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Research article

How the designed processing parameters affect the liquid mixture density and viscosity of the tretinoin-loaded niosomes at different temperatures?

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ABSTRACT

The current study was conducted to present novel thermophysical data on tretinoin-loaded niosomes paired with a combination of span 60 and tween 80. Measurements were carried out to analyze the liquid mixture density and viscosity of the mentioned multilayered structures for the first time, with consideration given to the diverse molecular weights of surfactants and various stabilizers at different temperatures. Through the application of equations of state, this study has the ability to set the stage for thermodynamic modeling of solutions that involve niosomes, presenting a promising avenue for further research. So, tretinoin-loaded formulations were prepared by investigating the effects of different co-surfactants, including cholesterol or dodecanol, as well as the impact of surfactant molecular weight limited to 650.525–1090.175 g mol^{-1}. This novel investigation was conducted to assess the superior stabilizing capabilities of dodecanol in comparison to cholesterol, with a specific emphasis on optimized vesicle size, highest incorporation efficiency, and lowest zeta potential. In particulars, the response surface methodology (RSM) was applied to optimize the operative factors and the number of experiments. The experimental evidence clearly indicates that the use of dodecanol in the manufacturing process significantly improves the stability of niosomes, while the inclusion of cholesterol leads to higher liquid mixture density and viscosity in the prepared niosomes.

1. Introduction

Design of the recently developed carriers shows a significant aim of the medical investigations. Vesicles, including niosomes and liposomes, are one of the nanostructures, providing access to enhanced medication local efficiency. However, their role as a skin drug delivery system is debatable, with varying effects observed depending on the category of vesicles and their content. As a consequence, throughout the last twenty years, new kinds of vesicles have been produced to maximize the drug delivery through the skin [1–4].

Non-ionic surfactant vesicles known as niosomes have been researched as a substitute for conventional liposomes in the latest days. Particularly, in comparison to phospholipid vesicles, they provide more chemical stability, lower prices, and better surfactant class accessibility [5–8]. Niosomes, especially, appear to be a promising drug delivery mechanism for dermatological treatments [9].

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Actually, therapeutically effective niosomes can prolong medication residency duration in the epidermis and stratum corneum while lowering drug absorption in the bloodstream. So, they are supposed to enhance the characteristics of the horny layer by decreasing transepidermal water loss and enhancing softness through refilling depleted skin lipids [10,11].

Niosomes are obviously prepared via non-ionic surfactants, co-surfactants, organic solvents, water, and drugs. In detail, non-ionic surfactants include spans, tweens, brijs, etc. Moreover, organic solvents contain methanol, chloroform, dichloromethane, etc. Also, the main co-surfactants are cholesterol and dodecanol that are used as membrane stabilizers. It should be noted, cholesterol is more commonly used in the formulation of niosomes compared to dodecanol. Although there has been lots of investigations in the case of cholesterol usage as a stabilizer in niosomes formulation, there has been the minimum studies in the case of dodecanol [12–14]. For illustration, Gutiérrez et al. [13] investigated a number of formulations for iron-entrapped niosomes with better entrapment stability. For niosome preparation, they chose a modified ethanol injection approach. They made niosomes using food-grade surfactants, containing polyglyceryl-3 dioleate, glycerol monoleate, or sorbitan monooleate, as well as dodecanol as a membrane stabilizer. It had iron encapsulation efficiencies of 72–84 percent. Furthermore, in the case of cholesterol application, Poustforoosh et al. [15], used niosomes structures in a new and effective way. The binding affinity of various biomolecules, including amino acids and carbohydrates, was evaluated in relation to their associated transporters through a molecular docking approach. Those biomolecules exhibiting the highest binding affinity were chosen for conjugation to niosomes. Following the conjugation of these biomolecules to cetyl alcohol, bio-conjugated vesicles were prepared with a non-ionic surfactant (Span 60) and cholesterol using the thin-film hydration method. The authors demonstrated that computational methodologies serve as effective techniques for identifying suitable targeting agents. In addition, trehalose emerged as a promising candidate for bio-conjugation, enhancing the transport of niosomes across the blood-brain barrier.

Obviously, The majority of dodecanol studies have been done in food industry, and the lack of research is perceived on skin drug delivery via niosomes. Among various medicines of skin treatment, all-trans retinoic acid or tretinoin (TRA) is an operative vitamin that has attracted lots of consideration in recent years [16–20]. It is also used for ichthyosis, acne vulgaris, and psoriasis [21,22]. However, the serious adverse effects of taking TRA orally make it unsuitable. Local tretinoin therapy is beneficial for dermatological problems while minimizing systemic toxicity and exposure [23,24]. Nevertheless, various disadvantages limit its topical utilization, including relatively poor water solubility, skin irritation, and photostability [25,26]. Tretinoin niosomal and liposomal preparations have been investigated to circumvent all of these drawbacks [27–34]. For instance, Manconi et al. [28] reported that tretinoin (0.02 %) may be carried via liposomes, and it also can produce higher efficiency and more stable formulations using niosomes. Furthermore, Manconi et al. [29] examined the ability of niosomes as topical vehicles able to increase the stability of photosensitive medications by comparing the chemical stability of TRA in methanol and vesicular solutions subjected to synthetic daylight and UV. They prepared the tretinoin-loaded niosomes from brij 30, span 40 or span 60, and a commercial mixture of Triton CG 110. Likewise, Rahman et al. [33] created a retinol liposomal gel formulation with a higher clinical impact and a decreased risk of skin irritation.

As a carrier for transdermal medication delivery, niosomes appear to be very hopeful [35,36]. Niosomes have been discovered to reduce TRA toxicity, change its bioavailability and pharmacokinetics, and enhance its photostability. It has been begun searching on retinoid niosomal compositions in the hopes of creating novel TRA-friendly topical application forms.

Clearly, the effects of formulation including the molar ratio of surfactant:co-surfactant, and the kind of drugs have been investigated widely in cutaneous niosomes preparation along with cholesterol. Unfortunately, insufficient literature exists so far on the impacts of other processing parameters about such structures; these influences are crucial to the formation of these vesicles as effective delivery carriers. Hence, other significant processing factors involving the hydration and solvent evaporation temperature, pH, ultrasonic time, ultrasonic bath temperature, kind of organic solvent, type of stabilizer, molecular weight of surfactant should be studied sufficiently in the case of cutaneous drugs. Consequently, the lack of a comprehensive reference that can assess the impact of operational parameters with minimal testing and cost is obvious in the delivery of dermatological drugs such as tretinoin via niosomes. Also, another problem is related to the lack of data in the case of liquid mixture density and viscosity of TRA-loaded niosomes. Once the thermophysical data is obtained, the thermodynamic modeling of the multilayer structures can be easily accomplished through the use of the equations of state.

This research was carried out to broaden the thermophysical data on tretinoin-loaded niosomes, focusing on skin delivery niosomes in conjunction with a combination of span 60 and tween 80. So, the thin film hydration method was firstly applied as a proper procedure to prepare the TRA-loaded niosomes supplied with multi-layer vesicles. In detail, ultrasonic time, surfactant molecular weight, and type of co-surfactants were considered as the most effective parameters. The category of co-surfactants included cholesterol or dodecanol, which were being studied to determine the advantages of dodecanol over cholesterol as a stabilizer that was not as commonly used. The response surface methodology was utilized as a statistical method to adjust the operative factors and the number of experiments. Subsequently, the morphology, size distribution, zeta potential, and entrapment efficiency were checked. Following the preparation of niosomes using diverse processing parameters, the density and viscosity of the mixture were assessed for each structure. This meticulous evaluation aimed to establish an appropriate methodology for studying the thermodynamic modeling of niosomes as efficacious pharmaceutical structures. Indeed, these thermophysical properties were measured for different molecular weight of surfactants mixture and various co-surfactants at a constant sonication time equal to 17 min.

Table 1
Formulation of the different TRA-loaded niosomes. Three central points were selected. The central points were not repeated in the Table.

Sample Number	Molecular Weight of Surfactant ($g.mol^{-1}$)	HLB	Molar ratio of Tween 80: Span 60	Sonication Time (min)	Kind of Co-Surfactant
CH-1-1	650.525	7.275	0.25 : 0.75	13	cholesterol
CH-1-2	650.525	7.275	0.25 : 0.75	15	cholesterol
CH-1-3	650.525	7.275	0.25 : 0.75	17	cholesterol
CH-2-1	870.350	9.850	0.50 : 0.50	13	cholesterol
CH-2-2	870.350	9.850	0.50 : 0.50	15	cholesterol
CH-2-3	870.350	9.850	0.50 : 0.50	17	cholesterol
CH-3-1	1090.175	12.425	0.75 : 0.25	13	cholesterol
CH-3-2	1090.175	12.425	0.75 : 0.25	15	cholesterol
CH-3-3	1090.175	12.425	0.75 : 0.25	17	cholesterol
D-1-1	650.525	7.275	0.25 : 0.75	13	dodecanol
D-1-2	650.525	7.275	0.25 : 0.75	15	dodecanol
D-1-3	650.525	7.275	0.25 : 0.75	17	dodecanol
D-2-1	870.350	9.850	0.50 : 0.50	13	dodecanol
D-2-2	870.350	9.850	0.50 : 0.50	15	dodecanol
D-2-3	870.350	9.850	0.50 : 0.50	17	dodecanol
D-3-1	1090.175	12.425	0.75 : 0.25	13	dodecanol
D-3-2	1090.175	12.425	0.75 : 0.25	15	dodecanol
D-3-3	1090.175	12.425	0.75 : 0.25	17	dodecanol

2. Experimental section

2.1. Materials and methods

2.1.1. Materials

The two consumed non-ionic surfactants have been tween 80 and span 60. Span 60 and tween 80 were used as a hydrophobic and hydrophilic substances, respectively. Polyoxyethylen (20) sorbitanmono-oleate (tween 80) was kindly provided from íPanreac AppliChem (Panreac Química SLU, ITW Reagents, Spain). Also, sorbitanmono-stearate (span 60) was prepared from íMerck Millipore Corporation. Furthermore, cholesterol and dodecanol were provided by Sigma-Aldrich and Merck Millipore Corporation, respectively as membrane stabilizer. Besides, tretinoin was obtained from Sepidaj Pharmaceutical Co (Iran, Tehran). Methanol was generously supplied from Ameretat Shimi Pharmaceutical Co, and dichloromethane was purchased via Merck Millipore Corporation. Likewise, the hydration process was performed via deionized water. Deionized water was purchased from the Semnan Azma laboratory.

2.2. Methods

2.2.1. Response surface methodology (RSM)

Statistically, the RSM indicates the relations of numerous descriptive variables and one or further response variables [37,38]. As a beneficial experimental design plan, the Central Composite Design (CCD) offers high-quality correlations of quadratic and linear interaction impacts of factors affecting the procedure [39]. So, Design Expert software (version 11.1.1.0) was applied for analyzing the reaction parameters via the RSM and CCD. The CCD was applied to estimate the optimal circumstances in the case of maximum IE %, the minimum particle size, and the lowest zeta potential. Two numerical parameters along with one categorical factor were used with three input levels for experiments design. The numerical factors contain the molecular weight of surfactants ($g.mol^{-1}$) and sonication time (min). Moreover, the kind of co-surfactant is utilized as categorical factor.

Also, Table 1 has listed 18 different TRA niosome formulations. This Table contains the hydrophilic lipophilic balance (HLB) of surfactants mixture calculated as below:

$$HLB_{mix} = \sum x_i HLB_i \tag{1}$$

in which, x_i represents the mole fraction of surfactant i. Index mix shows the mixture.

It is important to highlight that the appendix provides a detailed explanation of the molar mass calculation of the surfactant mixture, as well as the HLB value of the mixture.

The ultrasonic time was set between 13 and 17 min to ensure the solution's uniformity and homogeneity, free of solid particles. Since Span 60 has strong hydrophobic properties, it requires a longer ultrasonic time, typically above 10 min. However, the most significant effects of ultrasonication occur in the initial stages, so intervals of 2 min were considered for precision. While different ratios of Span 60 and Tween 80 have not yet been explored, a molar mass range of 650.525–1090.175 g mol^{-1} was selected for this study. Additionally, dodecanol was investigated as a potential new stabilizer to replace traditional cholesterol.

2.2.2. Tretinoin-loaded niosomes preparation

Tretinoin niosomes were prepared using the thin film hydration method [40] via the rotary evaporator (HAHNSHIN SCIENTIFIC CO, HAHNVAPOR, HS-2005V-N, South Korea) based on the presented formulation in Table 1. Multilamellar vesicles (MLVs) were

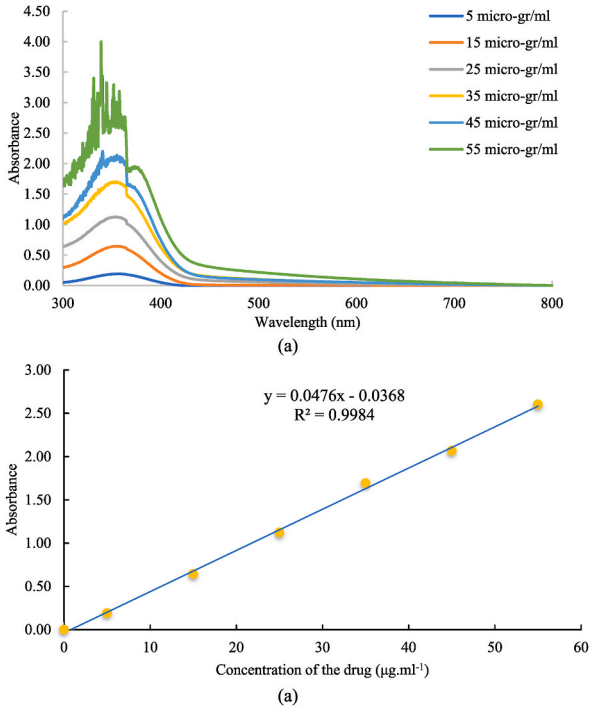


Fig. 1. Standard calibration curve of tretinoin at 357 nm.

provided in a constant molar ratio of surfactant to co-surfactant equal to 3:1 (total molarity of 10 mM). A mixture of span 60 and tween 80 with a molecular weight of 650.525, 870.350, and 1090.175 g mol^{-1} was used to make the consumed surfactant. The HLB of manufactured niosomes were 7.275, 9.850, and 12.425 for 650.525, 870.350, and 1090.175 g mol^{-1}, respectively. Dodecanol and cholesterol were also utilized as co-surfactants. Hence, a mixture of surfactants in a given molecular weight, one of the noted co-surfactants, and tretinoin (5 mg) were dissolved in 10 ml of dichloromethane. Subsequently, the organic solvent was vacuum evaporated at 313.15 K and 80 rpm for 120 min. The attained solid phase was then hydrated via 60 ml of deionized water at 313.15 K and 80 rpm. The water was added to the system in four steps for 40 min. Eventually, the hydrated solution was sonicated using an ultrasonic cleaner (ALEX machine, Japan) at a working frequency of 32 kHz and ambient temperature. The sonication time was adjusted to 13, 15, and 17 min.

2.2.3. Surface charge, particle size, and polydispersity index (PDI) measurements

The zeta potential, mean diameter and polydispersity index of tretinoin-loaded niosomes were examined via dynamic light scattering apparatus (Malvern, Zetasizer, Nano series, ZS90 with a serial number of MAL1236033, United Kingdom). Moreover, the device can measure the particle size in the range of 0.3 nm up to 5 μm.

2.2.4. High resolution scanning electron microscopy test

The structure of vesicles was determined via scanning electron microscopy (HR-SEM). A droplet of the manufactured niosomes was poured on a copper grid and vacuumed for nearly 10 min. The samples were subsequently analyzed and photographed using a HR-SEM QUANTA 200, America at an accelerating voltage of 0.2–30 kV.

2.2.5. Incorporation efficiency (IE%) determination

The loaded TRA was separated from unincorporated TRA in niosomes structures via centrifugation (Bench top high speed centrifuge HS 18500 R, FAR TEST, FARZANEH ARMAN Co, Iran) at 11000 rpm and 4 °C for 30 min. The supernatant was separated from the precipitate. Consequently, unincorporated TRA was examined using UV absorbance (UV Visible spectrophotometer, SHIMADZU UV-1650PC, Kyoto, Japan) at λ_{357} nm utilizing 1 cm quartz cell along with empty niosome as blank. Hence, the incorporation efficiency (IE) was calculated as below [41]:

$$IE\% = \frac{Entire\ drug\ quantity - Unloaded\ drug\ quantity}{Entire\ drug\ quantity} \times 100 \quad (2)$$

The unloaded drug quantity was computed applying Beer–Lambert law as follow [42–44]:

$$A = \varepsilon bC \quad (3)$$

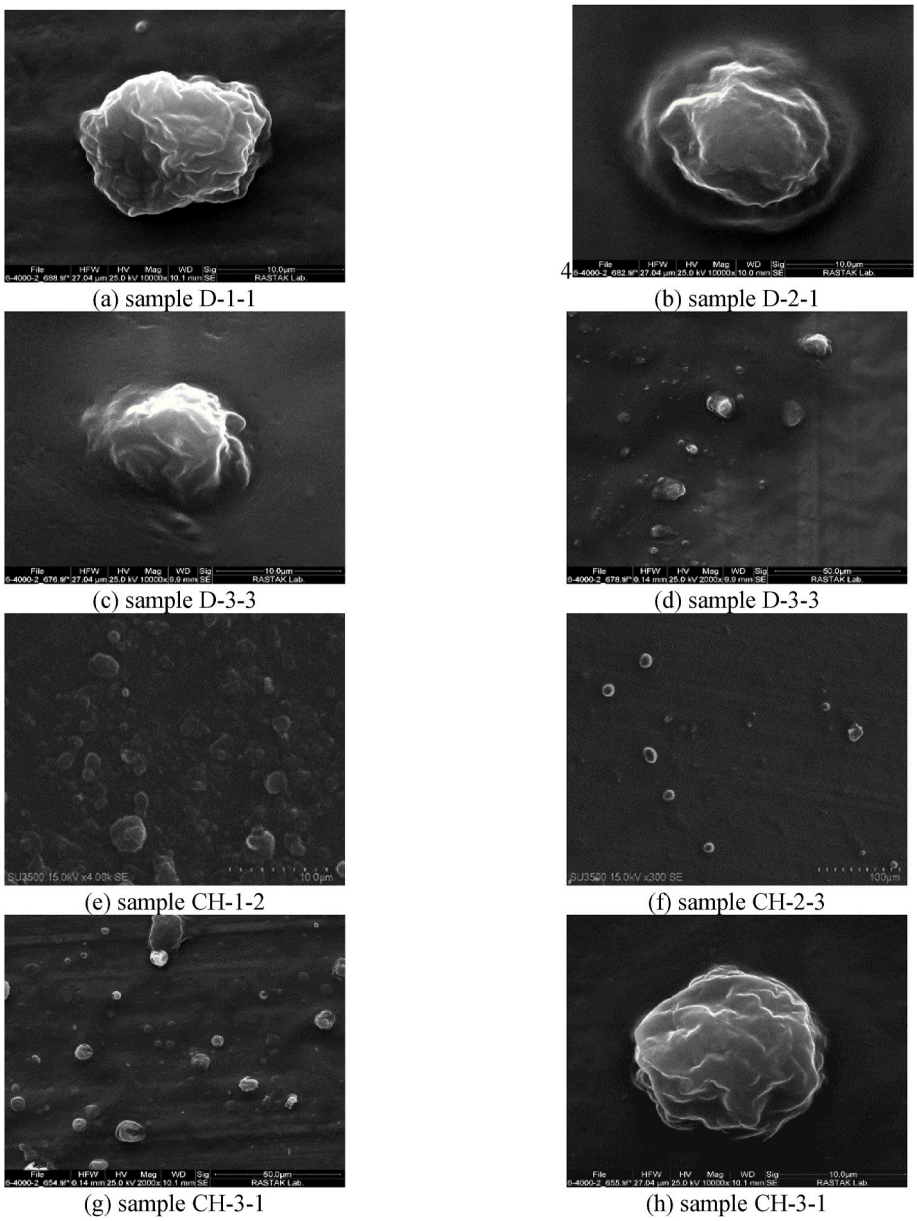


Fig. 2. HR-SEM of TRA loaded niosomes which were prepared using dodecanol and cholesterol. (a) sample D-1-1, (b) sample D-2-1, (c), (d) sample D-3-3, (e) sample CH-1-2, (f) sample CH-2-3, (g) and (h) sample CH-3-1.

in which, A is the Absorbance at λ_{max} obtained from UV spectrophotometer. In addition, b indicated the optical path length in cm wchich is equal to 1 cm. Besides, C and ε are concentration ($\mu g.ml^{-1}$) and molar absorptivity ($ml.cm.\mu g^{-1}$), respectively. So, knowing A, b, and ε, the unloaded drug (C) will be calculated. The molar absorptivity (ε) of TRA-loaded niosomes have obtained by plotting the calibration curve and using the empty niosomes as blank. Fig. 1 has illustrated the absorbance data versus wavelength (a) and concentration (b).

During the calibration procedure, a stock solution with a concentration of 150 $\mu g\ L^{-1}$ was prepared using the drug and methanol as the solvent. Additionally, a drug-free niosome was formulated, incorporating Tween 80, Span 60, and a co-surfactant with a defined molar mass. Subsequently, the drug-free niosome underwent centrifugation under the above-mentioned conditions. Various volumes of the stock solution were then introduced to the centrifuged niosome to achieve different drug concentrations within the niosome. The resulting solutions were subjected to UV spectrophotometric analysis, with absorbance measurements taken across a wavelength range of 200–800 nm. It is important to highlight that the drug-free and centrifuged niosome served as the blank solution. The absorbance data indicated that the peak absorption occurred at a wavelength of 357 nm, which was thus identified as the optimal wavelength. Furthermore, the molar absorptivity (ε) was calculated from the slope of the absorption versus concentration graph during the

Table 2
Particle size, PDI, IE, and zeta potential of RSM designed formulations of TRA-loaded niosomes.

Sample Number	M_s (g. mol^{-1})	Sonication Time (min)	Co-Surfactant	Particle Size (nm) (average ± SU)	PDI (average ± SU)	IE (%) (average ± SU)	Zeta Potential (mV) (average ± SU)
CH-1-1	650.525	13	cholesterol	303.50 ± 10.1	0.57 ± 0.021	59.64 ± 0.51	−15.50 ± 0.50
CH-1-2	650.525	15	cholesterol	286.63 ± 8.60	0.58 ± 0.011	59.40 ± 0.32	−15.20 ± 0.40
CH-1-3	650.525	17	cholesterol	260.16 ± 9.30	0.59 ± 0.012	59.18 ± 0.44	−14.90 ± 0.45
CH-2-1	870.350	13	cholesterol	222.11 ± 5.40	0.72 ± 0.009	78.94 ± 1.10	−13.90 ± 0.96
CH-2-2	870.350	15	cholesterol	220.33 ± 5.10	0.70 ± 0.007	70.87 ± 0.20	−12.16 ± 0.87
CH-2-3	870.350	17	cholesterol	219.36 ± 4.60	0.65 ± 0.010	70.70 ± 0.36	−11.56 ± 0.65
CH-3-1	1090.175	13	cholesterol	502.63 ± 15.2	0.98 ± 0.022	70.31 ± 0.26	−18.46 ± 0.24
CH-3-2	1090.175	15	cholesterol	474.53 ± 13.1	0.97 ± 0.031	70.01 ± 0.35	−18.16 ± 0.36
CH-3-3	1090.175	17	cholesterol	407.56 ± 14.6	0.95 ± 0.029	69.12 ± 0.23	−17.46 ± 0.48
D-1-1	650.525	13	dodecanol	293.10 ± 7.40	0.63 ± 0.016	77.59 ± 1.23	−12.10 ± 0.11
D-1-2	650.525	15	dodecanol	269.30 ± 6.60	0.64 ± 0.019	73.76 ± 2.12	−12.60 ± 0.15
D-1-3	650.525	17	dodecanol	265.40 ± 4.20	0.67 ± 0.021	67.07 ± 1.36	−12.96 ± 0.22
D-2-1	870.350	13	dodecanol	239.63 ± 6.80	0.79 ± 0.008	79.48 ± 0.56	−19.50 ± 0.26
D-2-2	870.350	15	dodecanol	228.53 ± 5.20	0.82 ± 0.007	76.85 ± 0.42	−19.65 ± 0.13
D-2-3	870.350	17	dodecanol	220.66 ± 4.30	0.87 ± 0.011	73.76 ± 0.98	−19.90 ± 0.32
D-3-1	1090.175	13	dodecanol	413.66 ± 12.1	0.66 ± 0.032	77.69 ± 1.10	−16.50 ± 0.46
D-3-2	1090.175	15	dodecanol	386.53 ± 4.10	0.62 ± 0.039	72.44 ± 0.65	−17.02 ± 0.31
D-3-3	1090.175	17	dodecanol	382.15 ± 3.60	0.56 ± 0.036	71.81 ± 0.56	−17.16 ± 0.12

calibration, yielding a value of 0.047.

It should be mentioned that the molecular weight, sonication time, and the type of co-surfactants do not affect the ε in calibration process, and this data is constants in all calculations. Neither dodecanol nor cholesterol peak in this range. This was analyzed by UV spectra of dodecanol and cholesterol in the same blank. Also, the standard deviation to data points in the calibration curve is ±0.05.

2.2.6. Density measurement

An oscillating U-tube digital densitometer Anton Paar-DMA 4500 M was operated to evaluate the liquid mixture density of TRA-loaded niosomes. This device can measure the liquid density between 0 and 3 g cm^{-3} at the temperature range 273.15–363.15 K.

2.2.7. Viscosity measurement

A rolling-ball micro viscometer Anton Paar-Lovis 2000 M was utilized to estimate the liquid mixture viscosity of TRA-loaded niosomes. This apparatus can determine the liquid viscosity between 0.3 and 10000 mPa s at the temperature range 278.15–373.15 K.

3. Results and discussion

In this work, a set of designed experiments were done to optimize the particle size, IE %, and zeta potential of the tretinoin-loaded niosomes, and then measure the liquid mixture density and viscosity of these formulations. As previously stated, the RSM was used to determine the best conditions and reduce the number of experiments. For the experiment design, three levels were applied with two numerical parameters and one categorical factor. The molecular weight of surfactants and the sonication time were the numerical factors. In addition, the type of co-surfactant was selected as a categorical component. Now, the results of performed experiments and the coded equations of the RSM optimization are presented in the niosomes characterization part and RSM optimization section, respectively. The most important features of TRA-loaded niosomes were HR-SEM, paricle size, PDI, zeta potential, and IE %. Following this, an analysis of the liquid mixture density and viscosity was conducted in these formulations and outlined in the niosomes characterization section, with the intention of introducing a fresh perspective to the thermodynamic modeling of these structures. Some of these characteristics were modeled with the RSM to investigate the interactions of parameters. The related results were discussed in the RSM optimization part.

3.1. Niosomes characterization

3.1.1. HR-SEM test

As mentioned previously, using the RSM design, various formulations were prepared in the case of TRA-loaded niosomes. So, it is vital to determine whether the RSM designed formulations of niosomes were formed or not. The formation ability of TRA-loaded vesicles was investigated by HR-SEM test in all mentioned formulations. Fig. 2 has indicated a few photomicrographs of the provided niosomes via dodecanol and cholesterol. Evidently, the spherical MLV structures were shaped.

In earlier studies, a wrinkle-like shape resembling a SEM has been observed in niosomes. This phenomenon has been attributed to the interaction of surfactants with varying carbon chain lengths, alongside the disparities in their hydrophilic and hydrophobic properties, which subsequently influence the surface free energy [45,46].

Table 3
The density and viscosity values of TRA-loaded niosomes at different temperatures and molecular weights.

		dodecanol		cholesterol	
Temperature (K)	Molecular weight (g.mol^{-1})	Density (g.cm^{-3})	Viscosity (mPa.s)	Density (g.cm^{-3})	Viscosity (mPa.s)
293.15	650.525	0.99912	1.0859	0.99915	1.0846
	870.350	0.99927	1.0890	0.99939	1.1127
	1090.175	0.99949	1.1052	0.99951	1.0498
298.15	650.525	0.99793	0.9661	0.99795	0.9700
	870.350	0.99808	0.9729	0.99820	1.0038
	1090.175	0.99830	0.9864	0.99838	0.9313
303.15	650.525	0.99650	0.8599	0.99640	0.8769
	870.350	0.99665	0.8756	0.99677	0.9211
	1090.175	0.99687	0.8874	0.99695	0.8611
308.15	650.525	0.99484	0.7708	0.99482	0.7897
	870.350	0.99499	0.7894	0.99512	0.8533
	1090.175	0.99520	0.7922	0.99532	0.7597
313.15	650.525	0.99297	0.7198	0.99303	0.7152
	870.350	0.99309	0.7407	0.99325	0.7311
	1090.175	0.99334	0.7372	0.99345	0.6939

[1] The standard uncertainty values for dodecanol: SU (density) = 1.20E-04 (g cm^{-3}), and SU (viscosity) = 9.00E-03 (mPa s).
[2] The standard uncertainty values for Cholesterol: SU (density) = 2.40E-04 (g cm^{-3}), and SU (viscosity) = 1.10E-02 (mPa s).

3.1.2. Vesicles particle size, polydispersity index, zeta potential, and incorporation efficiency

The four significant parameters of niosomes characterization have been summarized in Table 2 using 18 designed formulations. These factors contain mean particle size, polydispersity index, zeta potential, and incorporation efficiency. Also, the standard uncertainty (SU) has been listed for all measured properties.

According to the presented data and fixing the sonication time at a constant value, as the molecular weight of mixed surfactants increases, the mean particle size tends to a minimum value, and it consequently increases for both co-surfactants. The minimum particle size occurs at the molar ratio of 0.5:0.5 of tween 80:span 60 for both co-surfactants (samples D-2 and CH-2 correspond to the molecular weight of 870.350 g mol^{-1} and HLB of 9.850). The HLB of surfactants mixture has the middle value in this molecular weight, and so, it is guessed that this molar ratio has had the best equivalence for decreasing the contrasting effects of hydrophilic-lipophilic balance. Consequently, the minimum particle size will be formed.

Formerly, it has been reported that the increment of the HLB and surface energy can increase the particle size of niosomes [47–50]. Also, longer alkyl chains and smaller head groups of surfactants resulted in the configuration of bigger vesicles [51–57]. So, when HLB is below 9.850, the smaller head group (low molecular weight) and reduced hydrophilic to lipophilic head group area cause surface energy increment and formation the thicker vesicles size. Moreover, high HLBs and longer alkyl chains lead to balance perturbation and consequently surface energy increment. Hence, at higher HLBs the, average size becomes more. Subsequently, the minimum average size occurs at the optimized HLB and surface energy.

Likewise, comparing cholesterol and dodecanol, cholesterol has produced bigger vesicles. Although both substances are hydrophobic, cholesterol has more lipophilicity. Therefore, for the same reason as mentioned above, cholesterol will make larger bilayers and higher surface energy [52].

Furthermore, increasing the molecular weight at a constant sonication time, the IE percent has been augmented up to a maximum value, and it decreased subsequently for both co-surfactants. Hence, the maximum IE has occurred at the minimum particle size. The same reason has been offered for this phenomenon.

Encapsulation of non-ionic vesicles is actually a result of the synthesis method, dispersion stability, and bilayer intrinsic properties. Another important parameter in IE % increment is related to niosome membrane stability [58]. Indeed, existing the optimal condition between hydrophilic and hydrophobic interactions leads to equilibrium development, membrane stability and therefore, maximizing the amount of drug intake. Moreover, cholesterol has had smaller IE % in comparison with dodecanol due to more surface energy as described above.

Additionally, when it comes to investigating the zeta potential and obtaining its relation with molecular weight increment, zeta potential becomes more positive and more negative for cholesterol and dodecanol, respectively, and it changes its trend afterwards. It means that the zeta potential has the highest and lowest values at a constant sonication time for cholestrol and dodecanol at 870.350 g mol^{-1}, respectively.

In more detail, zeta potential demonstrates the electrical potential at the slipping surface. This interface can separate mobile fluid from the fluid that remains attached to the surface. In the case of negative zeta potentials, the bilayer has been surely formed without charged species [52]. This occurs due to the preferred adsorption of OH^- ions or counters ion adsorption. So, zeta potential should be the most negative one in dodecanol and at the best stability due to the more preferred adsorption of OH^- ions. Hence, in the case of the minimum paricle size (870.350 g mol^{-1}), dodecanol can place near the interface due to its more hydrophilicity and OH group, and subsequently it can cause more negative charge (the lowest value of zeta potential). This situation is vice versa for Cholesterol.

Changing our viewpoint and setting the molecular weight at a constant value equal to 650.525, 870.350, or 1090.175 g mol^{-1}, as the sonication time increases, the mean particle size and IE % tend to a minimum value, for both co-surfactants. The results are similar to the previous studies [59]. In addition, at a conatant molecular weight, zeta potential has became more negative and positive for

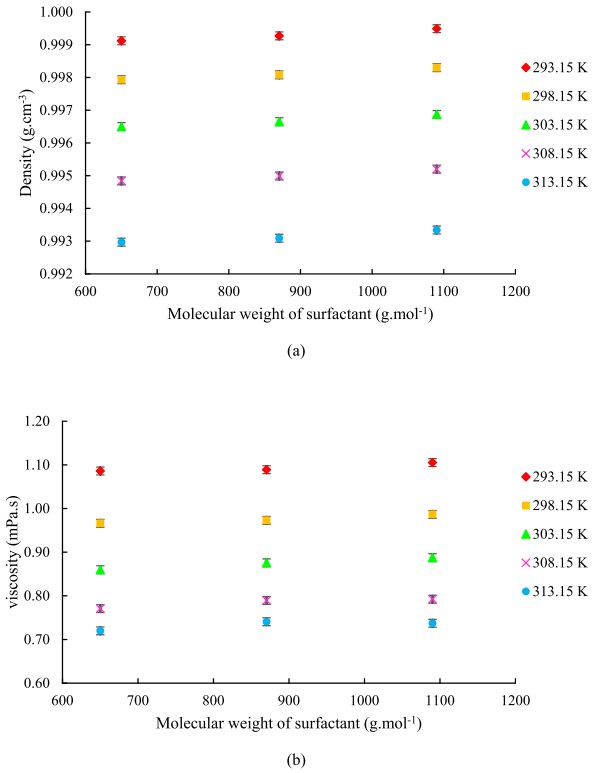


Fig. 3. Measured density (a) and viscosity (b) data as a function of molecular weight at constant temperatures for dodecanol.

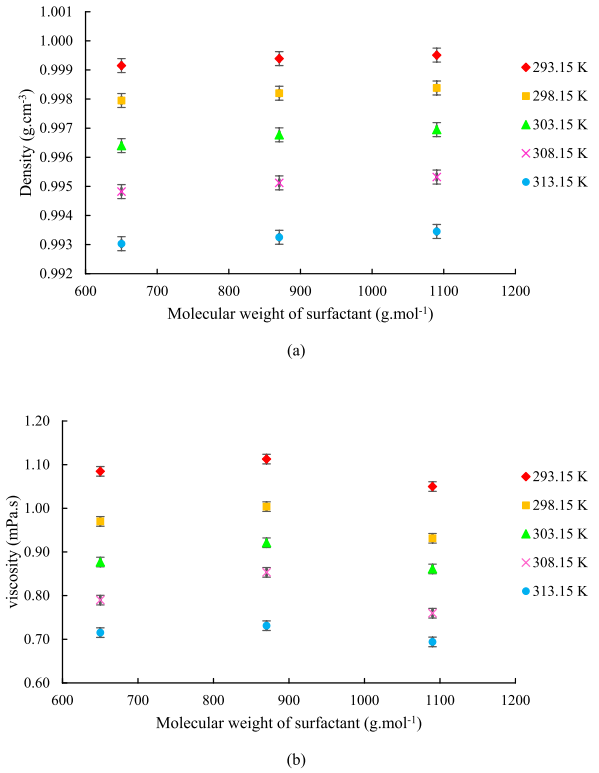


Fig. 4. Measured density (a) and viscosity (b) data as a function of molecular weight at constant temperatures for cholesterol.

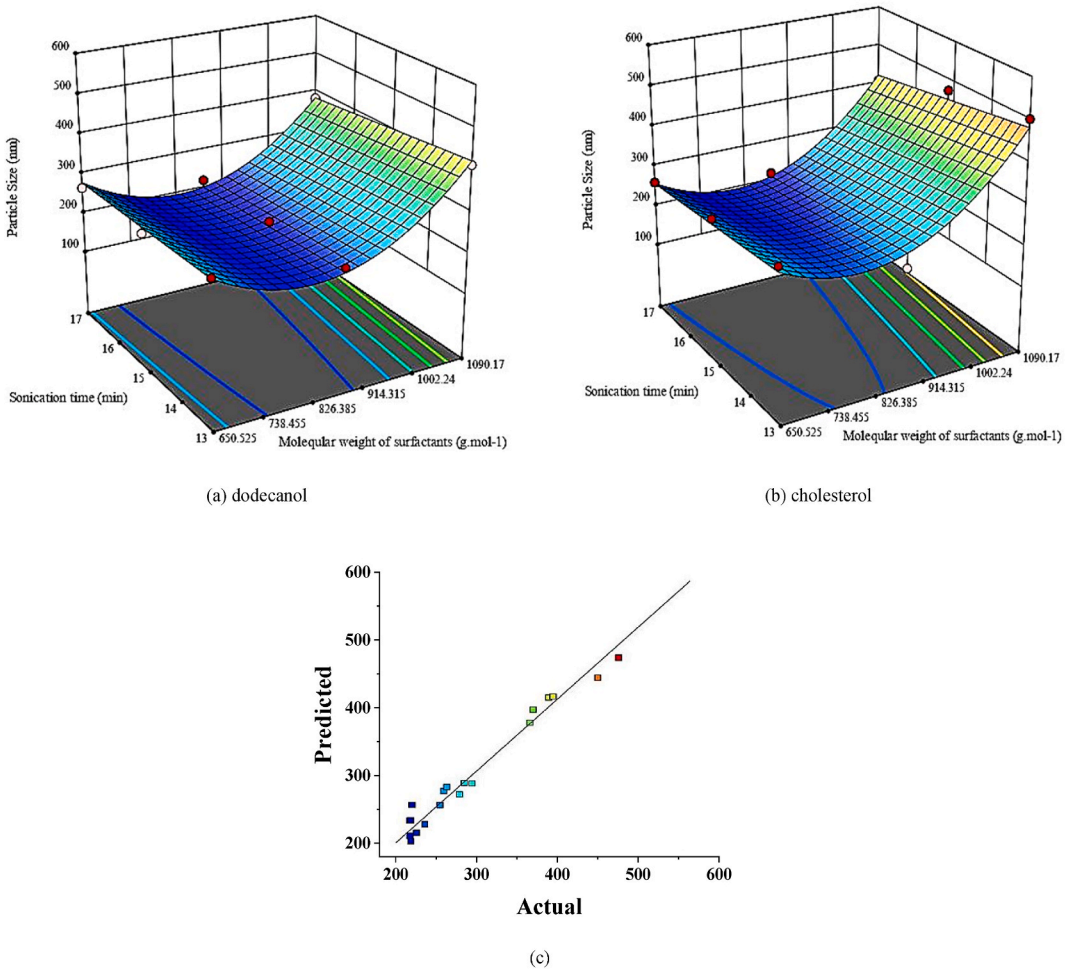


Fig. 5. Interactive effects of the sonication time (min) and molecular weight of the surfactant (g.mol^{-1}) for (a) dodecanol, and (b) cholesterol in the case of particle size (nm), as well as (c) the diagnosis of the model for the predicted values vs the actual values of particle size (nm).

dodecanol and Cholesterol, respectively by increasing the sonication time. These results are consistent with the above description.

3.1.3. Vesicles density and viscosity

The density and viscosity data of TRA-loaded niosomes were measured at five various temperatures. The temperatures range 293.15–313.15 K with 5 K intervals at the soniction time set as 17 min. The rationale for choosing this ultrasonic time is the confirmed stability outlined in the earlier section. Also, the vesicles density and viscosity were measured for different molecular weight of surfactants mixture changed between 650.525 and 1090.175 g mol^{-1}. The tests were performed for both dodecanol and cholesterol. Table 3 has summarized the density and viscosity data at various temperatures and molecular weights for both co-surfactants. Moreover, the standard uncertainty values of vesicles density are 1.20E-04 and 2.40E-04 (g cm^{-3}) for dodecanol and cholesterol, respectively. Also, the standard uncertainty values of vesicles viscosity are 9.00E-03 and 1.10E-02 (mPa s) for dodecanol and cholesterol, respectively. Likewise, Figs. 3 and 4 have illustrated the density and viscosity data as a function of molecular weight of surfactants at constant temperatures for dodecanol, and cholesterol, respectively along with related error bars.

Obviously, as the temperature increases, the density and viscosity of the vesicles decrease. Increasing the temperature, the volume increases, and as a result, the density of the mixture decreases [60]. In the case of viscosity reduction, since intermolecular forces and molecules adhesion decrease, the vesicles viscosity is reduced via temperature increment [61,62]. The solute-solvent or solvent–solvent interactions are a consequence of hydrogen bonds. So, they can be destroyed at higher temperatures by thermal motions [62].

It has also been observed, increasing the molecular weight of surfactants and HLB causes an increment of the vesicles density. Increasing the molecular weight leads to mass increment at a constant mol, which subsequently leads to density increment. Because of the same reason, the density of the niosomes containing cholesterol is more than dodecanol.

In addition, increasing the molecular weight of surfactant causes increment of the liquid mixture viscosity in the case of dodecanol. Furthermore, in the case of cholesterol, the viscosity increases with molecular weight increment, and it subsequently decreases. It

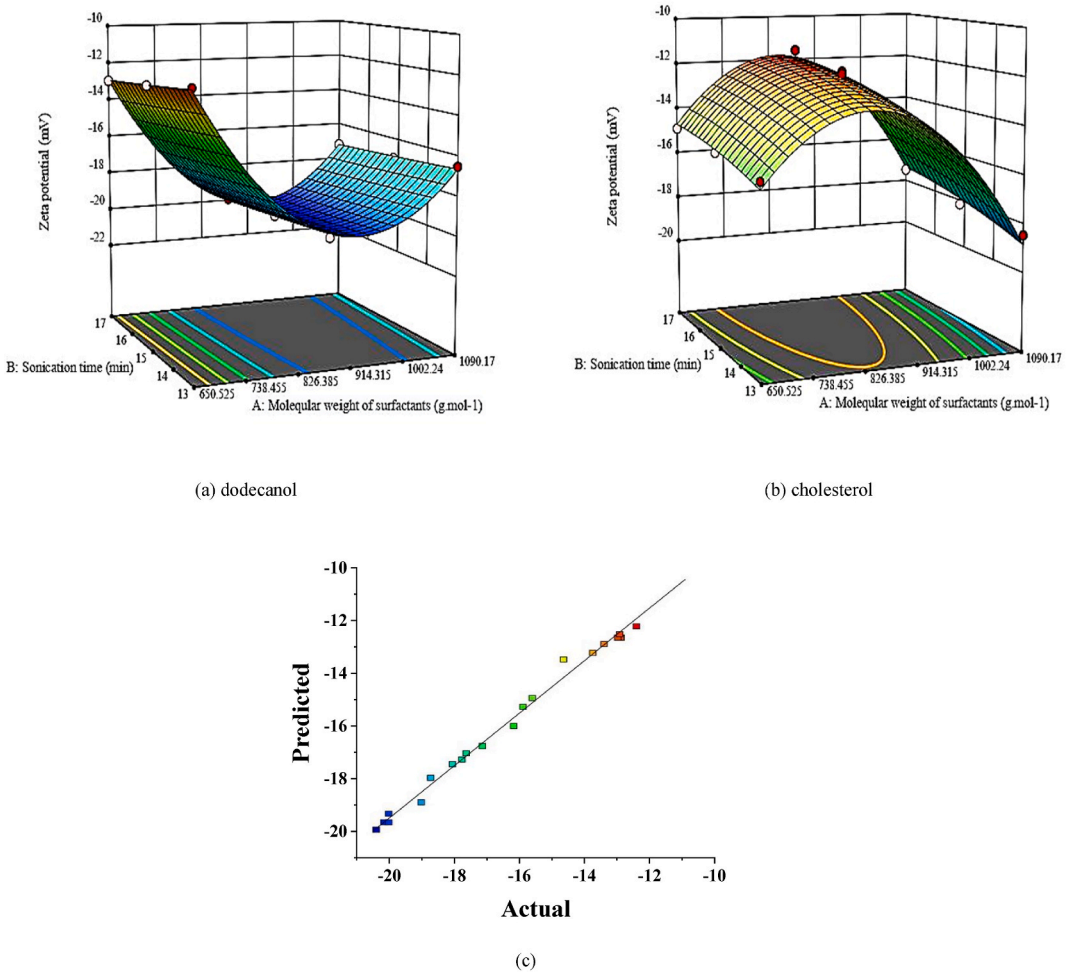


Fig. 6. Interactive effects of the sonication time (min) and molecular weight of the surfactant (g.mol^{-1}) for (a) dodecanol, and (b) cholesterol in the case of zeta potential, as well as (c) the diagnosis of the model for the predicted values vs the actual values of zeta potential (mV).

means that the viscosity of the niosomes, including cholesterol, has the highest value at the molecular weight of 870.35 g mol^{-1}. Actually, The average size, lipid content, and niosomes composition are the basic parameters affecting the niosomes viscosity [62].

Indeed, molecular weight increment is correspond with increasing the Tween 80 content, alkyl chain, and hydration of niosomes. So, in the case of dodecanol, the molecular weight increment leads to surfactant/lipid content that consequently causes the increment of the intermolecular forces, molecules adhesion, and viscosity. Also, the hydration increment can multiply the efficient volume fraction and viscosity [62]. When it comes to cholesterol, it is gussed that the hydration process and the solvent-vehicles interactions decrease at molecular weight of 1090.175 g mol^{-1} due to the nature of cholesterol and its more hydrophobicity in comparison with dodecanol.

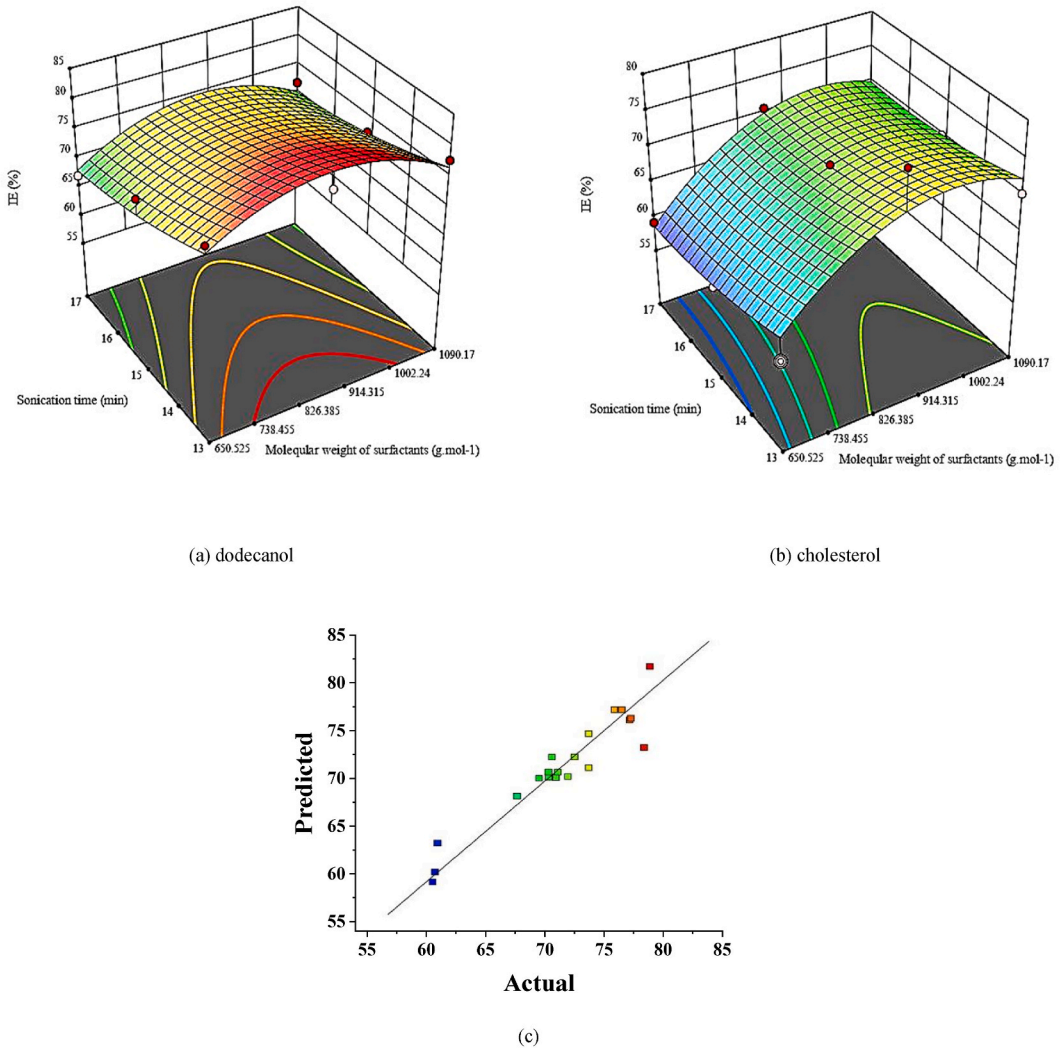
3.2. RSM optimization

As it mentioned above, the RSM was applied to estimate the optimal circumstances in the case of maximum IE %, the minimum particle size, and the lowest zeta potential. Two numerical parameters along with one categorical factor were used with three levels for experiments design. The numerical factors contain the molecular weight of surfactants (A) and sonication time (B). Moreover, the kind of co-surfactant is utilized as categorical factor (C). Likewise, average particle size, zeta potential, and IE % were selected as response of the RSM.

3.2.1. Particle size

According to Table 2, particle size of TRA-loaded niosomes has changed between 219.330 and 502.630 nm. Also, a quadratic model was used to optimize the solution of the RSM. In addition, the coded equation of average size by the RSM is as below:

$$\text{Particle Size (nm)} = 224.69 + 74.08A - 18.28B - 9.45C - 6.94AB - 14.99AC + 5.25BC + 128.97A^2 + 0.1575B^2 \quad (4)$$



(a) dodecanol

(b) cholesterol

(c)

Fig. 7. Interactive effects of the sonication time (min) and molecular weight of the surfactant (gr/mol) for (a) dodecanol, and (b) cholesterol in the case of IE %, as well as (c) the diagnosis of the model for the predicted values vs the actual values of IE %.

Additionally, Fig. 5 has indicated the interactive effects of the sonication time and molecular weight of the surfactant for (a) dodecanol, and (b) cholesterol in the case of particle size (nm). All the explained results in previous parts have illustrated here. For example, as the molecular weight of mixed surfactants increases, the mean particle size tends to a minimum value, and it consequently increases for both co-surfactants. Besides, as the sonication time increases, the mean particle size tends to a minimum value, for both co-surfactants. Moreover, Fig. 5 (c) has depicted the predicted particle size versus actual particle size. Obviously, the model can reproduced the experimental data as well.

3.2.2. Zeta potential

Unlike the particle size, a reduced cubic model was applied to evaluate the solution of the RSM. This model was selected because of its better convergence toward experimental data. Additionally, the zeta potential was coded as follow:

$$\text{Zeta Potential} = -15.98 - 1.79A + 0.1683B - 3.67C + 0.075AB - 0.3783AC - 0.4883BC + 0.3761A^2 - 0.0939B^2 - 0.025ABC + 4.53A^2C + 0.1146B^2C \quad (5)$$

In more detail, Fig. 6 has demonstrated the interactive effects of the sonication time and molecular weight of the surfactant for (a) dodecanol, and (b) cholesterol in the case of zeta potential (mV). All the explained results in previous parts have been approved here. For instance, zeta potential becomes more positive and more negative for cholesterol and dodecanol, respectively, and it changes its trend afterwards. In addition, at a conatant molecular weight, zeta potential has became more negative and positive for dodecanol and cholesterol, respectively by increasing the sonication time. Besides, Fig. 6 (c) has shown the predicted zeta potential vs. actual zeta

Table 4
The RSM solutions for optimizing the IE%, zeta potential, and particle sizes of TRA-loaded niosomes.

Number	M_S of Surfactants	Sonication Time	Kind of Co- Surfactant	IE	SE of IE	Particle Size	SE of Particle Size	Zeta Potential	SE of Zeta Potential	PDI	SE of PDI	Desirability
1	**916.171**	**13.973**	**dodecanol**	**79.480**	**1.226**	**240.642**	**10.026**	**−19.722**	**0.215**	**0.805**	**0.016**	**0.989**
2	913.848	13.981	dodecanol	79.481	1.225	239.363	10.021	−19.722	0.216	0.806	0.016	0.989
3	1084.272	13.000	cholestrol	72.472	1.969	473.392	16.104	−18.592	0.338	0.980	0.025	0.743
4	1082.522	13.000	cholestrol	72.524	1.959	470.637	16.018	−18.516	0.336	0.977	0.025	0.740
5	1084.380	13.036	cholestrol	72.410	1.944	473.006	15.901	−18.575	0.333	0.980	0.024	0.740
6	1084.579	13.100	cholestrol	72.301	1.903	472.342	15.565	−18.547	0.324	0.980	0.024	0.736
7	1088.193	13.953	cholestrol	71.037	1.629	465.025	13.324	−18.253	0.275	0.980	0.020	0.685
8	1090.175	14.456	cholestrol	70.476	1.611	460.469	13.174	−18.109	0.278	0.978	0.020	0.661
9	1090.175	14.659	cholestrol	70.312	1.608	457.380	13.153	−18.023	0.279	0.976	0.021	0.652
10	1090.175	14.820	cholestrol	70.196	1.608	454.926	13.148	−17.958	0.280	0.974	0.021	0.645

potential with good agreement. The color of the points can separate the various zeta potentials. For illustration, the red color is related to −13 to −12 mV.

3.2.3. Incorporation efficiency

Correspondingly, to optimize the RSM solution, a quadratic model was utilized for IE percent. Moreover, the coded equation of IE % is as follow:

$$IE\ \% = 74.07 + 2.90A\ - 2.67B + 3.42C + 0.4888AB - 2.31AC - 1.02BC\ - 5.77A^2 + 1.05B^2 \quad (6)$$

Besides, Fig. 7 has indicated the interactive effects of the sonication time and molecular weight of the surfactant for (a) dodecanol, and (b) cholesterol in the case of IE %. Clearly, as the sonication time increases, the IE % tends to a minimum value, for both co-surfactants. Furthermore, increasing the molecular weight at a constant sonication time, the IE percent has been augmented up to a maximum value, and it decreased subsequently for both co-surfactants. Also, Fig. 7 (c) has shown the predicted IE percent vs. actual IE percent in greater detail.

3.2.4. RSM solution

After optimization process, the RSM has offered some solutions for maximizing the IE % and minimizing the zeta potential. Also, it has been suggested that the particle size be in range. The ten better RSM solutions have been presented in Table 4. Molecular weight of 916.171 g mol^{-1}, sonication time of 13.973 min, and dodecanol have been optimized as the best solution.

4. Conclusion

The study was carried out to introduce fresh thermophysical findings on tretinoin-containing niosomes combined with span 60 and tween 80. Measurements were performed to analyze the density and viscosity of the liquid mixture in the mentioned multilayered structures for the first time, considering the diverse molecular weights of surfactants and various stabilizers at different temperatures. By employing equations of state, this investigation possesses the capability to establish the foundation for thermodynamic modeling of solutions containing niosomes, thereby offering a potential avenue for future research. The preparation of tretinoin-loaded formulations involved a study on the influence of different co-surfactants, like cholesterol or dodecanol, and the restriction of surfactant molecular weight within the range of 650.525–1090.175 g mol^{-1}. In order to determine the superior stabilizing abilities, this novel research aimed to compare dodecanol with cholesterol. The study specifically emphasized the optimization of vesicle size, the achievement of maximum incorporation efficiency, and the reduction of zeta potential. The RSM was employed to optimize the operational variables and determine the optimal number of experiments, focusing on specific details. The experimental evidence unambiguously demonstrates that the use of dodecanol during the manufacturing process greatly improves the stability of niosomes. In contrast, the inclusion of cholesterol in the prepared niosomes leads to higher density and viscosity of the liquid mixture.

Statements and declarations

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CRediT authorship contribution statement

Azam Shadloo: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kiana Peyvandi:** Writing – review & editing, Validation, Project administration. **Abolfazl Shojaeian:** Supervision. **Sheida Shariat:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

In this part of the document, the methodology for determining the molar mass of the surfactant's mixture and their HLB will be outlined.

Consider a formulation that includes the surfactants Span 60 and Tween 80. In this formulation, the molar fraction of Tween 80 is 0.25, while the molar fraction of Span 60 is 0.75. Given that the molar mass of Span 60 is 430.62 g mol^{-1} and that of Tween 80 is 1310

g mol^{-1}, the following calculations can be derived.

$$M_{mix} = \sum x_i M_i = (0.25 \times 1310) + (0.75 \times 430.7) = 327.5 + 323.025 = 650.525 \ \frac{gr}{mol} \quad \text{(A1)}$$

here, M shows the molecular weight of surfactant. Moreover, x_i represents the mole fraction of surfactant i. Index mix displays the mixture.

Similarly, the HLB for this mixture is determined using the following method. It is important to highlight that the HLB values for Span 60 and Tween 80 are 4.7 and 15, respectively.

$$HLB_{mix} = \sum x_i HLB_i = (0.25 \times 15) + (0.75 \times 4.7) = 3.75 + 3.525 = 7.272 \ \frac{gr}{mol} \quad \text{(A2)}$$

The calculations for all the numbers displayed in Table 1 have been conducted using this method.

List of symbols	
M_s	Molecular weight of sursactant (g.mol^{-1})
ε	Molar Absorptivity (ml.cm.μg^{-1})
b	Optical Path Length (1 cm)
C	Concentration ($\mu g.ml^{-1}$)
A	Absorbance
Abbreviations	
IE	Incorporation Efficiency
PDI	Polydispersity Index
HR-SEM	High Resolution Scanning Electron Microscopy
TRA	Tretinoin
RSM	Response Surface Methodology
CCD	Central Composite Design
Subscripts	
w	Water
s	Surfactant
mix	Mixture
i	Component i

References

[1] Jafar Akbari, Majid Saeedi, Reza Enayatifard, Katayoun Morteza-Semnani, Seyyed Mohammad Hassan Hashemi, Amirhossein Babaei, Seyyed Mobin Rahimnia, Seyyed Sohrab Rostamkalaei, Nokhodchi Ali, Curcumin Niosomes (curcusomes) as an alternative to conventional vehicles: a potential for efficient dermal delivery, J. Drug Deliv. Sci. Technol. 60 (2020) 102035.

[2] A.M. Alenzi, S.A. Albalawi, S.G. Alghamdi, R.F. Albalawi, H.S. Albalawi, M. Qushawy, Review on different vesicular drug delivery systems (VDDSs) and their applications, Recent Pat. Nanotechnol. 17 (1) (2023) 18–32.

[3] Pranav Shah, Rinkal Jariwala, Swinal Kapadiya, Vidya P. Sabale, Priya Patel, Pallavi M. Chaudhari, Niosomes: a novel Nanometric vesicular system for drug delivery, in: Nanocarriers: Drug Delivery System, Springer, Singapore, 2021, pp. 201–226.

[4] C. Richard, S. Cassel, M. Blanzat, Vesicular systems for dermal and transdermal drug delivery, RSC Adv. 11 (1) (2021) 442–451.

[5] P. Ghasemiyeh, F. Moradishooli, S. Daneshamouz, R. Heidari, U. Niroumand, S. Mohammadi-Samani, Optimization, characterization, and follicular targeting assessment of tretinoin and bicalutamide loaded niosomes, Sci. Rep. 13 (1) (2023) 20023.

[6] M. Moghtaderi, K. Sedaghatnia, M. Bourbour, M. Fatemizadeh, Z. Salehi Moghaddam, F. Hejabi, F. Heidari, S. Quazi, B. Farasati Far, Niosomes: a novel targeted drug delivery system for cancer, Med. Oncol. 39 (12) (2022) 240.

[7] A. Kauslya, P.D. Borawake, J.V. Shinde, R.S. Chavan, Niosomes: a novel carrier drug delivery system, J. Drug Deliv. Therapeut. 11 (1) (2021) 162–170.

[[8]] M.G. Umbarkar, Niosome as a novel pharmaceutical drug delivery: a brief review highlighting formulation, types, composition and application, Indian Journal of Pharmaceutical Education & Research 2 (2021) 55.

[9] Aryana Radmard, Majid Saeedi, Katayoun Morteza-Semnani, Seyyed Mohammad Hassan Hashemi, Nokhodchi Ali, An eco-friendly and green formulation in lipid nanotechnology for delivery of a hydrophilic agent to the skin in the treatment and management of hyperpigmentation complaints: arbutin niosome (Arbusome), Colloids Surf. B Biointerfaces 201 (2021) 111616.

[10] B. Kheilnezhad, A. Hadjizadeh, Factors affecting the penetration of niosome into the skin, their laboratory measurements and dependency to the niosome composition: a review, Curr. Drug Deliv. 18 (5) (2021) 555–569.

[11] Maria Chiara Cristiano, Francesca Froiio, Antonia Mancuso, Michelangelo Iannone, Massimo Fresta, Serena Fiorito, Christian Celia, Donatella Paolino, In vitro and in vivo trans-epidermal water loss evaluation following topical drug delivery systems application for pharmaceutical analysis, J. Pharmaceut. Biomed. Anal. 186 (2020) 113295.

[12] María Matos, Daniel Pando, Gemma Gutiérrez, Nanoencapsulation of food ingredients by niosomes, in: Lipid-based Nanostructures for Food Encapsulation Purposes, Academic Press, 2019, pp. 447–481.

[13] G. Gutiérrez, M. Matos, P. Barrero, D. Pando, O. Iglesias, C. Pazos, Iron-entrapped niosomes and their potential application for yogurt fortification, Lebensm. Wiss. Technol. 74 (2016) 550–556. .

[14] Noelia D. Machado, Pablo García-Manrique, Mariana A. Fernández, Maria Carmen Blanco-López, María Matos, Gemma Gutiérrez, Cholesterol free niosome production by microfluidics: comparative with other conventional methods, Chem. Eng. Res. Des. 162 (2020) 162–171.

[15] A. Poustforoosh, S. Farmarz, M.H. Nematollahi, H. Hashemipour, A. Pardakhty, Construction of Bio-conjugated nano-vesicles using non-ionic surfactants for targeted drug delivery: a computational supported experimental study, J. Mol. Liq. 367 (2022) 120588.

[16] Shobharaj Malavi, Popat Kumbhar, Arehalli Manjappa, John Disouza, Jayesh Dwivedi, Emulgel for improved topical delivery of Tretinoin: formulation design and characterization, in: Annales Pharmaceutiques Françaises, vol. 80, Elsevier Masson, 2022, pp. 157–168, 2.

[17] Saba Khoshbakht, Farzin Asghari-Sana, Anahita Fathi-Azarbayjani, Yaeghob Sharifi, Fabrication and characterization of tretinoin-loaded nanofiber for topical skin delivery, Biomater. Res. 24 (2020) 1–7.
[18] A. Gupta, S. Singh, N.G. Kotla, T.J. Webster, Formulation and evaluation of a topical niosomal gel containing a combination of benzoyl peroxide and tretinoin for antiacne activity [retraction], Int. J. Nanomed. 16 (2021) 4195–4196.
[19] A. Waheed, A. Ahad, D.K. Gupta, M. Aqil, F.I. Al-Jenoobi, A.M. Al-Mohizea, Nanovesicles for the Treatment of Skin Disorders, In Applications of Nanovesicular Drug Delivery Academic Press, 2022, pp. 285–302.
[20] S.S. Omar, H. Hadi, Advancement of all-trans retinoic acid delivery systems in dermatological application, Cosmetics 9 (6) (2022) 140.
[21] Aniseh Samadi, Zohre Sartipi, Saman Ahmad Nasrollahi, Behjat Sheikholeslami, Mansour Nassiri Kashani, Mohammad Reza Rouini, Rassoul Dinarvand, Alireza Firooz, Efficacy assessments of tretinoin -loaded nano lipid carriers in acne vulgaris: a double blind, split-face randomized clinical study, Arch. Dermatol. Res. (2021) 1–9.
[22] Mahsa Sabouri, Aniseh Samadi, Saman Ahmad Nasrollahi, Effat Sadat Farboud, Bahareh Mirrahimi, Hournaz Hassanzadeh, Mansour Nassiri Kashani, Rassoul Dinarvand, Alireza Firooz, Tretinoin loaded nanoemulsion for acne vulgaris: fabrication, physicochemical and clinical efficacy assessments, Skin Pharmacol. Physiol. 31 (2018) 316–323.
[23] D. Milosheska, R. Roškar, Use of retinoids in topical antiaging treatments: "a focused review of clinical evidence for conventional and nanoformulations.", Adv. Ther. 39 (12) (2022) 5351–5375.
[24] J.H. Saurat, O. Sorg, Retinoids. InEuropean Handbook of Dermatological Treatments, Springer International Publishing, Cham, 2023, pp. 1741–1761.
[25] David J. Elbaum, Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne, J. Am. Acad. Dermatol. 19 (1988) 486–491.
[26] Paul A. Lehman, John T. Slattery, Thomas J. Franz, Percutaneous absorption of retinoids: influence of vehicle, light exposure, and dose, J. Invest. Dermatol. 91 (1988) 56–61.
[27] Maria Manconi, Bianca Maria Baroli, Anna Maria Fadda, C. Sinico, D. Valenti, Liposomes and niosomes for the photoprotection of tretinoin, in: 26th International Symposium on Controlled Release of Bioactive Materials, 1999.
[28] Maria Manconi, Chiara Sinico, Donatella Valenti, Giuseppe Loy, Anna M. Fadda, Niosomes as carriers for tretinoin . I. Preparation and properties, Int. J. Pharm. 234 (2002) 237–248.
[29] Maria Manconi, Donatella Valenti, Chiara Sinico, Francesco Lai, Giuseppe Loy, Anna M. Fadda, Niosomes as carriers for tretinoin : II. Influence of vesicular incorporation on tretinoin photostability, Int. J. Pharm. 260 (2003) 261–272.
[30] Maria Manconi, Chiara Sinico, Donatella Valenti, Francesco Lai, Anna M. Fadda, Niosomes as carriers for tretinoin : III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin, Int. J. Pharm. 311 (2006) 11–19.
[31] G. Ioele, E. Cione, A. Risoli, G. Genchi, G. Ragno, Accelerated photostability study of tretinoin and isotretinoin in liposome formulations, Int. J. Pharm. 293 (2005) 251–260.
[32] Salwa Abdel Rahman, Nevine Shawky Abdelmalak, Alia Badawi, Tahany Elbayoumy, Nermeen Sabry, Amany El Ramly, Tretinoin -loaded liposomal formulations: from lab to comparative clinical study in acne patients, Drug Deliv. 23 (2016) 1184–1193.
[33] Salwa Abdel Rahman, Nevine Shawky Abdelmalak, Alia Badawi, Tahany Elbayoumy, Nermeen Sabry, Amany El Ramly, Formulation of tretinoin-loaded topical proniosomes for treatment of acne: in-vitro characterization, skin irritation test and comparative clinical study, Drug Deliv. 22 (2015) 731–739.
[34] Andreia Ascenso, Mariana Cruz, Carla Euletério, Filomena A. Carvalho, Nuno C. Santos, Helena C. Marques, Sandra Simoes, Novel tretinoin formulations: a drug-in-cyclodextrin-in-liposome approach, J. Liposome Res. 23 (2013) 211–219.
[35] F. Nigro, C.D. Cerqueira Pinto, E.P. dos Santos, C.R. Mansur, Niosome-based hydrogel as a potential drug delivery system for topical and transdermal applications, International Journal of Polymeric Materials and Polymeric Biomaterials 71 (6) (2022) 444–461.
[36] W. Zhang, X. Zhao, G. Yu, M. Suo, Optimization of propofol loaded niosomal gel for transdermal delivery, Journal of Biomaterials Science 32 (7) (2021) 858–873. *Polymer Edition*.
[37] Soumasree Chatterjee, Atul Kumar, Srabanti Basu, Susmita Dutta, Application of response surface methodology for methylene blue dye removal from aqueous solution using low cost adsorbent, Chem. Eng. J. 181 (2012) 289–299.
[38] Anna Witek-Krowiak, Katarzyna Chojnacka, Daria Podstawczyk, Anna Dawiec, Karol Pokomeda, Application of response surface methodology and artificial neural network methods in modelling and optimization of biosorption process, Bioresour. Technol. 160 (2014) 150–160.
[39] Willis A. Jensen, Response surface methodology: process and product optimization using designed experiments, J. Qual. Technol. 49 (2017) 186.
[40] Alec D. Bangham, Malcolm M. Standish, Jeff C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 13 (1965), 238-IN27.
[41] Ahmed A. Aboelwafa, Ahmed El-Setouhy Doaa, Aliaa Nabil Elmeshad, Comparative study on the effects of some polyoxyethylene alkyl ether and sorbitan fatty acid ester surfactants on the performance of transdermal carvedilol proniosomal gel using experimental design, AAPS PharmSciTech 11 (2010) 1591–1602.
[42] Ankush Gupta, Monica Gulati, Narendra Kumar Pandey, A validated UV spectrophotometric method for simultaneous estimation of tretinoin and benzoyl peroxide in bulk and semisolid dosage form, Rasayan J Chem 2 (2009) 649–654.
[43] Johann Heinrich Lambert, Photometria sive de mensura et gradibus luminis, colorum et umbrae, sumptibus vidvae, E. Klett, typis CP Detleffsen, 1760.
[44] Beer, August, and P. Beer. "Determination of the absorption of red light in colored liquids." Annalen der Physik und Chemie. 86 (1852) 78-88. .
[45] M.M. El-Sayed, A.K. Hussein, H.A. Sarhan, H.F. Mansour, Flurbiprofen-loaded niosomes-in-gel system improves the ocular bioavailability of flurbiprofen in the aqueous humor, Drug Dev. Ind. Pharm. 43 (2017) 902–910. .
[46] R. Raychaudhuri, A. Pandey, A. Hegde, S.M. Abdul Fayaz, D.K. Chellappan, K. Dua, S. Mutalik, Factors affecting the morphology of some organic and inorganic nanostructures for drug delivery: characterization, modifications, and toxicological perspectives, Expet Opin. Drug Deliv. 17 (2020) 1737–1765. .
[47] Lorena Tavano, Pasquale Alfano, Rita Muzzalupo, Bruno de Cindio, Niosomes vs microemulsions: new carriers for topical delivery of capsaicin, Colloids Surf. B Biointerfaces 87 (2011) 333–339.
[48] Mahmoud Elsayed, Saleh Abd El Rasoul, Amal K. Hussein, Response surface methodology as a useful tool for development and optimization of sustained release ketorolac tromethamine niosomal organogels, Journal of Pharmaceutical Innovation 15 (2020) 664–677.
[49] F. Nowroozi, A. Almasi, J. Javidi, A. Haeri, S. Dadashzadeh, Effect of surfactant type, cholesterol content and various downsizing methods on the particle size of niosomes, Iran. J. Pharm. Res. (IJPR): Int. J. Psychol. Res. 17 (2018) 1.
[50] B. Kheilnezhad, A. Hadjizadeh, Factors affecting the penetration of niosome into the skin, their laboratory measurements and dependency to the niosome composition: a review, Curr. Drug Deliv. 18 (2021) 555–569.
[51] D.N. Ala'a, N.A. Rajab, Formulation and characterization of niosomes for controlled delivery of tolmetin, J. Pharm. Negat. Results 13 (2022) 159–169.
[52] C. Laslau, D. Gologan, C. Ott, B. Balanuca, R. Stan, Niosomes loaded with ascorbic acid—influence of surfactants on stability and morphology, UPB Sci. Bull. Ser. B Chem. Mater. Sci 82 (2020) 101–112. .
[53] M. Yaghoobian, A. Haeri, N. Bolourchian, S. Shahhosseni, S. Dadashzadeh, The impact of surfactant composition and surface charge of niosomes on the oral absorption of repaglinide as a BCS II model drug, Int. J. Nanomed. (2020) 8767–8781. .
[54] R. Sharma, J.S. Dua, D.N. Parsad, An overview on Niosomes: novel Pharmaceutical drug delivery system, J. Drug Deliv. Therapeut. 12 (2022) 171–177. .
[55] Abbas Pardakhty, Jaleh Varshosaz, Abdolhossein Rouholamini, In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin, Int. J. Pharm. 328 (2007) 130–141.
[56] Ghada Abdelbary, Nashwa El-Gendy, Niosome-encapsulated gentamicin for ophthalmic controlled delivery, AAPS PharmSciTech 9 (2008) 740–747.
[57] Zerrin Sezgin Bayindir, Nilufer Yuksel, Characterization of niosomes prepared with various nonionic surfactants for paclitaxel oral delivery, J. Pharmaceut. Sci. 99 (2010) 2049–2060.
[58] P. García-Manrique, N.D. Machado, M.A. Fernández, M.C. Blanco-López, M. Matos, G. Gutiérrez, Effect of drug molecular weight on niosomes size and encapsulation efficiency, Colloids Surf. B Biointerfaces 186 (2020) 110711. .
[59] Ebtessam A. Essa, Effect of formulation and processing variables on the particle size of sorbitan monopalmitate niosomes, Asian J. Pharm.: Free full text articles from Asian J Pharm. 4 (2014).

[60] Ehsan Mohajeri, Gholamreza Dehghan Noudeh, Effect of temperature on the critical micelle concentration and micellization thermodynamic of nonionic surfactants: polyoxyethylene sorbitan fatty acid esters, E-Journal of Chemistry 9 (2012) 2268–2274.
[61] Zhou Mingzheng, Guodong Xia, Li Jian, Chai Lei, Zhou Lijun, Analysis of factors influencing thermal conductivity and viscosity in different kinds of surfactant solutions, Exp. Therm. Fluid Sci. 36 (2012) 22–29.
[62] Hamdy Abdelkader, Zimei Wu, Raida Al-Kassas, Raid G. Alany, Niosomes and discomes for ocular delivery of naltrexone hydrochloride: morphological, rheological, spreading properties and photo-protective effects, Int. J. Pharm. 433 (2012) 142–148.

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