometry, was found to be 71.6 ± 0.4%, indicating efficient incorporation within the gel matrix. A UV spectrophotometric method developed for the quantitative analysis of *Hypericum perforatum* oil has been validated in accordance with the ICH Q2(R1) guidelines. The analysis was performed at a wavelength of 268 nm using methanol as the solvent, and the method was found to be linear in the concentration range of 0.5–10 µg/mL (R^2 = 0.9995). Accuracy was evaluated at 80%, 100%, and 120% concentration levels, yielding recovery values between 99.1% and 101.3%. The method demonstrated reliable precision with intra-day and inter-day relative standard deviations (RSD) of 1.2% and 1.5%, respectively. The limit of detection (LOD) was calculated to be approximately 0.1 µg/mL, and the limit of quantification (LOQ) was 0.3 µg/mL. Specificity tests showed no interference from other excipients present in the formulation at the absorbance peak of the analyte. The method was found to be robust under small variations in wavelength (±2 nm), solvent composition, and temperature. Stability testing showed that the sample solution remained stable for 24 hours, with absorbance changes not exceeding ±2%. These findings confirm that the method is suitable for routine quantitative analysis.

***In vitro* release profile of *Hypericum perforatum* oil from the HPMC-based gel formulation**

The release rate of *Hypericum perforatum* oil from the optimized niosomal gel formulation was compared to that of the non-niosomal HPMC gel containing only *Hypericum perforatum* oil, and the comparative release profile is presented in Figure 2.

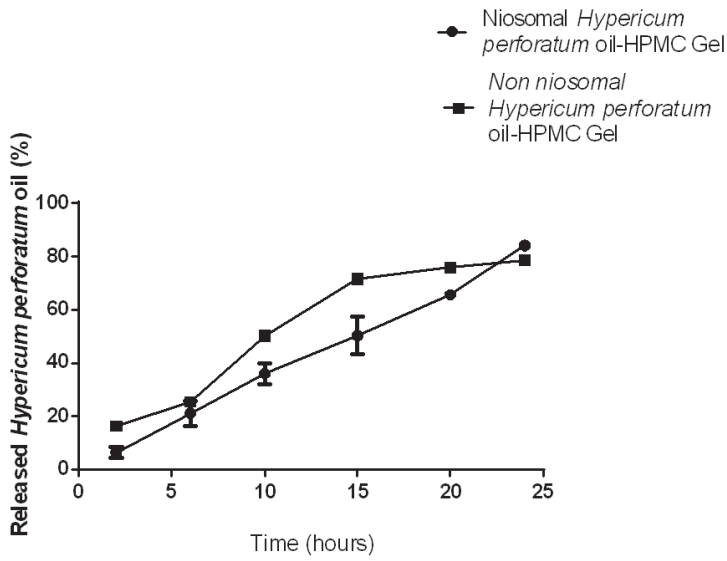

Figure 2. Comparative *in vitro* release profile of *Hypericum perforatum* oil from niosomal and non-niosomal HPMC gels

Stability of niosomal *Hypericum perforatum* HPMC gel

The stability conditions for the prepared formulation were selected according to ICH Q1A(R2) guidelines. This was done to evaluate the physical and chemical stability of the niosomal gel formulation during long-term and accelerated storage. To evaluate the physicochemical stability of the optimized niosomal HPMC gel formulation, a systematic stability study was conducted following ICH Q1A(R2) guidelines. The gel samples were stored under two different conditions: accelerated (40 ± 2 °C / 75 ± 5% RH) temperature for three months. Analyses were performed at predetermined intervals: 0, 15, 30, 60, and 90 days.

At each time point, samples were evaluated for pH, viscosity, physical appearance, *Hypericum perforatum* oil content, and zeta potential value. All measurements were taken in triplicate, and the results were expressed as mean ± SD. Specifically, pH and viscosity remained within ±5% of initial values, the color and homogeneity of the gel were preserved, and the drug content and release behavior stayed within acceptable pharmaceutical limits. The results of stability tests are summarized in Table 3.

Table 3. Stability parameters of optimized niosomal HPMC gel

Duration (day)	Physical appearance	pH (mean±SD)	Viscosity (mean±SD (cP)	*Hypericum perforatum* oil content (mean±SD) (%)	Particle size (mean±SD) (nm)	PDI (mean±SD)	Zeta potential (mean±SD) (mV)
0	opaque white odorless	5.3±0.3	17000 ± 52	71.6±0.4	190.8±0.1	0.205±0.1	-27.30±0.1
15	opaque white odorless	5.1±0.3	16200 ± 47	70.4±0.7	194.8±0.3	0.222±0.3	-20.40±0.2
30	opaque white odorless	5.2±0.1	16600 ± 22	70.1±0.5	189.8±0.5	0.192±0.3	-22.40±0.5
60	opaque white odorless	5.5±0.2	17200 ± 41	68.7±0.4	192.1±0.2	0.208±0.1	-26.10±0.4
90	opaque white odorless	5.6±0.1	16800 ± 32	69.7±0.2	190.2±0.3	0.231±0.2	-24.50±0.2

DISCUSSION

The current research introduces a novel niosomal vaginal gel containing *Hypericum perforatum* oil, encapsulated in an HPMC matrix, for controlled release and enhanced mucosal delivery. The results, including particle size, encapsulation efficiency (EE%), *in vitro* release, and stability over 90 days, verified the value of the niosomal system over regular gels.

The optimized formulation (F2; Span 60 : Tween 80 = 1:1) yielded vesicles with an average diameter of approximately 190 nm, low PDI (PDI < 0.3), and a moderately negative zeta potential (~–27 mV), all collectively indicative of colloidal dispersion stability and little likelihood of aggregation. This finding agrees with recent literature reporting increased vesicle stability and homogeneity by way of equilibrated surfactant ratios (Yasamineh et al., 2022).

The EE% of the optimized preparation was 72%, reflecting good entrapment of the lipophilic Hypericum oil. High EE% values have also been reported in current niosomal gel systems with herbal extracts, which supports the viability of employing niosomal carriers for hydrophobic actives (Mahale et al., 2021). *In vitro* release profiles were remarkably different between the niosomal gel and the control non-niosomal HPMC gel: prolonged release up to 24 h with significantly less burst release was recorded for the niosomal preparation, whereas the plain gel released ~90% of the oil over the same period. The sustained release behavior seen for the niosomal gel is consistent with findings demonstrating that vesicular systems can be used as diffusion barriers and hence alter release kinetics and enhance retention (Ali et al., 2020b).

The HPMC gel demonstrated desirable physical properties: it had a viscosity of ~17,000 cP and pH 5.5, within the physiological range of the vagina, and is easy to apply, ensuring proper residence at the mucosal surface. Such rheological properties are related to some recent reports on the importance of viscosity for sustaining drug residence time and permitting controlled delivery (Gaynanova et al., 2025).

Three-month accelerated stability testing at accelerated (40 ± 2 °C / 75% RH) revealed very low pH variations (≤0.1 units), viscosity (<2% change), and Hypericum oil content loss (≤3%), with no measurable appearance change. These results indicate stable physicochemical integrity, which is an achievement that is generally lost with non-vesicular systems (Yasamineh et al., 2022).

Collectively, the results suggest that the addition of niosomal vesicles to an HPMC gel matrix enhances formulation stability, determines release behavior, and preserves physico-biological compatibility. The approach is an encouraging vehicle for vaginal delivery of lipophilic herbal actives and merits additional exploration in *in vivo* and clinical studies.

CONCLUSION

In this study, an innovative vaginal gel dosage form was developed through the encapsulation of *Hypericum perforatum* oil-loaded niosomes into an HPMC gel system. Among the three developed formulations of varying Span®60:Tween®80 ratios, the F2 formulation (1:1) displayed the most suitable physicochemical characteristics in the form of optimum particle size, narrow PDI, and stable zeta potential values. The niosomal formulation was optimized and entrapped in the gel matrix to produce a homogenous, stable formulation with acceptable viscosity and pH for vaginal use. The drug release pattern exhibited a sustained and controlled release profile in the niosome-loaded gel compared to the conventional formulation, suggesting that encapsulation effectively modulates the release kinetics of Hypericum oil. Furthermore, stability studies conducted over 90 days supported the stability of the formulation under 40 ± 2 °C / 75% RH condition. In summary, the findings highlight the promise of niosomal gels as appropriate vehicles for localized vaginal administration of lipophilic herbal medications like *Hypericum perforatum* oil. The approach could lead to enhanced bioavailability, diminished irritation, and prolonged therapeutic effect, an indication that such systems can be further developed in clinical and translational research in the future.

AUTHOR CONTRIBUTION STATEMENT

Hypothesis development, literature research, experimentation, data analysis, and preparation and correction of the manuscript were carried out by EDÖ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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