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Synthesis and Characterization of Nanostructured Lipid Carriers Containing Tretinoin and Grape Seed Oil Using Hot Homogenization and Ultrasonic Waves

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A B S T R A C T

Background: This study focuses on the formulation and characterization of nanostructured lipidcarriers (NLCs) containing tretinoin, a vitamin A derivative known for its anti-aging and anti-acne properties. The primary objective was to enhance drug loading capacity and reduce skin irritation while prolonging the duration of action.

Materials and Methods: Utilizing hot homogenization and ultrasound methods, 26 different formulations were developed, varying lipid and surfactant percentages, as well as ratios of Tween to Span. The optimized NLCs were assessed for morphology, particle size, and zeta potential, revealing that increasing lipid and tretinoin concentrations led to larger sizes and a consistent zeta potential. Morphological analysis confirmed the spherical nature of the nanoparticles, and FTIR spectroscopy indicated successful drug encapsulation.

Results: The optimal formulation comprised 2% lipid, 2% surfactant, and 0.01% tretinoin, achieving a particle size of 253.4 nm and a polydispersity index (PDI) of 0.241.

Conclusion: Overall, these results underscore the promise of NLCs in advancing topical drug delivery systems, paving the way for future clinical applications and further research into their efficacy and safety in diverse dermatological conditions.

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Introduction

The synthesis and characterization of lipid carriers at the nanoscale have garnered significant interest in drug delivery and cosmetic formulations, particularly through the development of nanostructured lipid carriers (NLCs). These systems combine the advantages of solid lipid nanoparticles (SLNs) and liquid lipid formulations, representing a significant advancement over SLNs. NLCs offer several advantages, including higher drug loading capacity, reduced drug expulsion, improved drug release profiles, enhanced stability, and superior tolerance to processing conditions [1]. Tretinoin (all-trans retinoic acid) is well-known for its effectiveness in addressing several skin issues, such as acne, photoaging, and hyperpigmentation. However, its therapeutic potential is often compromised by its limited stability and low solubility in water, which can reduce its effectiveness in topical treatments. By encapsulating tretinoin in lipid carriers, especially NLCs, its stability and solubility can be improved, leading to better therapeutic results. The lipid matrix in NLCs not only safeguards the active compound from degradation but also promotes controlled release, resulting in prolonged therapeutic effects [2, 3].

Grape seed oil (GSO) is an appealing lipid phase for NLC formulations due to its rich composition of polyunsaturated fatty acids and bioactive compounds, such as proanthocyanidins and tocopherols. These components provide antioxidant properties, which can synergistically enhance the stability of tretinoin within the NLCs. The incorporation of GSO not only serves as a lipid carrier but also contributes to the overall efficacy of the formulation, making it particularly suitable for dermatological applications [4-7].

The aim of this study was to encapsulate tretinoin in NLC using hot homogenization followed by ultrasonic treatment. The particle size of the NLC systems was analyzed. Additionally, the encapsulation efficiency (%EE) of the NLC was examined, and fourier transform infrared spectroscopy (FTIR) was employed. This provided insights into potential strategies for enhancing the stability and bioavailability of tretinoin for future use in cosmetic formulations. Moreover, the optimization of the formulation was achieved using response surface methodology (RSM), a statistical approach that facilitates the evaluation of multiple variables and their interactions, ensuring a robust and effective NLC formulation [8]. This comprehensive study underscores the potential of NLCs as a promising vehicle for tretinoin delivery, paving the way for advanced therapeutic solutions in skin care.

Materials and Methods

Materials

Tween 20, Sorbitan monostearate (Span), and stearic acid were obtained from Merck Chemical Co. (Darmstadt, Germany). Tretinoin and GSO were purchased from Sepidaj Pharmaceutical Company, Iran, and Ferdows Juice Manufacturing Company, respectively.

Preparation of NLCs

The synthesis of NLCs was conducted using the hot homogenization method combined with ultrasound waves. Briefly, tretinoin ($C_{20}H_{28}O_2$, MW= 300.442 g.mol^{-1}, and melting point=180 ˚C) was dissolved in liquid lipid (GSO), and the mixture was added to the melted solid lipid (stearic acid). The lipid phase was then heated to 70 ˚C (the water bath set point, which was 5 ˚C higher than the melting point of the solid lipid). Next, the hot aqueous surfactant solution containing different concentrations of water and Tween (Table 1), with the same temperature as the melted lipid mixture, was added dropwise to the lipid phase under homogenization (Silent Crusher M, Heidolph, Nuremberg, Germany) at 20000 rpm for 45 minutes, followed by exposure to ultrasonic waves for an additional 3 minutes. The hot o/w nanoemulsion was cooled down to room temperature, resulting in lipid phase recrystallization and the ultimate formation of NLCs [9].

Characterization of NLCs

Particle size, zeta potential, and polydispersity index (PDI) analysis

The particle size, zeta potential, and PDI of RG-NLC were assessed using photon correlation spectroscopy (PCS) using a Zetasizer (Malvern, UK) at 25 ˚C, employing disposable plain folded capillaries. Before the measurements, all samples were diluted with distilled water and vortexed for 30 seconds to achieve an appropriate scattering intensity [10].

Morphological examination

The morphology of the NLCs was assessed using scanning electron microscopy (SEM). Samples were prepared by placing them onto a carbon tape and subjecting them to gold-palladium coating under vacuum. Images were captured to analyze the surface characteristics and shape of the nanoparticles [11].

Table 1. Formulations containing various percentages of stearic acid, tretinoin, and surfactant

No.	Lipid (%W/W)	Tretinoin (%W/W)	Ratio of Lipid	Ratio of Surfactant (Tween/Span)	Surfactant (%W/W)	Size	Zeta Potential	PDI
1	2	0.03	4	1.25	2	325.27.42	-22.33.21	0.3150.081
2	3	0.05	4	0.50	1	549.97.3	-23.83.92	0.5910.073
3	2	0.03	3	1.25	1	382.34.32	-22.24.81	0.3650.041
4	1	0.01	4	2	3	2314.81	-23.95.35	0.2820.047
5	1	0.05	2	2	3	262.33.95	-23.24.85	0.3570.058
6	1	0.01	2	0.5	1	252.37.49	-24.36.83	0.2900.053
7	3	0.03	3	1.25	2	503.96.82	-25.23.20	0.5620.087
8	1	0.05	4	0.5	3	273.26.10	-24.36.31	0.2930.052
9	1	0.03	3	1.25	2	258.35.61	-23.33.55	0.2400.064
10	2	0.03	3	1.25	3	251.23	-24.25.66	0.3390.045
11	2	0.03	3	1.25	2	331.23.11	-22.53.20	0.3310.073
12	3	0.05	2	2	1	541.13.95	-24.37.38	0.5630.068
13	2	0.01	3	1.25	2	253.45.38	-25.23.20	0.2410.071
14	1	0.05	4	2	1	262.39.36	-22.88.38	0.2530.093
15	2	0.03	3	1.25	2	338.34.94	-24.93.92	0.3100.04
16	2	0.03	3	2	2	315.28.22	-22.36.75	0.3220.059
17	3	0.01	4	0.5	3	493.53.81	-23.47.90	0.5450.064
18	3	0.05	2	0.5	3	521.87.32	-25.34.25	0.5830.038
19	2	0.01	3	1.25	2	269.34.88	-24.26.94	0.3010.060
20	2	0.03	3	0.5	2	322.35.30	-23.93.54	0.3980.075
21	2	0.05	3	1.25	2	3534.32	-21.26.43	0.4520.069
22	2	0.03	3	1.25	2	325.35.90	-23.35.48	0.4080.031
23	2	0.03	2	1.25	2	319.24.35	-22.87.35	0.411.058
24	2	0.03	3	1.25	2	302.54.85	-24.36.92	0.4150.062
25	3	0.01	4	2	1	501.93.84	-22.34.92	0.5820.035
26	3	0.01	2	2	3	495.26.31	-25.26.51	0.5020.62

Drug loading efficiency

The drug loading capacity of the NLCs was measured using an indirect method. To assess the amount of tretinoin entrapped in the NLCs, a certain volume of NLC suspension was centrifuged at 6000 rpm for 30 minutes using Amicon filter devices with a 10 kDa cutoff. Subsequently, the supernatant was analyzed to determine the concentration of unencapsulated tretinoin using UV-Vis spectroscopy. Calibration curves were generated using various concentrations of tretinoin dissolved in DMSO to facilitate quantification at a wavelength of 340 nm [12, 13].

FTIR analysis

The infrared spectra were recorded using an FTIR spectrophotometer (IRAffinity-1S, Shimadzu, Tokyo, Japan) with a sample-to-KBr ratio of 1:10, at a resolution of 4 cm^{-1}. Scans were conducted within the frequency range of 4000 to 500 cm^{-1}, with one scan performed for each individual outcome [14].

Response surface methodology (RSM)

RSM was employed to optimize the formulation parameters of NLCs containing tretinoin and GSO. The methodology involved the following steps: A central composite design (CCD) was used to systematically vary the independent variables, which included the concentration of lipid, tretinoin, surfactant, and the ratio of surfactant. The design matrix was generated using Design Expert software version 13.0.5.0, resulting in a set of experimental runs to evaluate the effects of these parameters on the responses. Each experimental run was conducted according to the design matrix, and data were collected on the following responses: Particle size, zeta potential, and PDI. The collected data were analyzed using Design Expert software to fit a second-order polynomial model, which describes the relationship between the independent variables and the responses. The software was utilized to determine the optimal formulation conditions that maximize %EE while minimizing particle size.

Statistical analysis

All the experiments were carried out in triplicate and represented as the Mean±SD. Data were analyzed using the Design Expert software, which employs RSM to optimize the formulation parameters and predict interactions between independent variables and their effects on responses [15].

Results

Preparation and optimization of NLCs

Design of experiments

The NLC formulations were synthesized based on a systematic design using the RSM with a total of 26 different formulations. The primary variables included percentages of lipid, surfactants (tween and span), and tretinoin. The specific formulations involved variations, seeking to optimize the %EE and stability of the NLCs. Table 1, designed using RSM software, comprises five columns that represent the initial data for formulating the desired combination. The DLS data were compared with the predicted data from the software, leading to the identification of formulation number 13 as the optimal formulation by RSM. This formulation contains 0.01% Tretinoin, 2% surfactant, a stearic acid to GSO ratio of 3%, and a Tween to Span ratio of 1.25. The particle characterization, including IR spectroscopy and morphology evaluation using electron microscopy, was performed on the optimal formulation. In the optimal formulation, the size, PDI, and zeta potential were 253.4 nm, 0.241, and -25.2 mV, respectively, while the predicted values for size, PDI, and zeta potential from the software were 247.8 nm, 0.230, and -24.8 mV, respectively.

Error percentage calculation

The error percentages were calculated based on the Equation 1:

$$1.\text{Error percentage}=\frac{\text{DLS data}-\text{predicted data}\times 100}{\text{DLS data}}$$

This analysis demonstrated the reliability of the RSM predictions in formulating the desired product, with acceptable error margins across the measured parameters (Table 2).

Characterization of NLCs

Particle size analysis

The sizes of the NLCs were determined using a particle size analyzer. The results for the optimized formula are summarized in Table 1. Notably, the size and PDI showed a notable variation with changing concentration levels of lipids and surfactants. For example, a formulation containing 2% lipid and a surfactant ratio of 1.25:1 produced a particle size of 253.4±5.38 nm with a PDI of 0.241±0.064. The DLS data revealed that as the lipid concentration increased, the particle size also increased, with the optimal formulation being characterized as follows:

Zeta potential

The zeta potential, an important indicator of the stability of nanoparticle dispersions, was measured for each formulation. The optimal formulation exhibited a zeta potential of -25.2 mV, indicating sufficient stability for pharmaceutical use. According to the literature, a zeta potential threshold of ±30 mV is commonly indicative of good stability; hence, the observed value supports the potential clinical applicability of the developed NLCs.

Table 2. Error percentages of the data

Variables	DLS Data	Predicted Data	Error (%)
Size	253.4	247.8	2.20
PDI	0.241	0.230	4.5
Zeta potential	-25.2	-24.8	1.5

PDI

The PDI values were analyzed to determine the homogeneity of the formulations. All formulations were within the acceptable range (0-1), with the optimal formulation showing a PDI of 0.241. A lower PDI value suggests a uniform distribution of particle sizes, which is desirable for consistent drug delivery.

Morphological characterization

The morphology of the NLCs was assessed through SEM. The images captured (Figure 1) illustrated that the NLCs had a spherical shape, which is beneficial for penetration into the skin's stratum corneum and enhances the potential for controlled release (Figure 1).

Drug loading efficiency

To evaluate the drug loading efficiency, a calibration curve was constructed by measuring the absorbance of various concentrations of tretinoin in DMSO using UV-Vis spectroscopy. The proportion of encapsulated tretinoin was calculated based on the concentration present in the supernatant after centrifugation. The results indicated an %EE of approximately 90%, suggesting that a significant proportion of the drug was successfully loaded into the NLCs.

FTIR spectroscopy

FTIR was employed to confirm the incorporation of tretinoin in the NLC matrix. The FTIR spectra of both the pure tretinoin and the NLC formulation were compared, revealing characteristic peaks of the drug in the NLC spectrum, which suggests successful encapsulation. The spectra showed that the characteristic functional groups of tretinoin were preserved after formulation, although some peaks were diminished due to interactions between the drug and the lipid matrix (Figure 2).

Discussion

The treatment of acne, a chronic inflammatory disorder that predominantly affects adolescents and young adults, requires effective and targeted therapeutic interventions [16]. Acne is characterized by the formation of comedones, papules, pustules, and sometimes scarring,

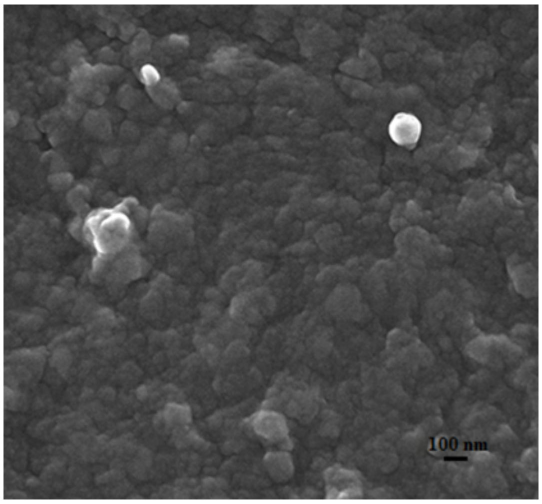

Figure 1. SEM image of the NLCs encapsulating tretinoin

The image illustrates the morphology and uniformity of the NLCs, highlighting their spherical shape and size distribution, which are conducive to enhanced skin penetration and drug delivery efficiency.

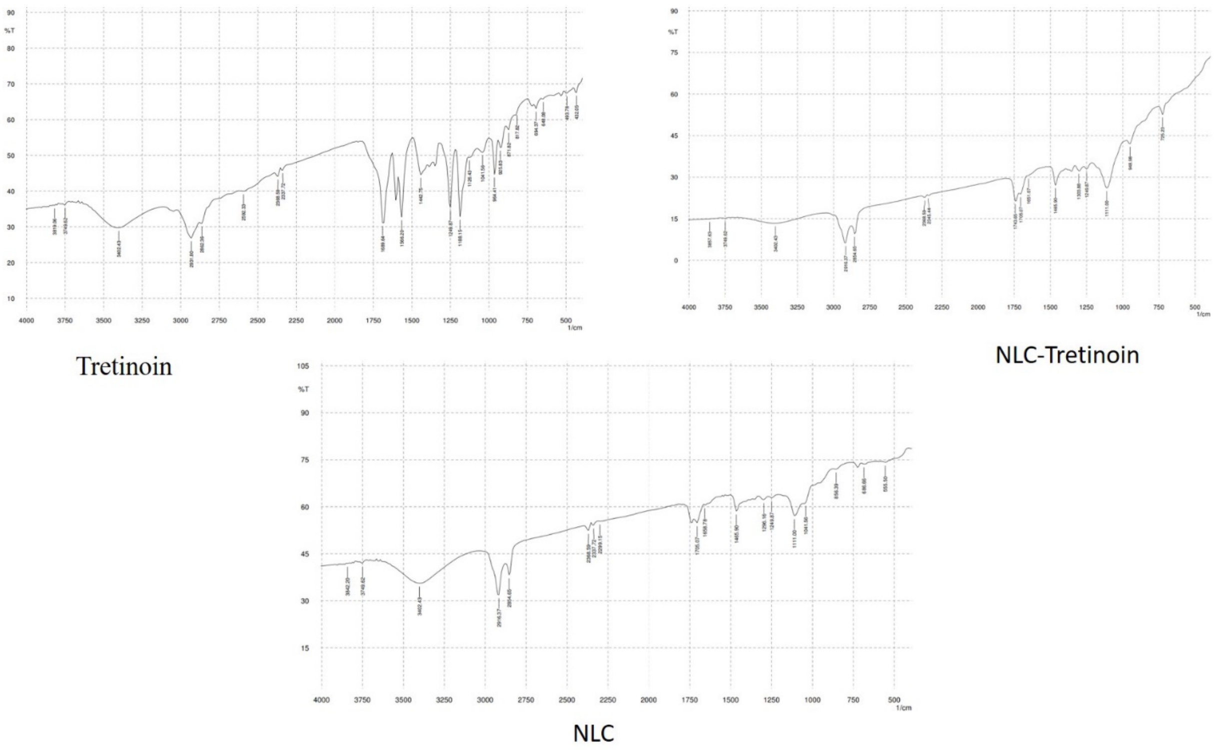

Figure 2. FTIR analysis of tretinoin

Tretinoin-NLC, and NLC, illustrating the characteristic absorption bands and chemical interactions among the compounds.

primarily due to the inflammation of pilosebaceous units in the skin [17]. Recent statistics reveal that approximately 85% of individuals between the ages of 12 and 25 experience some form of acne, although it can persist well into adulthood [18]. The impact of acne transcends physical symptoms; it can lead to significant psychological distress, including anxiety, depression, and low self-esteem. Notably, managing acne effectively is vital not only for physical health but also for emotional well-being [19]. Among various treatment modalities, topical retinoids—particularly tretinoin, the active metabolite of vitamin A—have emerged as one of the most effective options for managing acne vulgaris [20]. Tretinoin exerts its anti-acne action through several mechanisms, including normalization of keratinization, reduction of sebum production, and anti-inflammatory properties. However, despite its therapeutic benefits, tretinoin's clinical use is often hampered by its limited skin penetration, high irritation potential, and instability. These challenges necessitate innovative approaches, such as formulation in nanosized delivery systems, which can enhance the bioavailability, stability, and overall efficacy of the drug while minimizing potential side effects [21]. In response to these challenges, the development of NLCs presents a promising solution. NLCs combine solid lipids and liquid lipids to improve the %EE of lipophilic drugs, such as tretinoin, and facilitate controlled release. Additionally, NLCs can protect the active ingredient from environmental degradation and improve overall stability, which is crucial for maintaining therapeutic efficacy [22, 23].

The primary objective of this research was to design and synthesize NLCs containing tretinoin and GSO using a combination of hot homogenization and ultrasonication techniques. The incorporation of GSO as a lipid phase in NLC formulations adds another layer of efficacy. GSO, rich in linoleic acid and antioxidants, was selected for this study due to its beneficial properties for skin health, including its moisturizing effects and potential anti-inflammatory activity, which can complement the action of tretinoin [24, 25].

A previous study has shown that oils rich in linoleic acid, like GSO, can improve skin hydration and may possess anti-inflammatory properties, further supporting its use in formulations aimed at acne treatment [26]. Therefore, in this study, GSO was used as the liquid lipid in the formulation. We utilized hot homogenization followed by ultrasonic method to encapsulate tretinoin in NLCs, building on findings from a 2013 study by Nasrollahi et al., which indicated that the use of a high-pressure homogenizer to produce SLNs of tretinoin resulted in a formulation with a slower and longer-lasting effect compared to tretinoin cream, suggesting that the SLN formulation has better skin tolerance and could serve as a more effective option in topical treatments [27]. The resulting formulations were characterized for particle size and %EE. The morphological analysis via SEM revealed spherical nanoparticles, which is beneficial for ensuring uniform penetration across the skin barrier [28]. Additionally, FTIR was employed to analyze the interactions between the lipid matrix and the encapsulated drug. FTIR analysis revealed characteristic peaks associated with the presence of tretinoin within the lipid matrices, suggesting successful encapsulation within the NLCs. The FTIR spectrum of pure tretinoin exhibited several significant peaks corresponding to specific functional groups in its molecular structure. Key peaks included a peak at 2931 cm^{-1} attributed to the stretching vibrations of the (—C—H) group, peaks at 1188 cm^{-1} and 1249 cm^{-1} corresponding to the stretching vibrations of the (C-O) group, and a peak at 1689 cm^{-1} associated with the stretching vibrations of the (C=O) bond.

Additionally, the trans configuration of the vinyl group was represented by a peak at 1964 cm^{-1}, while the stretching vibrations of (C=C) coupled with the carbonyl group were observed at 1566 cm^{-1} with high intensity. As illustrated, the FTIR spectrum of pure tretinoin displays sharp and intense peaks, indicating the presence of various functional groups. Furthermore, the FTIR results for the blank sample and the NLC formulation containing tretinoin showed striking similarities, with notable reductions in peak intensity for the functional groups in the NLC formulation. This reduction suggests successful encapsulation of tretinoin within the nanoparticles. Importantly, peaks associated with functional groups were absent in formulations lacking the active pharmaceutical ingredient, further confirming the effective incorporation of tretinoin into the NLC. Overall, these results indicate not only successful encapsulation of tretinoin but also optimal particle sizes that enhance skin penetration, thereby supporting the potential efficacy of the NLC formulation in topical applications.

Conclusion

The research successfully developed and characterized NLCs for the effective delivery of tretinoin. The optimized formulation demonstrated a suitable particle size, zeta potential, and %EE, indicating its potential for enhanced skin permeation and reduced irritation compared to traditional formulations. The morphological analysis confirmed the uniformity and stability of the nanoparticles, highlighting their suitability for topical applications. Future studies should focus on in vivo evaluations to assess the pharmacokinetics and therapeutic efficacy of the optimized NLC formulation in acne treatment. Additionally, exploring the incorporation of other active ingredients within the NLC system could enhance multifunctional properties, targeting not only acne but also signs of aging. Investigating alternative methods for large-scale production and stability studies under various storage conditions will also be crucial for the eventual commercialization of this formulation. Finally, patient-centric clinical trials will be essential to validate the safety and effectiveness of the NLCs in real-world applications.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Zabol University of Medical Sciences, Zabol, Iran (Code: IR.ZBMU.REC.1400.005).

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Authors contribution's

Data collection and data analysis: Rokhsaneh Balouchi; writing the original draft: Mahdie Arefi; Conceptualization, supervision and validation: Sara Daneshmand; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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