




Original Article



Green Formulation of Menadione-Loaded Niosome as a Skin-Lightening Preparation: *In Vitro/In Vivo* Safety Evaluation on Wistar Rat

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Article info

Article History:

Received: January 24, 2024

Revised: August 2, 2024

Accepted: September 8, 2024

Published: September 15, 2024

Keywords:

Green technique, Anti-melanogenesis, Menadione, Niosome, Cosmetic

Abstract

Purpose: In the present research, a green technique (an ultrasonic method) was used to synthesize menadione sodium bisulfite (MSB) niosome (Menasome) which is used to improve dermal delivery and increase anti-melanogenesis activities.

Methods: Various cholesterol: surfactant (Chol: Sur) ratios were investigated to optimize the Menasomes. Photon correlation spectroscopy, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), transmission electron microscopy (TEM), and differential scanning calorimetry (DSC) were employed to characterize the solid state of MSB in nanoparticle form. Additionally, the optimized formulation was used to investigate ex-vivo skin absorption, *in vivo* skin irritation, *in vitro* cell survival, and anti-melanogenesis activity.

Results: The results exhibited that increasing cholesterol declined the average size of the Menasomes from 653.766 ± 25.171 nm to 298.133 ± 8.823 nm and increased entrapment efficiency $30.237 \pm 3.4204\%$ to $83.616 \pm 2.550\%$. The rat skin permeation study indicated that Menasome gel administered more MSB in dermal layers (439.000 ± 36.190 $\mu\text{g}/\text{cm}^2$ or $23.827 \pm 1.964\%$) than MSB plain gel (286.200 ± 22.6 $\mu\text{g}/\text{cm}^2$ or $15.53 \pm 1.227\%$). In both the *in vivo* skin irritation test and the *in vitro* cytotoxicity experiment, the extended-release behavior of the enhanced Menasome demonstrated a minimal side effect profile. Furthermore, optimum Menasome inhibited melanin formation ($37.426 \pm 1.644\%$ at $15\mu\text{M}$) greater than free MSB ($57.383 \pm 1.654\%$) considerably ($P < 0.05$). Furthermore, Menasome 7 prevented L-dopa auto-oxidation in higher levels ($95.140 \pm 2.439\%$) than pure MSB solution ($83.953 \pm 1.629\%$).

Conclusion: According to the study's findings, the prepared Menasome could be employed as a viable nanovehicle for MSB dermal delivery, a promising solution for the management of human hyperpigmentation disorders.

Introduction

Frequently, cutaneous melanin is regarded as a widespread class of biological pigments.¹ The complex chemical mechanism involving multiple enzymes causes the melanin synthesis stage in epidermal melanocytes.² The primary enzyme involved in the synthesis of melanin is tyrosinase, also known as monophenol or o-diphenol oxygen oxidoreductase. It is an enzyme that contains copper.³

The most common skin diseases caused by abnormal melanocyte distribution, function, and structure are pigmentation conditions. For most populations, especially

women, the management of pigmentation problems has long been dissatisfying and difficult. A large proportion of middle-aged women frequently have aberrant facial pigmentation brought on by various endogenous and exogenous factors.⁴ Reduced skin pigmentation (hypopigmentation) and enhanced skin pigmentation (hyperpigmentation) are two kinds of pigmentation problems. These skin pigmentation disorders might have psycho-social impacts as well as cognitive, mental, and aesthetic challenges for the patients. Skin pigmentation disturbances are linked to common pigmentation diseases such as Melasma (Chloasma) and Ephelides (freckles).⁵

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Certain skin lighteners can inhibit the synthesis of melanin, which makes them potentially useful as a treatment for hyperpigmented complaints.⁶ Menadione sodium bisulfite (MSB) can prevent the production of melanin in a dose-dependent manner as a result of decreased tyrosinase activity.⁷ One of the products of menadione (vitamin K3), which has a high water solubility, is MSB.⁸

For hydrophilic drugs, like MSB, delivery systems development is crucial, but there are several problems in the way of hydrophilic and macromolecule drug delivery.² These obstacles include (a) unsuitable and low permeability skin penetration due to hydrophilic structure, (b) unsuitable half-life in the biological fluid, and (c) low bioavailability and hydrolytic degradation.⁹ To overcome this challenge, researchers have developed nano-formulations that encapsulate hydrophilic drugs.

Higher dosages of drugs could be successfully delivered through the skin using lipidic nanocarriers, which could release drugs at controlled rates.¹⁰ According to Manconi et al, niosomes are generally non-toxic and safe, have low production prices, and have sufficient stability over a prolonged duration. In 1975, niosomes were discovered and advanced for topical usage and after that, numerous fabrication industries have paid much attention to the technology of niosomes, containing the anti-aging Lancome product Niosome Plus.¹¹ According to Manconi et al, the skin depot reached via a liposomal platform yielded the lowest dermal concentration, whereas the niosomal formulation of tretinoin, manufactured utilizing the thin film hydration method, produced the highest dermal concentration.¹² In other research, To improve arbutin's cutaneous permeability, Span 60, a non-ionic surfactant, was used to create arbutin niosomes through thin film hydration.¹³ To increase arbutin's skin permeability, arbutin niosome (Arbusome) was made in a different study utilizing an ultrasonic technique and non-ionic surfactants (Tween and Span 20).¹⁴ In a previous study, it was discovered that the epidermal absorption capacity of ellagic acid niosomes produced by reverse phase evaporation was higher than that of ellagic acid solutions.¹⁵ To increase the skin's ability to absorb kojic acid, other research has produced different types of nanoparticles using alternate methods, for example, nanostructured lipid carriers and solid lipid nanoparticles.²

In comparison with other approaches (reverse phase evaporation and thin film hydration), the ultrasonic technique (as a green technique) has the advantage of not requiring the usage of an organic solvent.¹⁶ Furthermore, the background of alternative methods and the ultrasonic technique differ when considering the preparation of niosomes. The ultrasonic technique, also known as the high energy technique, creates niosomal structures by applying a high system energy input for a short period. High cavitation forces are produced by the ultrasonic approach, which effectively mixes the system.¹⁷

However, changes occur much more slowly during the multistep process of thin film hydration and reverse phase evaporation method.¹⁸

As far as we are aware, no study has been done to assess the MSB niosomal formulation employing an economical and environmentally friendly technique like ultrasonication as a carrier for topical administration. Therefore, as an eco-friendly and green alternative to conventional carriers for topical distribution, MSB-encapsulated niosomes (Menasome) were produced as cosmeceutical products (Menasome gel) utilizing ultrasonication. In order to achieve optimum skin penetration, the niosomal formulation can also be improved by changing the cholesterol to surfactant (Span 20 and Tween 20) ratio (Chol:Sur ratio). Furthermore, absence of investigation on the inhibitory impression of MSB niosome on the procedure of L-DOPA auto-oxidation and the quantification of melanin in the B16F10 cell line. Also, there is an absence of examination of cutaneous sensitivity produced by MSB-niosome on Wistar rats.

Materials and Methods

Materials

In this study, Tween 20 (Sharlua, Sharlab S.L., Spain), Span 20 (Samchun Pure Chemical Co., Ltd. Korea), Menadione sodium bisulfite (Sigma, Germany), and Cholesterol (Merck, Germany) were applied. Carbopol 941 was obtained from B-F Goodrich, USA.

Preparation of Menasome

An ultrasonic procedure was done to prepare the Menasome.^{14,16} Tween 20, Span 20, and Chol were combined in a crystal beaker using magnetic stirring at 70 °C (organic phase). In the aqueous medium, the temperature of MSB (1%)¹⁹ and water was raised to equal levels with the organic phase. A magnetic stirrer set to 600 rpm for 30 minutes with a hot plate was used to mix the two processes to create a pre-niosome. To create Menasome, the mixture was then rapidly frozen in an ice reservoir bath after being sonicated with a probing sonicator (Table 1). The RHLB value can be calculated using the following formula:

$$RHLB = f \times HLB \text{ (Tween 20)} + (1-f) \times HLB \text{ (Span20)} \quad (1)$$

Which *f* is a fraction of surfactant in a mixture of surfactants.

Menasome characterization

The average size of Menasome preparations and polydispersity index (PDI) was achieved at 25 °C using a Zetasizer Nano ZS system (Malvern Instruments Worcestershire, UK) and a dynamic light scattering (DLS) Mastersizer 2000 (Malvern Panalytical Technologies, UK). Laser Doppler electrophoresis was used to conclude the

Table 1. Component and physicochemical properties of investigated Menasome (Mean \pm SD, n=3)

Formulation	MSB (%)	Chol (%)	Span 20 (%)	Tween 20 (%)	Chol/Sur ratio	Water up to ml	Particle size nm	PDI	Zeta potential (mv)	EE (%)
Menasome 1	1	0	0.75	0.75	0:6	20	653.766 \pm 25.171	0.536 \pm 0.0136	-8.156 \pm 0.285	30.237 \pm 3.420
Menasome 2	1	0.25	0.75	0.75	1:6	20	516.300 \pm 12.522	0.513 \pm 0.006	-7.540 \pm 0.450	38.678 \pm 2.977
Menasome 3	1	0.5	0.75	0.75	2:6	20	470.600 \pm 15.070	0.493 \pm 0.010	-6.513 \pm 0.441	45.958 \pm 2.510
Menasome 4	1	0.75	0.75	0.75	3:6	20	427.766 \pm 13.136	0.4577 \pm 0.018	-5.740 \pm 0.557	43.477 \pm 2.537
Menasome 5	1	1	0.75	0.75	4:6	20	393.233 \pm 15.157	0.392 \pm 0.018	-5.520 \pm 0.501	65.420 \pm 3.300
Menasome 6	1	1.25	0.75	0.75	5:6	20	332.300 \pm 10.068	0.357 \pm 0.013	-4.746 \pm 0.363	73.3018 \pm 1.504
Menasome 7	1	1.5	0.75	0.75	6:6	20	298.133 \pm 8.823	0.3047 \pm 0.006	-3.056 \pm 0.797	83.616 \pm 2.5508

Abbreviations: Chol: Sur, cholesterol: surfactant; MSB, menadione sodium bisulfite ; PDI, polydispersity index; EE, Entrapment efficiency.

zeta potential of Menasomes.²⁰

Formulating of Menasome gel and MSB simple gel

To distribute 1.5% w/v of Carbopol 941, 1% w/w of Menasome was combined with 0.1% sodium benzoate-filtered preserved water, and the mixture was stored for 24 hours. The combination of carbopol in the Menasome dispersion was neutralized using 100 mg triethanolamine applying a propeller homogenizer that rotates at 400 rpm. MSB solution was combined using a propeller homogenizer operating at 400 rpm (1% w/w MSB) with 1.5% w/v Carbopol 941 to form an MSB simple gel.

Entrapment efficiency (EE %)

The prepared Menasomes were centrifuged (SIGMA; 3-30 KS: Germany) for 45 minutes at 18 000 rpm and then filtered with 0.22 m pore size to determine the EE%. After that, the amount of MSB in the filtrate (free drug) was measured using a UV spectrophotometer: JASCO V-630 (UV \pm Vis, UK) at 264 nm.

$$EE\% = ((W_{\text{initial}} - W_{\text{free}}) / W_{\text{initial}}) \quad (2)$$

Where Wfree indicates the total amount of material in the supernatant and Winitial indicates the amount of material added to the formulation.

Microscopic investigation

The morphology of the Menasome was examined using transition electron microscopy. After applying a gold coating, the sample was analyzed using a HITACHI S-4160 SEM (Japan) with a magnification of 15,000 and an acceleration voltage of 20.0 kV.

Differential scanning calorimetry (DSC) Assessment

DSC was used to examine the parts and Menasome powder (Pyris 6, PerkinElmer; USA). To create Menasome powder, the niosomes were separated from the dispersion using a centrifuge (3–30 ks Sigma centrifuge). The alpha 1-2 LDplus freeze drier was utilized to freeze-dry the separated niosomes at a lower pressure (Marin Christ, Osterode, Germany). MSB, cholesterol, and freeze-dried Menasome specimens totaling around 5 mg were quantified and placed

in aluminum pans. For thirty minutes, the covered pans were kept in an isothermal atmosphere at a temperature of 20 °C. Following equilibrium, the physical mixture, Menasome powder, and original drug components were subjected to a temperature of 25-300 °C at a heating rate of 20 °C/min in an environment of inert N₂ gas. The DSC spectra of these samples were then recorded. The DSC was calibrated with indium.²⁰

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) Spectroscopy

To examine the MSB–excipient interaction, a diamond ATR-equipped FTIR spectrophotometer Cary 630 (Agilent Technologies Inc., CA, USA) was used. MSB, Span 20, Tween 20, cholesterol, and Menasome (freeze-dried powder) were done for the ATR-FTIR analyses. The observed spectra ranged between 4000-400 cm⁻¹.²¹

Drug release test

Immersion cells with a cellulose acetate membrane (MWCO of 12 kDa) were used for the *in vitro* release study. The samples were positioned into the cells, and then the caps were placed on and an acetate cellulose membrane was placed over the cells. Within the USP Dissolution Apparatus II, the cells were installed.^{22,23} A dissolution medium volume of around 900 mL was produced for the release test at a speed of 100 rpm and 37.0 °C. At various time intervals (2, 4, 6, 8, and 24 hours), the dissolving medium (5 mL) was extracted and filtered using 0.22 micrometer filter paper. A UV light with a wavelength of 264 nm was used to determine the drug content of the samples. Following each sampling interval, pH 6.8 buffer phosphate (5 mL) was added to maintain a constant volume of the dissolving solution.

Ex-vivo dermal absorption investigations

The UK Animals (Scientific Procedures) Act 1986 and its related guidelines, the ARRIVE regulations, and EU Directive 2010/63/EU on animal research were all followed during the animal investigations. A mixture of 13 mg xylazine/kg 87 mg and ketamine/kg body weight was utilized to anesthetize male Wistar rats weighing 120-150 g, and electric razors were utilized to shave their abdomen

skin. Following a 48-hour time, the rats were put to sleep via breathing in chloroform, and surgically removed the skin of the abdomen. Before the diffusion investigations started, all subcutaneous lipids were cautiously eliminated, and for 24 hours at 4 °C, the skin was immersed in a saline solution. After that, the skin was put in Franz cells with a 3.8 cm² diffusional area to evaluate permeation. The removed skin was positioned with its dermis exposing the recipient fluids, situated between the donor and recipient compartments. After that, purified double-distilled water (pH 5.5 equivalent to normal skin pH) was placed within the diffusion cells, maintained at 32 ± 0.5 °C during the tests.^{24,25} Since the aim of this test is to examine the amount of medicine passing through the skin, the receptor should have the ability to dissolve the medicine completely. In other words, it should be able to maintain the sink condition according to USP pharmacopeia 43. For this reason, purified double-distilled water (pH 5.5) was chosen for the dissolution medium. The cell chamber was surrounded by a jacket that enabled water to circulate, and magnetic stirring rods were used to agitate the mixture at 150 rpm. 1 g of Menasome gel preparations including MSB (10 mg) and 1 g of simple MSB gel combinations, which contained the same value of MSB as the niosome formulations, were equally administered to the skin's trimmed dorsal portions in the donor chamber that were sealed off from the environment. At specific intervals (2, 4, 6, 8, 10, and 24 hours), the sample (5 mL) was taken out of the receiver medium and substituted with an equivalent amount of fresh water. A UV spectrophotometer with a 268 nm setting was applied to determine the amount of drug in the removed samples.

Skin MSB retention examinations

As previously stated, the skins were removed following the last stage of the permeation studies. Before determining the amounts of MSB deposited in the skin, they were washed with water 3 times followed by drying. Using scissors, the skin was sliced into tiny pieces. After being put into tubes, it was dissolved in water and subjected to an hour of sonication using a bath sonicator. The final solution was filtered using filter paper and a 0.22 µm syringe filter, and a UV spectrophotometer was used to quantify the concentration of the mixture at 264 nm.

Cell survival research

Human foreskin fibroblast (HFF) cells from the National Cell Bank (Pasteur Institute of Iran) were used to test the preparations' cell survival *in vitro*. Following that, a mass of 10⁵ cells were placed onto the Nunclon microplate base for a duration of 24 hours with different concentrations of MSB, niosome without medication, and Menasome (15, 10, 7.5, 5, 2.5, and 1 µM) or the carrier control. After the materials were removed, the cells were cleaned with PBS, and colorimetry formazan (MTT) was utilized to evaluate the cells' viability.²⁶ The cells were then incubated for

four hours at 37 °C after MTT (0.5 mg/mL) was added. Furthermore, after discarding the supernatant, the silt was dissolved in dimethyl sulfoxide (DMSO) (100µL) comprising formazan crystals. Following a 20-minute shake of the plates, the optical density at 560 nm was measured by applying a multi-walled spectrophotometer. Six more controls (the cells in the medium) were examined three times at various doses (15, 10, 7.5, 5, 2.5, and 1 µM) to evaluate the cell's viability. The vitality of the cells was measured as follows:

$$\% \text{Cell survival} = (\text{Abs Sample} - \text{Abs Blank}) / (\text{Abs Control} - \text{Abs Blank}) \quad (3)$$

L-DOPA auto-oxidation inhibitory test

Much of the previously stated calculation was used to determine the formulation and pure drug's preventive effect on L-DOPA auto-oxidation. To summarize, sodium phosphate buffer (0.1 M at pH 6.5) was prepared in 96-well plates, and different quantities of MSB and Menasome were prepared. A 96-well plate was filled with 300 µL of 10 mM L-DOPA and sodium phosphate buffer (0.1 M at pH 6.5). The blend was then incubated for 15 minutes at 37 °C. By measuring absorbance at 475 nm, activity was determined.¹⁹

Calculation of melanin amount of melanoma cells

Murine B16F10 melanoma cells were achieved at the Pasteur Institute.²⁷ Murine B16F10 melanoma cells were seeded in complete RPMI media supplemented (streptomycin (0.1 mg /mL), penicillin (100 units /mL), 10% fetal bovine serum (FBS; Gibco, USA)) in a 12-well plate overnight. Following a 24-hour incubation period, the cells were treated with MSB and Menasome. For thirty minutes at 100 °C, the cell pellets were dissolved in a 100 µL solution of NaOH (2 M). By measuring their absorptions at 405 nm, the melanin content of the samples was compared to the control group via a Microplate Reader (BioTek, Winooski, USA).

In vivo skin irritation investigations

The Institute Animal Ethical Board criteria were adhered to throughout the investigations. Wistar rats were shaved the day before the experiment to remove any hair from their dorsal regions. Group V was given a 0.8% (v/v) water solution of formalin as the standard irritant, Group I was the control, and Group IV received the free drug niosome. Menasome gel and MSB simple gel were given to groups II and III. For three days, Menasome gel, MSB simple gel, formalin solution, and a placebo were applied topically to the skin of rats. The affected area was examined visually throughout the time.^{19,28} This procedure is blinded for observers. After 72 hours, the sites of application were examined for edema and erythema and graded on a scale from zero to 4 based on visual assessment by the same blind investigator. The grading scale was as follows: 0 for

none, 1 for slight, 2 for well-defined, 3 for moderate, and 4 indicating scar or ulcer formation. Edema was graded similarly: 0 for none, 1 for slight, 2 for well-defined, 3 for moderate, and 4 for severe. Photographs were taken to document erythema observed at some application sites on rats. The primary irritancy index for each group was determined using a specific calculation formula.²⁹

$$\text{Primary irritancy index} = \text{Mean score of erythema} + \text{Mean score of edema} \quad (4)$$

The formulation was classified as non-irritant if (mean score < 2)

Statistical evaluation

The mean standard deviation was used to report the study's findings. GraphPad Prism 8 was utilized to examine the outcome. Statistical analysis was performed on the selected parameters using analysis of variance and the Tukey test LSD's post hoc test. It was discovered that the $P < 0.05$ has statistical significance ($n = 3$).

Results and Discussion

Characterization of Menasome

Niosomes composed of MSB were synthesized via ultrasonication. This technique used various ratios of Chol to a binary mixture of Tween 20 and Span 20, which is a nonionic surfactant (Chol:Sur 0:6, 1:6, 2:6, 3:6, 4:6, 5:6, 6:6 w/w). Table 1 lists the characteristics of the Menasome compositions. From 0:6 (Menasome 1) to 6:6 (Menasome 7), the Menasome's particle size decreased significantly from 653.766 ± 25.171 to 298.133 ± 8.823 nm ($P < 0.05$). It has been demonstrated that the presence of Chol enhances Menasome bilayer cohesion and rigidity.³⁰ Niosomes diminished in size as a result of this. Niosomes with more Chol may have a reduced radius, according to findings by Chaw and Kim³¹ and Akbari et al.³² Additionally, the probe's increased energy output may have contributed to the excessive heat generation that resulted in the decreased particle size. Furthermore, the characteristics of the MSB vesicles can be adjusted to reach an optimal value in terms of size by utilizing the dual combinations of surfactants (Tween 20 and Span 20), which control the amount of HLB.³³ Table 1 also displays each Menasome encapsulation efficiency (EE percent). Table 1 displays the range of percentages of MSB entrapped in Menasomes from 30.237 ± 3.420 to $83.616 \pm 2.550\%$. When the ratio of Chol to sur was raised to 6:6, the EE % increased due to the rise in bilayer rigidity and a decrease in drug leakage from the niosome membrane. It has been demonstrated that Chol influences EE % and membrane permeability, leading to less permeable niosomes.³⁴ Additionally, surfactants with low (Span 20 HLB value 8.6) and high (Tween 20 HLB 16.7 value HLB values could be dispersed in the oily and aqueous phases, respectively, therefore enhancing the physical stability at the interface of the

surfactant films. Also, for obtaining stable niosome and successful emulsified Chol, the ratio of surfactant (Span 20 and Tween 20) was considered constant (0.75%) to each other as mentioned in previous studies.^{14,32} In order to create a stable emulsion in water, it is important to have an HLB value above 10 (in this case 12.65) because this indicates that the HLB is more hydrophilic than lipophilic. Emulsifiers (Tween 20 with HLB 16.7) with an HLB value above 10 effectively stabilize oil-in-water emulsions, where water is the continuous phase and oil is the dispersed phase. These emulsifiers help to reduce the surface tension between the oil and water, thereby preventing the oil droplets from coalescing and ensuring a stable emulsion. In oil-in-water emulsions, using a hydrophilic emulsifier beyond a certain point becomes ineffective for high oil concentrations because these emulsifiers are primarily water-soluble. As the oil content increases, the hydrophilic emulsifier cannot sufficiently stabilize the larger oil droplets, as it tends to dissolve in the water phase. This imbalance leads to an inadequate reduction in the surface tension between oil and water, causing the emulsion to become unstable. Instead, a balance between hydrophilic and lipophilic properties often represented by an appropriate HLB value, is necessary to effectively stabilize the emulsion with higher oil content (in this case the required HLB is 12.65).³⁵

Additionally, Chol forms associations with the surfactant molecules, which alter their vesicular structures and physical properties through the control of bilayer cohesion and strength, ultimately leading to an increase in EE%.³⁴ Chol decreases the vesicles' gel-liquid transition temperature, making them less permeable to drug leakage and more stable, which can raise the EE%.³⁶ Mokhtar et al looked at how formulation factors, such as the amount of Chol, affected the entrapment of flurbiprofen-loaded niosomes.^{30,37} They discovered that when the ratio of Chol:Sur increased, the EE percent improved.³⁷

Table 1 shows that the higher concentrations of Chol decreased the surface charge of Menasome. For instance, the negative zeta potential decreased (-3.056 ± 0.797 mV) when the ratio of Chol:Sur was increased to 6:6, but it increased (-8.156 ± 0.285 mV) when the concentration of Chol was lower. Because of the electrostatic stabilization of the niosome, Menasome 1 with a maximum zeta potential of -8.156 ± 0.285 mV may be desirable since it offers high stability to the Menasome during storage and when combined with water. The distribution of free MSB in the aqueous state or the diffusion layer potential may be what causes the finding that the percentage of MSB entrapment reduced as the zeta potential of particles increased. These results are consistent with other studies.^{32,38} It was also noteworthy that the colloidal particles were surrounded by a negative zeta potential due to the usage of nonionic surfactants. This could be explained by the nonionic surfactants' ethoxy groups' dipole characteristics.³⁹

According to Taymouri and Varshosaz, the PDI value

normally ranged from 0 to 1, with values close to zero indicating a uniform and homogenous dispersal.⁴⁰ The PDI value ranged from 0.304 ± 0.006 to 0.536 ± 0.013 in Table 1. A wide distribution of particle size is usually indicated by PDI values greater than 0.7.⁴¹ Table 1 makes it clear that formulations with 1.5% of Chol had the lowest PDI value (0.304 ± 0.006). Conversely, Menasome 1 with the lowest level of Chol—showed the highest PDI value (0.536 ± 0.013). In addition to Chol concentration, other factors that affect the reduction in PDI value are surfactant type, surfactant HLB value, sonication power, and particle charge.^{18,21} Additionally, the Chol regularly decreases the radius and number of the bilayers as well as the chain order of gel state bilayers.⁴² Ultimately, the normalized distribution gets smaller due to the reduction in relation to the radius or number of bilayers, as per the thermodynamic scaling effect for soft self-assembling particles.⁴³

The absence of organic solvent requirements in the current niosome manufacturing process makes it a more desirable approach compared to other methods. The physical experience of traditional (such as thin-film hydration) and Ultrasonic methods is quite diverse when considering the construction of niosomes. The high-energy method known as the ultrasonic processor methodology produces niosomal structures by supplying a large amount of energy to the system during a short period. Higher cavitation forces produced by the ultrasonic processing method efficiently mix the material and produce smaller niosomes than the traditional approach. However, changes occur much more slowly throughout the multistep process of thin-film hydration. Niosomes are often made using traditional thin-film techniques, which frequently call for the addition of organic solvents at one or more stages throughout the procedure. Human health is at risk of solvents from these processes.¹⁸ Moreover, none of the studies examined the impact of the Chol: Sur w/w percent ratio—which is necessary to increase the efficiency of Menasomes. Particle size, PDI, zeta potential, and drug entrapment percentage of the ultrasonicated optimal Menasome 7 are 298.133 ± 8.823 nm, 0.304 ± 0.006 , -3.056 ± 0.797 mV, and $83.616 \pm 2.550\%$, respectively.

ATR-FTIR analysis

The chemical interaction between the drug and the excipients utilized to make the niosome was examined in ATR-FTIR spectroscopy. The ATR-FTIR spectra of MSB in pure form, Span 20, Tween 20, Chol, and Menasome 7 are shown in Figure 1. Specific peaks of MSB were detected at 1687 cm^{-1} (C=O, stretching), 1637 cm^{-1} (C=O, stretching), 1589 cm^{-1} (C-C, stretching), and 1050 cm^{-1} (S=O, stretching). Chol showed peaks at 1054 cm^{-1} (C-O stretching), 1463 cm^{-1} (C-H bending), $3000\text{--}2850\text{ cm}^{-1}$ (C-H stretching), and 3400 cm^{-1} (O-H stretching). Span 20 displays diagnostic peaks at 1738 cm^{-1} (C=O stretching), 2854 cm^{-1} (–CH– symmetric stretching)

2923 cm^{-1} (–CH– asymmetric stretching), and 3392 cm^{-1} (O–H stretching); and Tween 20 presented peaks at 1734 cm^{-1} (C=O stretching), 2860 cm^{-1} (–CH– symmetric stretching) 2920 cm^{-1} (–CH– asymmetric stretching), and 3488 cm^{-1} (O–H stretching). Figure 1 demonstrated that the ATR-FTIR spectra of Menasome 7 display specific peaks for MSB (1687 cm^{-1} (C=O, stretching), 1637 cm^{-1} (C=O, stretching) (is not considered as a distinctive peak), and 1589 cm^{-1} (C-C, stretching). Any chemical interactions between the excipient and the MSB were ruled out, as the ATR-FTIR showed.

DSC analysis

The DSC traces of pure MSB, Chol, and Menasome 7 are displayed in Figure 2. Thermogravimetric analysis using Chol and MSB indicated a single, strong endothermic peak at $150\text{ }^{\circ}\text{C}$, $122.5\text{ }^{\circ}\text{C}$, and exothermic $156.96\text{ }^{\circ}\text{C}$, respectively. These peaks showed their highly crystalline structure, fitting their melting temperatures. Also, glass transition temperature (T_g) Chol ($41\text{ }^{\circ}\text{C}$), MSB ($70\text{ }^{\circ}\text{C}$), and Menasome 7 ($41\text{ }^{\circ}\text{C}$) were shown in Figure 3.

MSB in the niosome is either molecularly dispersed within the niosome or in an amorphous state, according to the DSC thermograms of the niosome formulation under examination, which only showed the endothermic peak around the Chol melting point (Figure 2).

Transmission electron microscopy (TEM) analysis

These niosomes were spherical and distinct from each other, as demonstrated by the Menasome 7 TEM image

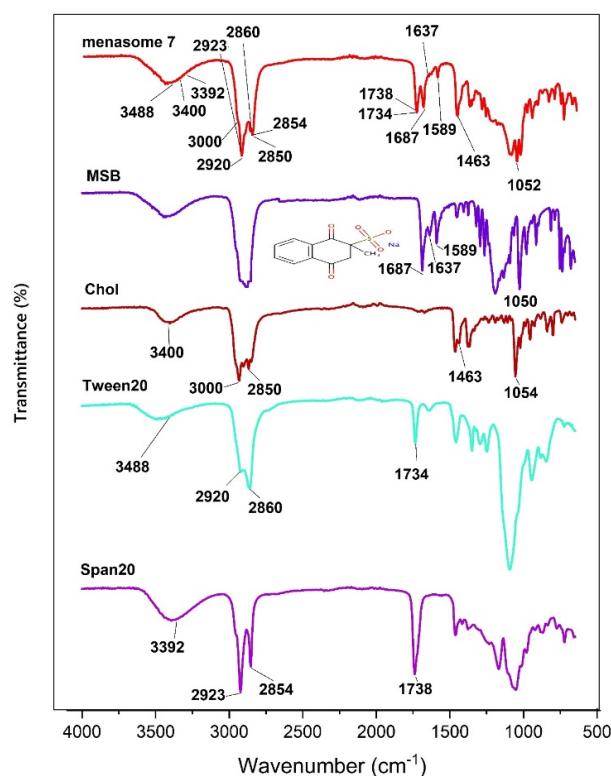


Figure 1. ATR-FTIR spectra of Menasome 7, MSB (menadione sodium bisulfite), Chol (cholesterol), Tween 20, and Span 20

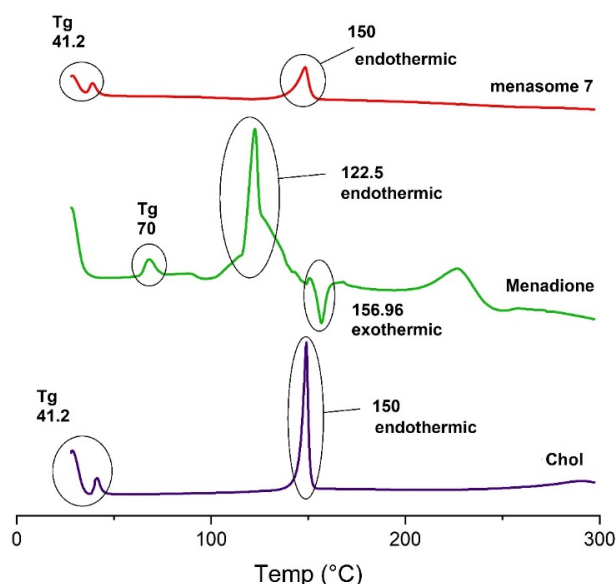


Figure 2. DSC traces of Menasome 7, MSB (menadione sodium bisulfite), and Chol (cholesterol)

(Figure 3). In addition, the micrograph showed that the Menasome's size is properly uniform, which is to be expected considering their morphological characteristics.

Drug release study

The release of a drug from a nanoparticle gel formulation is often studied over extended periods, such as 24 hours or more, to fully understand the release profile and ensure a comprehensive evaluation of the formulation's performance. Also, Regulatory agencies often require extended-release studies to ensure that the drug delivery system meets safety and efficacy standards over the intended duration of use, and these studies are also part of the quality control process to ensure consistency and reliability.⁴⁴

Adjusting the drug release from the niosome has been revealed to affect drug bioavailability.¹ The impact of simple gel and niosome formulation gel on the MSB release profile is depicted in Figure 4. The results indicated that the Menasome 7 gel released $14.920 \pm 2.789\%$ of the drug for up to 2 hours, and thereafter it displayed a continuous release profile for up to 24 hours. This was not the case for the MSB-containing simple gel, as over 94% of the drug was released in the first 8 hours.

The release profile of a hydrophilic drug from niosomes typically follows a biphasic pattern, which includes an initial burst release (release of drug after 8 hours is 41%) followed by a slower, sustained release (47% drug release after 24 hours). Extending the drug release study beyond 25 hours would allow for a comprehensive determination of the fate of the remaining drug in the niosome and gel matrix, potentially resulting in the complete release of the drug. It has been demonstrated that Chol can reduce drug mobility in niosomes relative to the use of simple gel, enabling a more extended and sustained drug release pattern. The drug release rate from niosomes is influenced

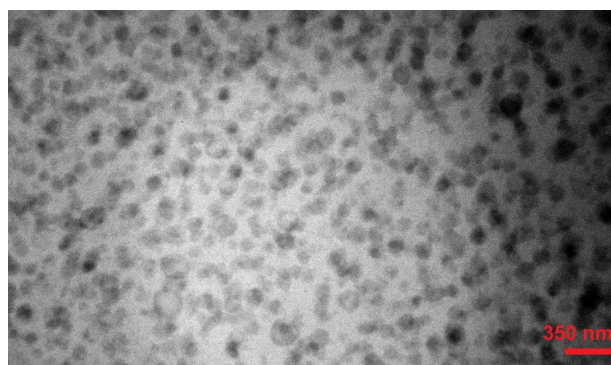


Figure 3. TEM image of Menasome 7 (optimum formulation)

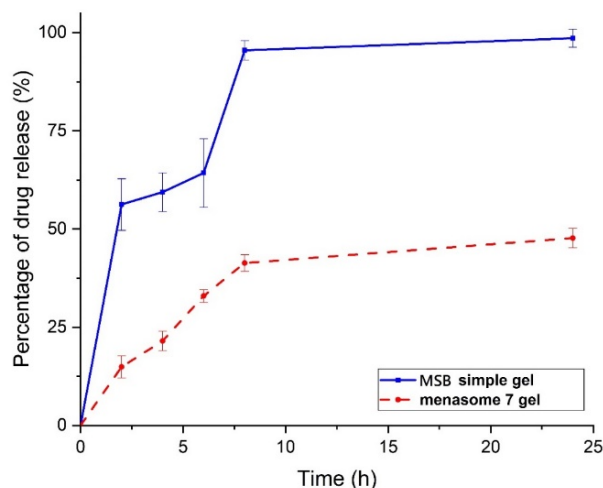


Figure 4. Profile of drug release from MSB (menadione sodium bisulfite) simple gel and Menasome 7 gel (mean \pm SD, n=3)

by several factors, but the most crucial ones include molecular size, matrix viscosity, high surface area, and high diffusion coefficient. If the drug is encapsulated in the niosome's inner core, it is delivered gradually and consistently.¹⁶ However, if the drug is placed onto the surface or outer shell, its release can occur extremely quickly.⁴⁵ Many efforts have been made to build niosome systems that can improve formulation effectiveness for improved drug delivery system parameters, as controlling drug release from niosomes is crucial.⁴⁶⁻⁴⁸ Saeedi et al have also provided information on the drug release from niosomes.⁴⁹

In vitro percutaneous absorption study

The cumulative amounts of MSB that permeated the rat skin (Figure 5, transdermal delivery) and the amount that infiltrated the skin layers (Figure 6, dermal delivery) for Menasome 7 gel and MSB simple gel are shown in Figures 5 and 6. When compared to MSB simple gel, the Menasome 7 gel (F8) showed a greater penetration across and into the epidermal layers, indicating that it was particularly suitable for transdermal delivery ($P < 0.05$). The highest MSB level identified in the compartment of the receptor for MSB simple gel ($15.53 \pm 1.227\%$ or $286.200 \pm 22.6 \mu\text{g}/\text{cm}^2$) was expressively lower than that of the Menasome 7 gel ($23.827 \pm 1.964\%$ or 439.000 ± 36.19

$\mu\text{g}/\text{cm}^2$) ($P < 0.05$). Also, the level of MSB that stayed in the cutaneous for Menasome 7 gel ($54.540 \pm 4.790\%$ or $1004.851 \pm 88.26514884 \mu\text{g}/\text{cm}^2$) was significantly larger than that for the MSB simple gel ($15.629 \pm 1.130\%$ or $287.948 \pm 15.727 \mu\text{g}/\text{cm}^2$) ($P < 0.05$).

According to Chakraborti et al, enhancing drug penetration from formulations also depends on the gelling qualities of carbopol polymers.⁵⁰ The niosome Chol concentration appeared to have an important impact on the skin's absorption of MSBs. According to Tavano et al, Chol can alter the persistence of membrane permeation, phase behavior, and fluidity which can improve drug absorption through the skin.³⁰ Topical permeability enhancers, such as Chol and its esters, are widely utilized.^{51,52} Research has demonstrated that niosome HLB value plays a significant role in determining drug cutaneous permeability.¹⁶ According to these results, a niosome formulation would be more effective than a conventional gel preparation for cutaneous and transdermal delivery.

MTT assay examination

Because of its ease of use, the MTT test is among the

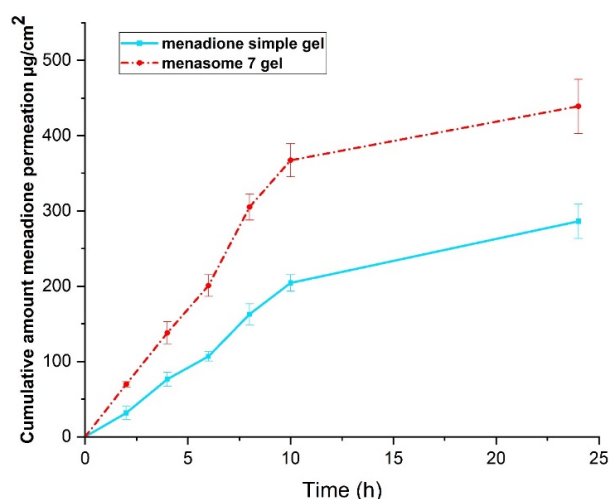


Figure 5. Cumulative MSB (menadione sodium bisulfite) amounts permeated across rat skin (mean \pm SD, $n=3$)

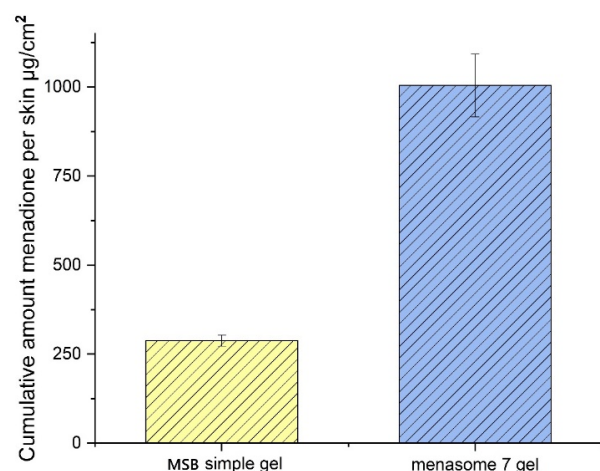


Figure 6. The cumulative MSB (menadione sodium bisulfite) amounts remained in rat skin

absorbance-based investigations that can be used to assess the metabolic activities of living cells. In this work, the Menasome effect on cell viability was investigated using MTT. As mentioned in section 2.9, several concentrations of Menasome, blank niosome, and free MSB solution at 1–15 μM were investigated.

HFF cells demonstrated concentration-dependent cytotoxicity following a 24-hour treatment with various concentrations of Menasome7, MSB solution, and bare niosome (Figure 7). After 24 hours of incubation with 400 μM blank niosome and Menasome 7, 96.020 ± 1.735 and $86.4705 \pm 1.304\%$ of cells remained, respectively; whereas this amount was $75.24 \pm 1.35\%$ with the MSB solution. These results show that entrapping MSB in a niosome increases cell viability. This is probably because Menasomes can sustain drug release, which lowers the concentration of drug deposited close to the cell and reduces the drug's cytotoxic effects. Furthermore, increased reactivities with proteins and cells derive from the charge density of the formulation. The prepared niosome showed a little zeta potential (-3.05). Consequently, there is less of a charge delivery around the particle and less toxicity.³⁸

Effect of Menasome on the synthesis of melanin

The final stage of the melanogenesis process is the synthesis of melanin. A reduction in the amount of melanin indicates that Menasome 7 is inhibiting tyrosinase, which ultimately results in a reduction in the melanin content of cells. Using B16F10 cells as the cell model, the anti-melanogenic activity of the produced niosome was determined. The findings indicated that the MSB solution was less effective than the Menasome in inhibiting the formation of melanin (Figure 8). When comparing the reduction in melanin synthesis ($37.426 \pm 1.644\%$) at 15 μM concentration to MSB solution ($57.383 \pm 1.654\%$), Figure 8 shows that Menasome 7 had the highest effect. MSB has been found to decrease melanin synthesis by activating the ERK (Extracellular signal-regulated kinase) pathway, a signaling system governing cellular processes.

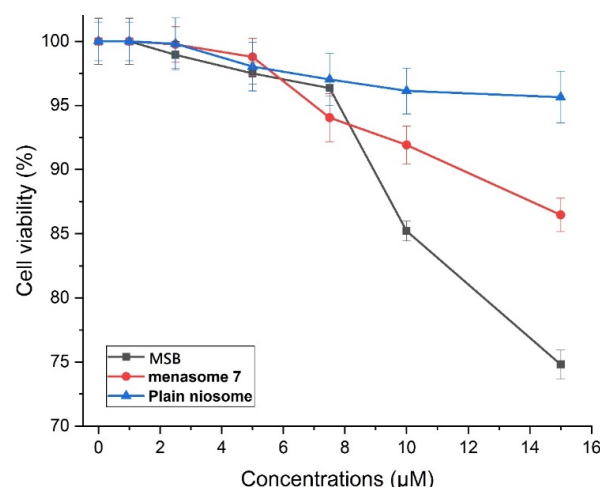


Figure 7. Viability of cells at diverse concentrations of MSB (menadione sodium bisulfite), Menasome 7, and plain niosome (mean \pm SD, $n=5$)

While ERK typically stimulates melanin production, MSB's presence alters ERK activation, leading to a reduction in melanin content. Also, The ERK pathway plays a key role in anti-melanogenesis by promoting the degradation of MITF (Microphthalmia-associated transcription factor), a critical transcription factor for melanogenic enzymes like tyrosinase. When activated, ERK phosphorylates MITF, leading to its degradation and subsequent reduction in melanin production. In the B16F10 melanoma cell line, MSB (vitamin K3) has been shown to generate reactive oxygen species (ROS), which activate the ERK pathway. This activation results in the phosphorylation and degradation of MITF, thereby decreasing tyrosinase expression and melanin synthesis.⁵³ The involvement of the ERK pathway in MSB's anti-melanogenic effect is confirmed through increased phosphorylated ERK levels, reduced melanogenesis upon menadione treatment, and the reversal of these effects by ERK pathway inhibitors.⁷ The exact mechanism of how MSB influences ERK signaling in melanocytes remains unclear but likely involves multiple steps and factors. Nevertheless, MSB shows potential as a hypopigmentary agent for treating skin pigmentation disorders.⁷

Inhibition of L-dopa auto-oxidation assay

Effective anti-melanogenic products are crucial because controlling unattractive hyperpigmentation is the most challenging test in dermatology and cosmetics.⁵⁴ The results of this study demonstrated that, in a concentration-dependent manner, Menasome 7 and MSB solution reduced the auto-oxidation of L-dopa. In the MSB solution, the highest inhibitory concentration was $83.953 \pm 1.629\%$ at $15 \mu\text{M}$, as shown in Figure 9. On the other hand, Figure 9 shows that an increase in Menasome concentration corresponds with an increase in inhibition ($94.806 \pm 2.411\%$). This profile shows that Menasome 7 activity is significantly inhibited at dosages ranging from 1 to $5 \mu\text{M}$. The release curve of vitamin K from hydrogel (Figure 1) explains this action.

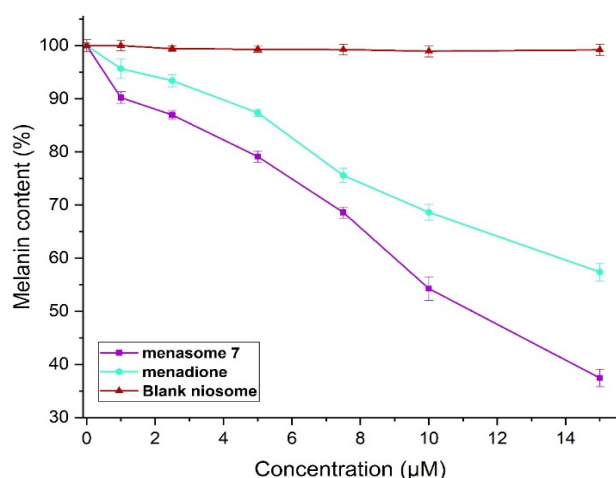


Figure 8. Melanin content of MSB (menadione sodium bisulfite), Menasome7, and plain niosome at various concentrations (mean \pm SD, $n=5$)

Compared to pure MSB (positive control) with an IC_{50} value of $9.187 \pm 0.48 \mu\text{M}$ ($P < 0.05$), Menasome 7 had a considerable inhibitory impact against L-DOPA auto-oxidation ($\text{IC}_{50} = 7.13 \pm 0.279 \mu\text{M}$). MSB performs as an antioxidant by diminishing the detrimental ROS and free radicals produced throughout the auto-oxidation of L-DOPA. MSB aids in safeguarding L-DOPA from oxidative destruction by diminishing ROS amounts, hence potentially amplifying its therapeutic efficacy.^{55,56} The outcomes suggest that adding MSB to the niosome could enhance the inhibitory auto-oxidation process, which could be useful for usage as a skin-lightening cosmetic.

Irritation test on the rat skin

In addition to administering the drug, the pharmaceutical delivery system should not significantly irritate the patient or have harmful or immunogenic effects. To be considered an appropriate preparation, the Menasome 7 preparation must not cause skin hypersensitivity because it is intended for topical application.⁵⁷ As a matter of fact, one of the most frequent adverse effects of dermal topical therapies is skin irritation, and it is directly related to the concentration of the active substance. Therefore, modifying the preparation's chemical release could reduce its adverse effects by increasing intra-follicular penetration and declining cutaneous uptake.⁵⁷ The impact of different preparations of MSB on the measurement of skin irritation (erythema and edema) is displayed in Table 2. Draize, Woodward, and Calvery have classified compounds with grade levels of 2 or lower as negative (no skin irritation). The edema and erythema rating of 0.333 for the Menasome 7 gel preparation was significantly lower than the value recorded for formalin and other MSB preparations (Table 2). This shows no irritation and improves the tolerance and dermal suitability of the Menasome 7 formulation.

This indicates the lack of sensitivity, hence improving the tolerance and compatibility of the Menasome

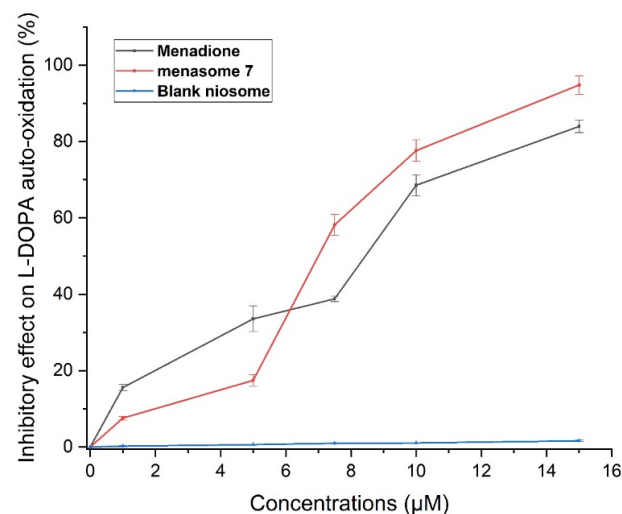


Figure 9. Inhibitory effect on L-dopa auto-oxidation of MSB (menadione sodium bisulfite), Menasome 7, and blank niosome (mean \pm SD, $n=5$)

Table 2. Cutaneous sensitivity values after local application

Rat No.	Control		Menasome 7 gel*		MSB plain gel		Blank niosomal gel		Formalin	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
1	0	0	0	0	0	0	1	0	4	3
2	0	0	0	0	1	0	0	0	3	4
3	0	0	0	0	1	1	1	1	4	3
4	0	0	0	1	1	0	1	0	4	4
5	0	0	0	0	0	1	1	0	3	3
6	0	0	1	0	2	2	0	0	3	3
Score	0		0.333		1.5		0.833		6.833	

* Significant compared with formalin MSB plain gel ($P < 0.05$).

formulation with the dermis. Further investigations have also observed comparable results in experiments that examined niosomal preparations of nortriptyline,⁵⁸ terbinafine,⁵⁹ kojic acid,³³ and venlafaxine.¹⁶

Conclusion

The hydrophilic drug MSB has been successfully added to niosomes made of Chol and nonionic surfactants. MSB in niosomes was in an amorphous state and did not interact chemically with the other niosome elements because of solid-state testing. The average encapsulation efficiency, particle size, PDI, and zeta potential of the adjusted niosome topical preparation were $83.616 \pm 2.550\%$, $298.133 \pm 8.823\text{nm}$, 0.304 ± 0.006 , and $-3.056 \pm 0.797\text{mV}$, respectively. Moreover, due to the developed Menasome's higher percentage of cell survival approximately 85%, the MTT investigation discovered no cell toxicity in HFFs. Besides, the applied Menasome 7 gel constituents were presented to be non-irritant in a dermal irritation test. When compared to free MSB sodium bisulfite and blank niosome, the anti-hyperpigmentation activity of niosome loaded with MSB showed the strongest effect, including reduction of melanin production and L-Dopa auto-oxidation inhibitory actions. This work has demonstrated the potential application of MSB-loaded green technology niosomes as preparation of anti-hyperpigmentation for use in cosmetic and pharmaceutical products.

Acknowledgments

The authors also wish to express their sincere and special appreciation to the research council of Mazandaran University of Medical Sciences, Sari, Iran, for their valuable and technical cooperation.

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Competing Interests

No conflicts of interest were reported by the authors.

Data Availability Statement

Data will be available upon request.

Ethical Approval

The ethical review board for animal studies at Mazandaran University of Medical Sciences approved the animal experiments, and the ethical code was IR.MAZUMS.REC.1400.10517.

Funding

The Research Council at Mazandaran University of Medical Sciences has funded the present study with grant number 10517.

References

1. Radmard A, Saeedi M, Morteza-Semnani K, Hashemi SM, Nokhodchi A. 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* 2021;201:111616. doi: [10.1016/j.colsurfb.2021.111616](https://doi.org/10.1016/j.colsurfb.2021.111616)
2. Khezri K, Saeedi M, Morteza-Semnani K, Akbari J, Rostamkalei SS. An emerging technology in lipid research for targeting hydrophilic drugs to the skin in the treatment of hyperpigmentation disorders: kojic acid-solid lipid nanoparticles. *Artif Cells Nanomed Biotechnol* 2020;48(1):841-53. doi: [10.1080/21691401.2020.1770271](https://doi.org/10.1080/21691401.2020.1770271)
3. Hashemi SM, Emami S. Kojic acid-derived tyrosinase inhibitors: synthesis and bioactivity. *Pharm Biomed Res* 2015;1(1):1-17. doi: [10.18869/acadpub.pbr.1.1.1](https://doi.org/10.18869/acadpub.pbr.1.1.1)
4. Silpa-Archa N, Kohli I, Chaowattanapanit S, Lim HW, Hamzavi I. Postinflammatory hyperpigmentation: a comprehensive overview: epidemiology, pathogenesis, clinical presentation, and noninvasive assessment technique. *J Am Acad Dermatol* 2017;77(4):591-605. doi: [10.1016/j.jaad.2017.01.035](https://doi.org/10.1016/j.jaad.2017.01.035)
5. Nicolaidou E, Katsambas AD. Pigmentation disorders: hyperpigmentation and hypopigmentation. *Clin Dermatol* 2014;32(1):66-72. doi: [10.1016/j.clindermatol.2013.05.026](https://doi.org/10.1016/j.clindermatol.2013.05.026)
6. Zhu W, Gao J. The use of botanical extracts as topical skin-lightening agents for the improvement of skin pigmentation disorders. *J Invest Dermatol Symp Proc* 2008;13(1):20-4. doi: [10.1038/jidsymp.2008.8](https://doi.org/10.1038/jidsymp.2008.8)

7. Kim EH, Kim MK, Yun HY, Baek KJ, Kwon NS, Park KC, et al. Menadione (vitamin K3) decreases melanin synthesis through ERK activation in Mel-Ab cells. *Eur J Pharmacol* 2013;718(1-3):299-304. doi: [10.1016/j.ejphar.2013.08.018](https://doi.org/10.1016/j.ejphar.2013.08.018)
8. Yuan G, Zhu X, Li P, Zhang Q, Cao J. New activity for old drug: In vitro activities of vitamin K3 and menadione sodium bisulfite against methicillin-resistant *Staphylococcus aureus*. *Afr J Pharm Pharmacol* 2014;8(17):451-54. doi: [10.5897/ajpp2013.3903](https://doi.org/10.5897/ajpp2013.3903)
9. Sharma V, Anandhakumar S, Sasidharan M. Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: an efficient carrier for cancer multi-drug delivery. *Mater Sci Eng C Mater Biol Appl* 2015;56:393-400. doi: [10.1016/j.msec.2015.06.049](https://doi.org/10.1016/j.msec.2015.06.049)
10. Khatoun M, Shah KU, Din FU, Shah SU, Rehman AU, Dilawar N, et al. Proniosomes derived niosomes: recent advancements in drug delivery and targeting. *Drug Deliv* 2017;24(Suppl 1):56-69. doi: [10.1080/10717544.2017.1384520](https://doi.org/10.1080/10717544.2017.1384520)
11. Azeem A, Anwer MK, Talegaonkar S. Niosomes in sustained and targeted drug delivery: some recent advances. *J Drug Target* 2009;17(9):671-89. doi: [10.3109/10611860903079454](https://doi.org/10.3109/10611860903079454)
12. Manconi M, Sinico C, Valenti D, Lai F, Fadda AM. Niosomes as carriers for tretinoin. III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. *Int J Pharm* 2006;311(1-2):11-9. doi: [10.1016/j.ijpharm.2005.11.045](https://doi.org/10.1016/j.ijpharm.2005.11.045)
13. Desnita R, Luliana S, Anggraini S. In vitro penetration of alpha arbutin niosome Span 60 system in gel preparation. *Pharmacia* 2017;7(2):249-56. doi: [10.12928/pharmacia.v7i2.6799](https://doi.org/10.12928/pharmacia.v7i2.6799)
14. Chang N-F, Tsai F-J, Zheng Y-M, Huang W-H, Lin C-C. Using a cellular system to directly assess the effects of cosmetic microemulsion encapsulated deoxyarbutin. *Int J Mol Sci* 2021;22(23):13110. doi: [10.3390/ijms222313110](https://doi.org/10.3390/ijms222313110)
15. Junyaprasert VB, Singhsa P, Suksiriworapong J, Chantasart D. Physicochemical properties and skin permeation of Span 60/Tween 60 niosomes of ellagic acid. *Int J Pharm* 2012;423(2):303-11. doi: [10.1016/j.ijpharm.2011.11.032](https://doi.org/10.1016/j.ijpharm.2011.11.032)
16. Hashemi SM, Enayatifard R, Akbari J, Saeedi M, Seyedabadi M, Morteza-Semnani K, et al. Venlafaxine HCl encapsulated in niosome: green and eco-friendly formulation for the management of pain. *AAPS PharmSciTech* 2022;23(5):149. doi: [10.1208/s12249-022-02299-5](https://doi.org/10.1208/s12249-022-02299-5)
17. Pereira-Lachataignerais J, Pons R, Panizza P, Courbin L, Rouch J, López O. Study and formation of vesicle systems with low polydispersity index by ultrasound method. *Chem Phys Lipids* 2006;140(1-2):88-97. doi: [10.1016/j.chemphyslip.2006.01.008](https://doi.org/10.1016/j.chemphyslip.2006.01.008)
18. Khan MI, Madni A, Hirvonen J, Peltonen L. Ultrasonic processing technique as a green preparation approach for diacerein-loaded niosomes. *AAPS PharmSciTech* 2017;18(5):1554-63. doi: [10.1208/s12249-016-0622-z](https://doi.org/10.1208/s12249-016-0622-z)
19. Rezanejad Gatabi Z, Rahimnia SM, Morteza-Semnani K, Yazdian-Robati R, Hashemi SM, Saeedi M. Vitamin K (Menadione)-incorporated chitosan/alginate hydrogel as a novel product for periorbital hyperpigmentation. *J Biomater Sci Polym Ed* 2024;35(7):967-88. doi: [10.1080/09205063.2024.2313825](https://doi.org/10.1080/09205063.2024.2313825)
20. Enayatifard R, Akbari J, Saeedi M, Morteza-Semnani K, Parvin S, Hashemi SM, et al. Investigating the effect of coated lipid nano particles of spironolactone with chitosan on their properties. *J Mazandaran Univ Med Sci* 2018;28(162):25-36. [Persian].
21. Rahimnia SM, Saeedi M, Akbari J, Morteza-Semnani K, Hedayatizadeh-Omran A, Yazdian-Robati R. Development, Optimization, and in vitro Evaluation of Silybin-loaded PLGA Nanoparticles and Decoration with 5TR1 Aptamer for Targeted Delivery to Colorectal Cancer Cells. *AAPS PharmSciTech* 2024;25(6):141. doi: [10.1208/s12249-024-02858-y](https://doi.org/10.1208/s12249-024-02858-y)
22. Saeedi M, Morteza-Semnani K, Siahposht-Khachaki A, Akbari J, Valizadeh M, Sanaee A, et al. Passive targeted drug delivery of venlafaxine HCl to the brain by modified chitosan nanoparticles: characterization, cellular safety assessment, and in vivo evaluation. *J Pharm Innov* 2023;18(3):1441-53. doi: [10.1007/s12247-023-09733-6](https://doi.org/10.1007/s12247-023-09733-6)
23. Rahimnia SM, Saeedi M, Morteza-Semnani K, Akbari J, Ghasemi M, Abootorabi S, et al. Green preparation, characterization, wound healing assessment, and histopathological evaluation of vitamin A encapsulated in niosome and solid lipid nanoparticle. *Pharm Sci* 2024;30(1):70-84. doi: [10.34172/ps.2023.23](https://doi.org/10.34172/ps.2023.23)
24. Saeedi M, Rezanejad Gatabi Z, Morteza-Semnani K, Rahimnia SM, Yazdian-Robati R, Hashemi SM. Preparation of arbutin hydrogel formulation as green skin lightener formulation: in vitro and in vivo evaluation. *J Dispers Sci Technol* 2024;1-13. doi: [10.1080/01932691.2024.2371954](https://doi.org/10.1080/01932691.2024.2371954)
25. Akbari J, Saeedi M, Morteza-Semnani K, Mousavi SN, Hashemi SMH, Rahimnia SM. Evaluation of an innovative, green, and eco-friendly baclofen-loaded niosome (Baclosome) formulation for pain management by transdermal delivery. *Nanomed J* 2025;12(1):59-69. doi: [10.22038/nmj.2024.79071.1941](https://doi.org/10.22038/nmj.2024.79071.1941)
26. Saeedi M, Morteza-Semnani K, Akbari J, Rahimnia SM, Ahmadi F, Choubdari H, et al. Development of kojic acid loaded collagen-chitosan nanoparticle as skin lightener product: in vitro and in vivo assessment. *J Biomater Sci Polym Ed* 2024;35(1):63-84. doi: [10.1080/09205063.2023.2268316](https://doi.org/10.1080/09205063.2023.2268316)
27. Oh MJ, Hamid MA, Ngadiran S, Seo YK, Sarmidi MR, Park CS. *Ficus deltoidea* (Mas Cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity in vitro and by suppressing tyrosinase gene expression in B16F1 melanoma cells. *Arch Dermatol Res* 2011;303(3):161-70. doi: [10.1007/s00403-010-1089-5](https://doi.org/10.1007/s00403-010-1089-5)
28. El-Ridy MS, Yehia SA, Mohsen AM, El-Awdan SA, Darwish AB. Formulation of niosomal gel for enhanced transdermal lornoxicam delivery: in-vitro and in-vivo evaluation. *Curr Drug Deliv* 2018;15(1):122-33. doi: [10.2174/1567201814666170224141548](https://doi.org/10.2174/1567201814666170224141548)
29. Draize JH. Methods for the study of irritation and toxicity of substances applied topically to the skin and the mucous membranes. *J Pharmacol Exp Ther* 1944;82:377-90.
30. Tavano L, Mazzotta E, Muzzalupo R. Innovative topical formulations from diclofenac sodium used as surfadrag: the birth of diclosomes. *Colloids Surf B Biointerfaces* 2018;164:177-84. doi: [10.1016/j.colsurfb.2018.01.030](https://doi.org/10.1016/j.colsurfb.2018.01.030)
31. Chaw CS, Kim KY. Effect of formulation compositions on niosomal preparations. *Pharm Dev Technol* 2013;18(3):667-72. doi: [10.3109/10837450.2012.672988](https://doi.org/10.3109/10837450.2012.672988)
32. Akbari J, Saeedi M, Enayatifard R, Morteza-Semnani K, Hashemi SM, Babaei A, et al. Curcumin niosomes (curculosomes) as an alternative to conventional vehicles: a potential for efficient dermal delivery. *J Drug Deliv Sci Technol* 2020;60:102035. doi: [10.1016/j.jddst.2020.102035](https://doi.org/10.1016/j.jddst.2020.102035)
33. Morteza-Semnani K, Saeedi M, Akbari J, Moazeni M, Babaei A, Negarandeh R, et al. Preparation and in-vitro evaluation of ketoconazole-loaded niosome (ketosome) for drug delivery to cutaneous candidiasis. *Pharm Sci* 2023;29(2):208-18. doi: [10.34172/PS.2022.34](https://doi.org/10.34172/PS.2022.34)
34. Nasserri B. Effect of cholesterol and temperature on the elastic properties of niosomal membranes. *Int J Pharm* 2005;300(1-2):95-101. doi: [10.1016/j.ijpharm.2005.05.009](https://doi.org/10.1016/j.ijpharm.2005.05.009)
35. Mekarun J, Treepet S, Rujiravanit R, Theeramunkong S, Watthanaphanit A. Caffeine-containing emulsion: influence of the HLB and mixing proportions, the oil's chemical composition, and the existence of caffeine on emulsion

- properties. *ACS Omega* 2024;9(2):2113-22. doi: [10.1021/acsomega.3c03674](https://doi.org/10.1021/acsomega.3c03674)
36. Rajabalaya R, Leen G, Chellian J, Chakravarthi S, David SR. Tolterodine tartrate proniosomal gel transdermal delivery for overactive bladder. *Pharmaceutics* 2016;8(3):27. doi: [10.3390/pharmaceutics8030027](https://doi.org/10.3390/pharmaceutics8030027)
 37. Mokhtar M, Sammour OA, Hammad MA, Megrab NA. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. *Int J Pharm* 2008;361(1-2):104-11. doi: [10.1016/j.ijpharm.2008.05.031](https://doi.org/10.1016/j.ijpharm.2008.05.031)
 38. Xu YQ, Chen WR, Tsosie JK, Xie X, Li P, Wan JB, et al. Niosome encapsulation of curcumin: characterization and cytotoxic effect on ovarian cancer cells. *J Nanomater* 2016;2016(1):6365295. doi: [10.1155/2016/6365295](https://doi.org/10.1155/2016/6365295)
 39. Rostamkalei SS, Akbari J, Saeedi M, Morteza-Semnani K, Nokhodchi A. Topical gel of metformin solid lipid nanoparticles: a hopeful promise as a dermal delivery system. *Colloids Surf B Biointerfaces* 2019;175:150-7. doi: [10.1016/j.colsurfb.2018.11.072](https://doi.org/10.1016/j.colsurfb.2018.11.072)
 40. Taymouri S, Varshosaz J. Effect of different types of surfactants on the physical properties and stability of carvedilol nano-niosomes. *Adv Biomed Res* 2016;5:48. doi: [10.4103/2277-9175.178781](https://doi.org/10.4103/2277-9175.178781)
 41. Morteza-Semnani K, Saeedi M, Akbari J, Eghbali M, Babaei A, Hashemi SM, et al. Development of a novel nanoemulgel formulation containing cumin essential oil as skin permeation enhancer. *Drug Deliv Transl Res* 2022;12(6):1455-65. doi: [10.1007/s13346-021-01025-1](https://doi.org/10.1007/s13346-021-01025-1)
 42. Shah P, Goodyear B, Haq A, Puri V, Michniak-Kohn B. Evaluations of quality by design (QbD) elements impact for developing niosomes as a promising topical drug delivery platform. *Pharmaceutics* 2020;12(3):246. doi: [10.3390/pharmaceutics12030246](https://doi.org/10.3390/pharmaceutics12030246)
 43. Min Y, Akbulut M, Kristiansen K, Golan Y, Israelachvili J. The role of interparticle and external forces in nanoparticle assembly. *Nat Mater* 2008;7(7):527-38. doi: [10.1038/nmat2206](https://doi.org/10.1038/nmat2206)
 44. Herdiana Y, Wathoni N, Shamsuddin S, Muchtaridi M. Drug release study of the chitosan-based nanoparticles. *Heliyon* 2022;8(1):e08674. doi: [10.1016/j.heliyon.2021.e08674](https://doi.org/10.1016/j.heliyon.2021.e08674)
 45. Harivardhan Reddy L, Murthy RS. Etoposide-loaded nanoparticles made from glyceride lipids: formulation, characterization, in vitro drug release, and stability evaluation. *AAPS PharmSciTech* 2005;6(2):E158-66. doi: [10.1208/pt060224](https://doi.org/10.1208/pt060224)
 46. Kenechukwu FC, Attama AA, Ibezim EC. Novel solidified reverse micellar solution-based mucoadhesive nano lipid gels encapsulating miconazole nitrate-loaded nanoparticles for improved treatment of oropharyngeal candidiasis. *J Microencapsul* 2017;34(6):592-609. doi: [10.1080/02652048.2017.1370029](https://doi.org/10.1080/02652048.2017.1370029)
 47. Devi R, Jain A, Hurkat P, Jain SK. Dual drug delivery using lactic acid conjugated SLN for effective management of neurocysticercosis. *Pharm Res* 2015;32(10):3137-48. doi: [10.1007/s11095-015-1677-3](https://doi.org/10.1007/s11095-015-1677-3)
 48. Pyo SM, Meinke M, Klein AF, Fischer TC, Müller RH. A novel concept for the treatment of couperosis based on nanocrystals in combination with solid lipid nanoparticles (SLN). *Int J Pharm* 2016;510(1):9-16. doi: [10.1016/j.ijpharm.2016.05.017](https://doi.org/10.1016/j.ijpharm.2016.05.017)
 49. Saeedi M, Morteza-Semnani K, Akbari J, Rahimnia SM, Babaei A, Eghbali M, et al. Eco-friendly preparation, characterization, evaluation of anti-melanogenesis/antioxidant effect and in vitro/in vivo safety profile of kojic acid loaded niosome as skin lightener preparation. *J Biomater Sci Polym Ed* 2023;34(14):1952-80. doi: [10.1080/09205063.2023.2201817](https://doi.org/10.1080/09205063.2023.2201817)
 50. Chakraborti CK, Sahoo S, Behera PK. Role of different biodegradable polymers on the permeability of ciprofloxacin. *J Adv Pharm Technol Res* 2014;5(3):140-6. doi: [10.4103/2231-4040.137434](https://doi.org/10.4103/2231-4040.137434)
 51. Tajbakhsh M, Saeedi M, Morteza-Semnani K, Akbari J, Nokhodchi A. Innovation of testosome as a green formulation for the transdermal delivery of testosterone enanthate. *J Drug Deliv Sci Technol* 2020;57:101685. doi: [10.1016/j.jddst.2020.101685](https://doi.org/10.1016/j.jddst.2020.101685)
 52. Kravchenko I, Yu B, Novikova N, Egorova A, Andronati S. Influence of cholesterol and its esters on skin penetration in vivo and in vitro in rats and mice. *Ukr Bioorg Acta* 2011;1:17-21.
 53. Ko GA, Cho SK. Phytol suppresses melanogenesis through proteasomal degradation of MITF via the ROS-ERK signaling pathway. *Chem Biol Interact* 2018;286:132-40. doi: [10.1016/j.cbi.2018.02.033](https://doi.org/10.1016/j.cbi.2018.02.033)
 54. Boo YC. p-Coumaric acid as an active ingredient in cosmetics: a review focusing on its antimelanogenic effects. *Antioxidants (Basel)* 2019;8(8):275. doi: [10.3390/antiox8080275](https://doi.org/10.3390/antiox8080275)
 55. Hörmann P, Delcambre S, Hanke J, Geffers R, Leist M, Hiller K. Impairment of neuronal mitochondrial function by L-DOPA in the absence of oxygen-dependent auto-oxidation and oxidative cell damage. *Cell Death Discov* 2021;7(1):151. doi: [10.1038/s41420-021-00547-4](https://doi.org/10.1038/s41420-021-00547-4)
 56. van Muiswinkel FL, Riemers FM, Peters GJ, LaFleur MV, Siegel D, Jongenelen CA, et al. L-Dopa stimulates expression of the antioxidant enzyme NAD(P)H:quinone oxidoreductase (NQO) in cultured astroglial cells. *Free Radic Biol Med* 2000;29(5):442-53. doi: [10.1016/s0891-5849\(00\)00328-2](https://doi.org/10.1016/s0891-5849(00)00328-2)
 57. Mahant S, Kumar S, Nanda S, Rao R. Microsponges for dermatological applications: perspectives and challenges. *Asian J Pharm Sci* 2020;15(3):273-91. doi: [10.1016/j.ajps.2019.05.004](https://doi.org/10.1016/j.ajps.2019.05.004)
 58. Akbari J, Saeedi M, Morteza-Semnani K, Sanaee A, Lotfi A, Rahimnia SM, et al. Green preparation, characterization, in vitro/in vivo safety assessment and in vivo pain management of nortriptyline HCl loaded in niosome (norosome) manufactured by ecofriendly green method. *J Dispers Sci Technol* 2024;45(4):819-31. doi: [10.1080/01932691.2023.2289625](https://doi.org/10.1080/01932691.2023.2289625)
 59. Morteza-Semnani K, Saeedi M, Akbari J, Hedayati S, Hashemi SM, Rahimnia SM, et al. Green formulation, characterization, antifungal and biological safety evaluation of terbinafine HCl niosomes and niosomal gels manufactured by eco-friendly green method. *J Biomater Sci Polym Ed* 2022;33(18):2325-52. doi: [10.1080/09205063.2022.2103626](https://doi.org/10.1080/09205063.2022.2103626)