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Development, characterization and evaluation of baicalein loaded niosome and niosomal gel

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ABSTRACT: This study focused on the formulation, in-vitro characterization, and evaluation of toxicity, antioxidant activity and skin penetration capabilities of baicalein-loaded niosome/niosomal gel as a dermal antioxidant. Baicalein-loaded niosomes were prepared using the thin film hydration method. Particular emphasis was placed on assessing the effects of different types of non-ionic surfactants (Tween60, Tween80) and various chloroform-to-methanol ratios (9:1, 7:3, 5:5 v/v) on formulation characteristics including particle size, polydispersity index, zeta potential, encapsulation efficiency, drug release. Cytotoxicity of different concentratons of baicalein and baicalein-loaded niosome/niosomal gel was evaluated on the L929 cells using the 3-(4,5-dimethyldiazol-2-yl)2,5-diphenyltetrazolium bromide(MTT) assay. F2 coded niosome, containing Tween60 and a chloroform:methanol ratio of 7:3 v/v, was identified as the optimal formulation due to its excellent encapsulation efficiency, small particle size, narrow size distribution, optimal zeta potential, and ability to achieve the highest and sustained release. F2-gel was prepared using Carbopol980, and its rheological properties were also examined. Cell permeation properties of both the niosome and the niosomal gel through L929 cells were investigated. Antioxidant efficacy of the baicalein from both formulations permeated through cells was assessed using 1,1-diphenyl-2-picrylhydrazyl(DPPH●) and (2,2-Azino-bis3-ethylbenzothiazoline-6-sulfonic acid)(ABTS●+) assays. The study demonstrated that surfactant type and solvent system used have a significant impact on the overall characteristics of niosomes. In conclusion, the high permeation of baicalein from both niosome and niosomal gel through cells, along with their strong capacity to scavenge DPPH● and ABTS●+ radicals, demonstrates that baicalein-loaded niosome/niosomal gel exhibit a potent antioxidant effect even in the deeper layers of the skin.

KEYWORDS: Niosome; niosomal gel; baicalein; antioxidant effect; drug carrier system; controlled release system; anti-aging.

1. INTRODUCTION

Baicalein is a naturally derived major flavonoid compound obtained from *Scutellaria baicalensis*, one of the important plants in Chinese Medicine. Baicalein also isolated from other Scutellaria species (*S. hypericifolia, S. amoena, S. lateriflora, S. barbata, S. viscidula*). It exhibits various pharmacological effects, including anticancer, antioxidant, anti-aging, anti-inflammatory, antimicrobial, cardioprotective, and wound-healing properties [1-3].

Currently, natural-derived compounds are attracting significant interest [4]. However, active ingredients obtained from natural sources often exhibit stability issues and pose challenges in formulation with conventional carrier systems. Therefore, incorporating these active ingredients into novel carrier systems is considered a highly effective approach. Baicalein, a naturally derived active compound, has low water solubility and stability issues due to its flavonoid structure [5, 6]. This study aims to enhance the stability of baicalein, extend its shelf life, improve its efficacy, and enable prolonged release for sustained effects by encapsulating it in niosomes, one of the novel carrier systems.

There are quite limited studies in the literature on carrier systems containing baicalein. Zhang et al. (2024) demonstrated that baicalein-loaded liposomes modified with hyaluronic acid and polyhexamethylene guanidine could be successfully used in methicillin-resistant *Staphylococcus aureus* infections [7]. Another study evaluated the effectiveness of baicalein-loaded liposomes in the treatment of pancreatic ductal adenocarcinoma [8]. Additionally, pseudo-ceramide liposomes have been shown to provide a more stable delivery system for the transdermal application of baicalein [9]. However, to the best of our knowledge, there are no studies in the literature investigating baicalein-loaded niosome/niosomal gel.

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Niosomes are spherical vesicular drug delivery systems developed as stable and cost-effective alternatives to liposomes, widely utilized in the pharmaceutical and cosmetic industries. They consist of biodegradable and non-toxic non-ionic surfactants and can be either unilamellar or multilamellar. Niosomes exhibit a molecularly organized structure of non-ionic surfactant vesicles and can be administered via oral, parenteral, ocular, dermal, transdermal, vaginal, and inhalation routes [10, 11]. Niosomes are known to increase drug permeation through the skin by many mechanisms, including alteration of the barrier function of the stratum corneum; increased hydration of the stratum corneum by reducing transepidermal water loss and loosening of its cellular structure [12].

Aging is a biological process that is influenced by a variety of internal and external factors. Oxidative stress is a major factor contributing to aging. It occurs when reactive oxygen species (ROS) accumulate in the body beyond the neutralizing capacity of antioxidant defense mechanisms. It has been associated with cellular deterioration, inflammatory responses, and the onset of age-related conditions, including cardiovascular diseases, neurodegenerative disorders, and skin aging [13-15].

Antioxidants serve a critical function in counteracting oxidative stress by scavenging free radicals and minimizing cellular damage. Recent studies highlight that both dietary intake and topical application of antioxidants may support skin vitality, enhance cognitive performance, and contribute to overall health and longevity [14-16].

The primary objective of this study is to enhance the stability and solubility of baicalein and develop a sustained-release topical antioxidant niosome and niosomal gel formulation designed for skin application to prevent oxidative damage caused by free radicals. Additionally, the study includes the formulation and in vitro characterization [encapsulation efficiency (EE), release, particle size (PS), polydispersity index (PDI), and zeta potential (ZP)], evaluation of antioxidant activity, cytotoxicity assessment of baicalein and baicalein-loaded formulations using the MTT assay, and cell penetration studies in L929 mouse fibroblast cells.

As a result, the well-characterized baicalein-loaded niosome/niosomal gel improves solubility, stability, and release properties, providing an effective, reliable, and stable formulation with a prolonged antioxidant effect on dermal tissue. In this way, free radicals are captured and stabilized, oxidation is slowed down or inhibited, protecting cells from oxidation-induced damage and ensuring a prolonged, more effective, and reliable anti-aging effect.

2. RESULTS AND DISCUSSION

2.1. UV-Visible Spectrophotometric Analysis of Baicalein

The maximum wavelength of baicalein was determined as 277 nm. The standard curve was established using linear regression ($y = 0.0659x + 0.0142$), demonstrating a strong linear relationship with a high correlation coefficient ($r^2 = 0.9969$).

2.2. Development and Characterization of Niosomes

Different baicalein-loaded niosomes were prepared using the thin-film hydration method. The gel formulated with the optimal niosome. All the characterization properties (PS, PDI, ZP, EE%) were presented in Table 1.

The emphasis on particle size and distribution in the FDA's "Guidance for Industry" for liposomal drug products, recognizing them as "critical quality attributes" and key factors in the stability assessment of these formulations, suggests that a similar evaluation should also be applied to niosomes [17, 18]. As the chloroform ratio increased and the methanol ratio decreased in the solvent mixture, the particle size decreased (Table 1). In niosomes containing Tween 60, increasing the chloroform:methanol ratio from 5:5 (v/v) to 7:3 (v/v) and 9:1 (v/v) resulted in a decrease in particle size from 1037 nm to 803 nm and 615 nm, respectively ($p>0.05$). In niosomes containing Tween 80, the particle size of niosomes with a chloroform:methanol ratio of 9:1 (v/v) increased from 591.4 nm to 780.1 nm and 832.1 nm, respectively (Table 1) ($p>0.05$). Chloroform has a higher vapor pressure than methanol, leading to a faster evaporation rate. This rapid evaporation can cause quicker lipid aggregation, resulting in larger niosome particles. Also, the polar nature of methanol helps disperse lipids more homogeneously in the solvent, leading to smaller vesicles. As chloroform becomes dominant, lipid dissolution and self-assembly behavior change, promoting the formation of larger particles [19, 20].

Table 1. Effect of formulation properties on characterization parameters (mean±SD)

Formulation Code	Surfactant	Cholesterol: surfactant (molar ratio)	Chloroform :Methanol (v/v)	PS (nm)	PDI	ZP (mV)	EE (%)
F1	Tween 60	1:1	9:1	615.3±8.2	0.084	-44.2±4.12	57.28±2.14
F2	Tween 60	1:1	7:3	803.0±10.1	0.124	-38.6±4.43	65.03±3.25
F3	Tween 60	1:1	5:5	1037.0±12.3	0.505	-34.6±3.27	51.40±2.16
F4	Tween 80	1:1	9:1	591.4±6.7	0.460	-12.6±2.44	48.89±2.05
F5	Tween 80	1:1	7:3	780.1±7.7	0.384	-12.7±2.25	54.57±252
F6	Tween 80	1:1	5:5	832.1±8.4	0.212	-31.9±3.39	55.63±2.85
F2-gel	Tween 60	1:1	7:3	705.7±8.0	0.193	-39.8±6.25	40.64±1.98

A smaller particle size is desirable for dermal drug delivery systems as it may enhance skin penetration. A smaller particle size is essential for dermal and transdermal drug delivery systems, as they can significantly enhance skin penetration, facilitating more effective drug absorption [21]. Hence, niosomes containing a chloroform:methanol ratio of 9:1 (v/v) or 7:3 (v/v) were favorable due to their smaller particle size. The PDI values of niosomes containing Tween 80 were generally higher than those of niosomes containing Tween 60. In lipid-based drug delivery systems such as niosomes, a PDI of 0.3 or less is considered suitable, indicating a well-distributed population of phospholipid vesicles [17]. Therefore, niosomes formulated with Tween 60 were considered more suitable due to their lower PDI values.

A zeta potential of 30 mV or higher indicates a high stability of drug delivery systems, as it suggests strong electrostatic repulsion between particles, preventing aggregation [22]. While the zeta potentials of all niosomes containing Tween 60 were higher than 30 mV, the zeta potentials of niosomes containing Tween 80, except for F6, were considerably lower ($p>0.05$). This proved that the stability of niosomes containing Tween 60 was superior.

The encapsulation efficiency ranged between 48.89% and 65.03%. Baicalein was incorporated into the niosomes with a significantly high encapsulation efficiency. Therefore, all niosomes were found to be appropriate in terms of encapsulation efficiency.

2.3. Cytotoxicity

The cell viability results obtained from the MTT assay are presented in Figure 1. The viability rate being over 50% proves that the samples do not have cytotoxic effects, so they are biocompatible [23]. Accordingly, the baicalein dose that resulted in cell viability above 50% was determined to be 50 µg/mL. Initially, baicalein was incorporated into the niosomes at a concentration of 50 µg/mL, as determined by the MTT assay. However, due to the encapsulation efficiency being around 50%, 100 µg/mL of baicalein was incorporated into the niosomes. As a result, as shown in Figure 1, the amount of baicalein loaded into the niosomes and released at the 24-hours remained below 50 µg/mL. Therefore, the niosome formulation was not found to be cytotoxic. It has been also proven that baicalein-loaded niosome and the niosomal gel is not cytotoxic (Figure 1). Statistical analysis revealed no significant differences in cell viability among the free baicalein (50 µg/mL), the corresponding niosomal formulation, and the niosomal gel containing the same dose ($p > 0.05$), indicating that all formulations were well tolerated and suitable for cellular applications (Figure 1).

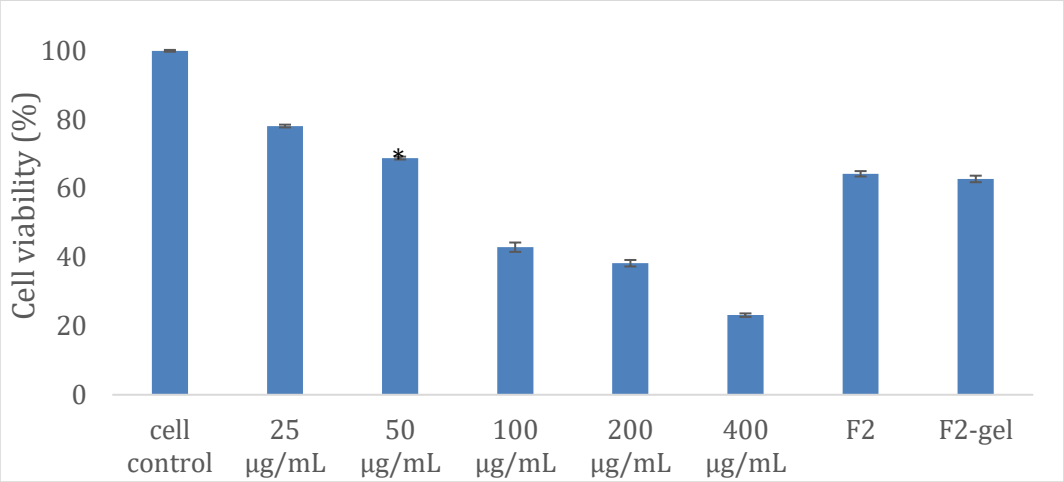

Figure 1. Effects on L929 cell viability of different concentrations of baicalein and baicalein loaded niosome/niosomal gel (error bars represent standard deviations, n = 6). Statistical analyses by one-way Anova comparison test. * $p<0.05$

2.4. Drug release

As can be seen from the Figure 2, niosomes containing Tween 60 showed similar or higher relase than niosomes containing Tween 80. Additionally, the release was higher in niosomes with chloroform:methanol ratios of 7:3. Besides, the lowest release was always observed in the formulation with chloroform:methanol ratios of 5:5. Considering all aspects, it can be concluded that niosomes containing Tween 80 and Tween 60 with chloroform:methanol ratios of 7:3 may be the most optimal formulations. The amount of baicalein released at 24 hours from niosomes containing Tween 60 (F2) was higher than that from niosomes containing Tween 80 (F5). In addition, due to the higher encapsulation efficiency of the niosomes containing Tween 60 (65.03%) compared to those containing Tween 80 (54.57%), F2 coded niosome was selected as the final ideal formulation.

Although numerous studies have investigated the impact of the surfactant type and the cholesterol-to-non-ionic surfactant ratio on niosome properties [24, 25], research examining the influence of solvent ratio or type remains limited. Solvent properties (the chloroform:methanol ratio) are to impact the fluidity and permeability of the niosomal bilayer. Baicalein is a flavonoid with limited water solubility, making its interaction with solvent systems crucial for effective delivery. A solvent mixture that optimally balances polarity can improve baicalein's solubility, enhancing its encapsulation efficiency and release rate from niosomes. In the study, various chloroform:methanol ratios (9:1 v/v, 7:3 v/v, and 5:5 v/v) were evaluated to determine their effect on baicalein release from niosomes (Figure 2). Chloroform and methanol differ significantly in polarity and hydrogen-bonding capabilities. Chloroform is a non-polar solvent, whereas methanol is polar and capable of hydrogen bonding. Furthermore, Gupta et al. (2015) demonstrated that specific ratios of methanol–chloroform binary solvent mixtures can influence solvent–solvent interactions via hydrogen-bonding networks, thereby affecting the solvation dynamics [26].

The 7:3 ratio exhibited the highest and sustained baicalein release, which can be attributed to the physicochemical properties of both the solvents and baicalein. The 7:3 (v/v) ratio may confer optimal membrane characteristics, enhancing baicalein release. In contrast, higher chloroform content (9:1 v/v) could lead to a more rigid, hydrophobic membrane, hindering drug release, while equal parts chloroform and methanol (5:5 v/v) might result in excessive membrane fluidity, compromising stability and controlled release.

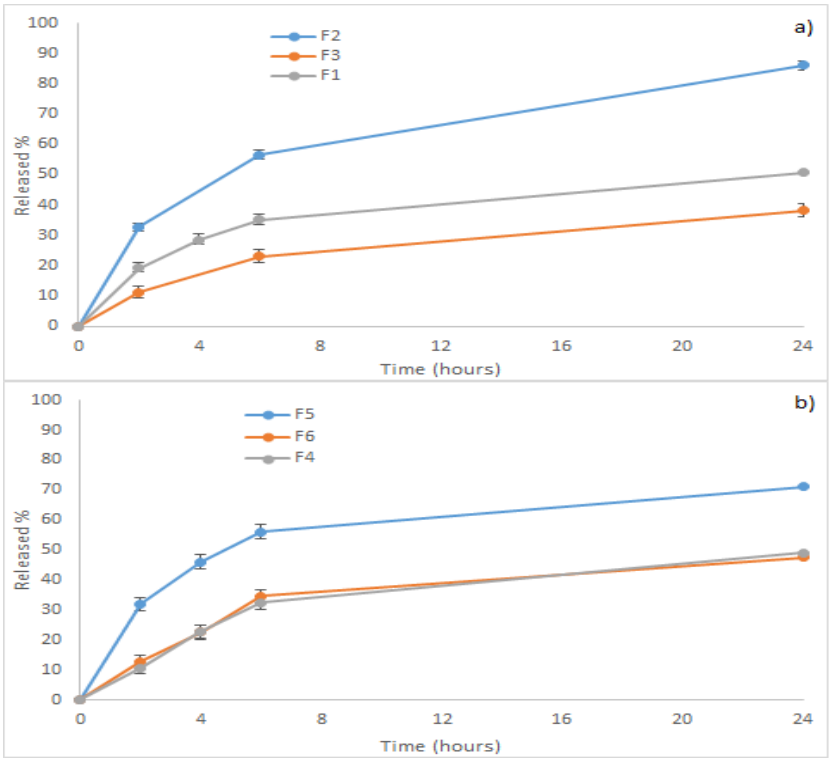

Figure 2. In vitro drug release from niosomes containing a) Tween 60 b) Tween 80

As a conclude, the type of surfactant used in the niosome formulation as well as the properties of the solvent system used have a significant impact on all the characterization properties of niosomes including the drug release profile.

Drug release of optimal niosome (F2) and niosomal gel (F2-gel) was shown in Figure 3. Although 86.1% of baicalein was released from niosomes within 24 hours, the release was reduced to 53.6% in the niosomal gel, presumably due to the increased viscosity of the gel matrix compared to the niosome. Nevertheless, the F2-gel exhibited satisfactory cellular permeation (49.8±3.5%) and retained sufficient antioxidant activity (Table 2).

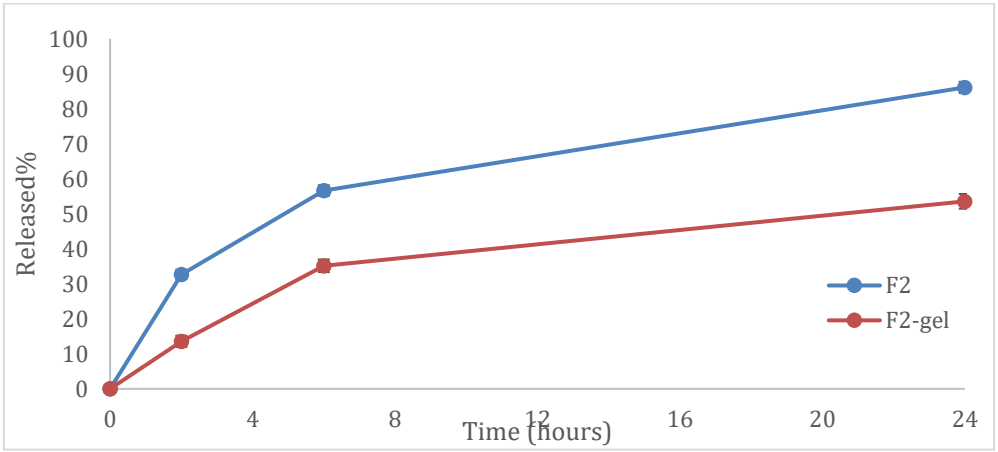

Figure 3. In vitro drug release from noisome (F2) and niosomal gel (F2-gel)

Table 2. Antioxidant activity of the baicalein-loaded niosome and the niosomal gel

	DPPH• (Inhibition% ± SD)	ABTS+• (Inhibition% ± SD)
F2-niosome	70.78 ± 1.59	47.38 ± 2.59
F2-niosomal gel	63.77 ± 1.03	40.75 ± 1.56

2.5. Viscosity

Viscosity of F2-gel as a function of shear rate at 25°C was shown in Figure 4.

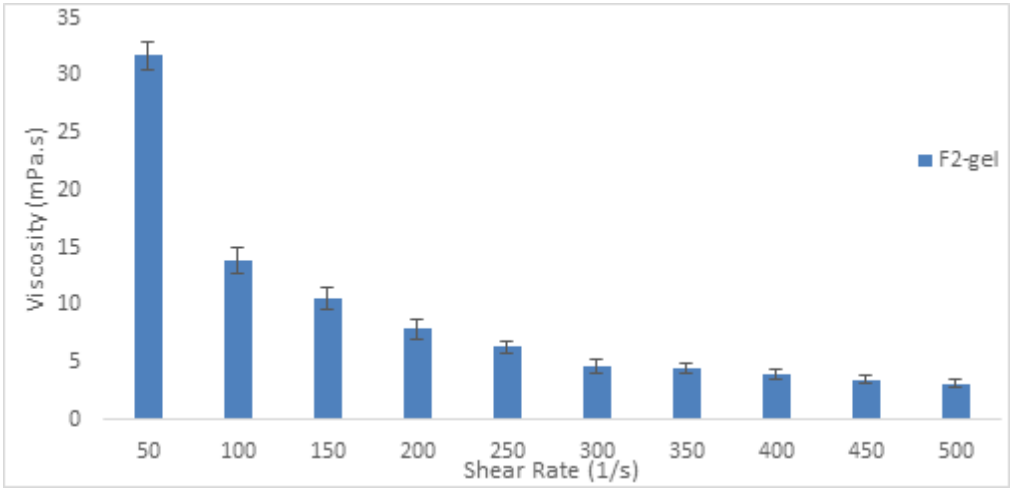

Figure 4. Viscosity of F2-gel as a function of shear rate at 25°C

The gel showed pseudoplastic behavior as a decrease in the viscosity of the gel was observed with the increase in shear rate. It was related to Carbopol 980, the gelling agent used [27, 28]. At low shear rates (e.g., 50 s^{-1}, where the viscosity is around 30 mPa·s), the gel exhibits a more viscous and dense structure. However, as the shear rate increases, particularly in the range of 100–500 s^{-1}, its viscosity decreases, indicating shear-thinning behavior. This property facilitates easier application, allowing the gel to spread more smoothly when dispensed from a tube or applied with fingers, demonstrating its favorable spreadability.

2.6. Permeation studies

Niosomes have demonstrated enhanced skin penetration capabilities, attributed to their ability to merge with skin cell membranes, facilitating deeper drug delivery [12]. Niosomes have superior properties to prevent baicalein's low solubility, low skin permeability and instability problems and to ensure effective passage through the skin. In our study, cell permeation percentages of baicalein from F2 coded niosome, and the F2-gel at the end of 24 hours were found to be 57.4±0.6% and 49.8±3.5%, respectively. These results indicate that baicalein exhibits notably high penetration into L929 mouse fibroblast cells, indicating that both the F2 and F2-gel are able to penetrate the skin effectively. There are a limited number of direct studies on the skin penetration of baicalein. However, there are studies showing that the skin penetration of similar flavonoid compounds can be enhanced using vesicular systems. In a study conducted by Yucel et al., aspasomes containing ferulic acid, a similar flavonoid compound, were proven to enable effective skin penetration [29]. In another study, it has been shown that the bioavailability, stability, antioxidant, antiaging, and wound-healing properties of baicalin [30], the glucuronic acid conjugate form of baicalein with higher water solubility, can be enhanced through transdermal administration using biovesicles.

Several studies have shown that niosomes can enhance the skin permeability of drugs, similar to our study [12]. Yen et al. demonstrated that the niosomal hydrogel increased the rate and amount of transport of diclofenac through the skin even compared to the commercial drug [31]. Soni et al. have shown that modifications in the formulation significantly impact the characterization of niosomes, and that well-formulated methotrexate-loaded niosomes may be effective for transdermal cancer treatment [32]. Another study has demonstrated that the use of niosomal gel improved drug penetration and, consequently, its

therapeutic efficacy compared to the conventional gel [33]. Considering all these factors, our study highlights the significance of successfully produced baicalein-loaded niosomes.

2.7. Antioxidant activity

Radical scavenging capabilities of the baicalein-loaded niosome and the niosomal gel are given in Table 2.

DPPH● radical scavenging activity above 50% is generally accepted as an indication of strong antioxidant potential [34]. As shown in Table 2, the DPPH● radical inhibition capacity of both the formulations was determined to be above 64%. Therefore, both the niosome and the niosomal gel demonstrated strong antioxidant activity. However, the ABTS+• radical inhibition effects of the formulations were found to be lower compared to their DPPH● inhibition effects ($p>0.05$). This difference may be attributed to the structural and physicochemical differences between the DPPH● and ABTS+• radicals, as well as the solubility characteristics of baicalein [35]. DPPH● is more lipophilic radical compared to ABTS+•, it tends to interact more effectively with lipophilic antioxidants. Since baicalein also has poor water solubility, it interacts more efficiently with the lipophilic DPPH● radical, leading to a higher observed inhibition compared to the ABTS+• assay. In addition, the lower radical scavenging activity of the niosomal gel could be due to the lower amount of drug loaded into the gel and the increase in viscosity caused by the gel matrix. Nevertheless, both the baicalein-loaded niosome and the niosomal gel demonstrated high inhibition of both DPPH● and ABTS+• radicals, indicating that the developed formulations possess strong antioxidant potential.

3. CONCLUSION

Baicalein-loaded niosomes were successfully prepared via the thin film hydration method. Niosomes generally exhibited an appropriate size with a narrow size distribution, as well as an ideal zeta potential close to -30 mV. Furthermore, their encapsulation efficiency was considered optimal, and they exhibited favorable in vitro release properties.

This study focused on the formulation, in vitro characterization, and evaluation of toxicity, antioxidant activity and skin penetration capabilities, with particular emphasis on assessing the effects of surfactant type and solvent property variations on formulation characteristics. Although niosomes containing Tween 60 generally exhibited larger particle sizes compared to those containing Tween 80, they had a narrower size distribution and better stability. The increase in the amount of chloroform in the solvent system also facilitated the formation of smaller particle sizes. Finally, the niosomes with a 7:3 chloroform: methanol ratio demonstrated superior in vitro release, and thus, the ideal formulation was determined to be the F2 formulation with Tween 60 and a 7:3 chloroform:methanol ratio. Afterward, baicalein-loaded niosomal gel was obtained using the F2 niosome formulation and Carbopol 980. These results indicate that the properties of the surfactant type and the solvent system used, have a significant impact on the overall characterization of niosomes including the drug release profile.

Cell culture studies have proven that baicalein-loaded niosome and the niosomal gel can be used safely, are biocompatible, penetrate into skin and have high antioxidant effects, thus accelerating skin vitality and providing a significant anti-aging effect by acting even in the lower layers of the skin.

As a conclude, these findings highlight the promising potential of baicalein-loaded niosomes/niosomal gel for transdermal drug delivery applications. Our study will contribute to the formulation of plant-derived active substances, which have several drawbacks such as stability and solubility limitations that restrict their use, into drug delivery systems, particularly baicalein-loaded niosomes/niosomal gels, and aid in their future market introduction.

4. MATERIALS AND METHODS

Baicalein, 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT), penicillin/streptomycin solution, fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM) and dimethyl sulfoxide (DMSO) for cell culture, chloroform and methanol were purchased from Sigma-Aldrich (USA). Carbopol 980 was kindly gifted from the Abdi İbrahim Pharmaceutical, Industry and Trade Company (Turkey). L929 mouse fibroblast cells were obtained from the American Type Culture Collection (ATCC® CCL-1™) (USA). Cell culture flasks and plates were purchased from Corning® (USA). Cedex, Trypan Blue solution and Smart Slides were acquired from Roche (Switzerland).

4.1. UV-Visible Spectrophotometric Analysis of Baicalein

The quantification of baicalein was performed using a UV-Vis spectrophotometer (Shimadzu 1800, Japan). The absorption spectrum of baicalein was recorded over a wavelength range of 200–600 nm. A series of baicalein solutions (1–15 µg/mL) were prepared from a stock solution. The standard calibration curve was generated using linear regression analysis, expressed by the equation: y = ax + b, where x represents the baicalein concentration (µg/mL) and y denotes the corresponding absorbance.

4.2. Niosome/niosomal gel

Niosomes were prepared using the thin-film hydration method [36]. This method is commonly used in niosome formulation involving the dissolution of surfactant and cholesterol in an appropriate organic solvent, such as chloroform, methanol, ethanol, or ether [10]. In the study, different formulations of cholesterol and non-ionic surfactants (molar ratio of 1:1) were prepared by accurately weighing the required amounts of each component and transferring them into a round-bottom flask. Subsequently, the solvent system (chloroform:methanol) was added at varying ratios (9:1, 7:3, and 5:5 v/v), and the mixture was subjected to rotary evaporation to form a thin film. Initially, the active ingredient was added during the hydration step; however, due to low encapsulation efficiency, it was instead dissolved in methanol and incorporated during the film formation process. After solvent evaporation, the resulting thin films were hydrated using an ultrasonic water bath.

To obtain a niosomal gel with the selected optimal niosome formulation, 1% w/w Carbopol 980 was used. The niosomes were incorporated into the gel, and a magnetic stirrer was used to ensure homogenization.

4.3. Cytotoxicity

The L929 mouse fibroblast cell line was cultured in DMEM enriched with 1% penicillin-streptomycin and 10% FBS in an incubator at 37 ◦C under 5% CO2 atmosphere. The medium was changed with fresh medium every 48 h. Cytotoxicity of baicalein solutions (400–25 µg/mL) and baicalein loaded optimum formulation on the L929 cell line were determined using MTT test. Cells (10.000 cells/well) were seeded in 96-well tissue culture plates for testing and incubated overnight in the incubator. After the medium was removed, 100 µL of the samples was added to the wells and incubated for 24 hours in the incubator. After the incubation, the contents of the wells were removed, and 100 µL of fresh DMEM and 13 µL of MTT solution was added. The plates were then tightly wrapped with aluminum foil and incubated for 2 hours at 37°C in a 5% CO_2 environment. At the end of the incubation period, the contents of the wells were discarded, and 100 µL of DMSO was added. The wells containing only the culture medium were used as the control group, with cell viability set at 100%, and the results were expressed as a percentage. The resulting purple color was measured spectrophotometrically at 570 nm using a microplate ELISA reader (Biotech Synergy HT, USA) [23].

4.4. Particle size, polydispersity index, zeta particle

The PS, PDI, and ZP of the niosomes were analyzed using a Zetasizer (Malvern, Nano ZS90) (n=3). The results were reported as mean ± standard deviation.

4.5. Encapsulation efficiency and drug release

The prepared niosomes were vortexed for 5 minutes to achieve homogeneity. 1 mL of the niosomal suspension centrifuged at 15.000 rpm for 30 minutes at 4°C. The supernatant was collected, and the amount of unencapsulated baicalein in the niosomes was analyzed using a UV spectrophotometer. Encapsulation efficiency was determined using the equation provided below (n=3). The results were reported as mean ± standard deviation.

Encapsulation Efficiency (EE%) = (Total baicalein amount−Unencapsulated baicalein in the supernatant)/Total baicalein amount X 100

In vitro release studies were conducted using the dialysis membrane method [25]. Baicalein-loaded niosomes were placed into a dialysis membrane and transferred into a tube. Then, 2 mL of distilled water was added, and the tube was placed in a shaking water bath set at 37°C with a shaking rate of 50 rpm. At predetermined time intervals (1, 2, 4, 8, and 24 hours), the release medium was collected and replaced with an equal volume of fresh distilled water to continue the measurement for the subsequent time points. The in vitro release of the active ingredient was analyzed using the collected samples. The amount of released active ingredient was determined using a validated quantification method with a UV spectrophotometer (n=3).

4.6. Viscosity of niosomal gel

The viscosity of the niosomal gel was measured using Brookfield DV3T Rheometer (USA) at 25°C with spindle CP-52, across a shear rate range of 50–500 s^{-1}. The results were reported as the mean ± standard deviation (n=3).

4.7. Cell permeation studies

L929 mouse fibroblast cells were used for the cell permeation studies. The cells were cultured as a monolayer on specialized six-well cell culture inserts with a pore size of 0.4 µm. Once the monolayer was established, the inserts were positioned between the apical and basolateral compartments of the diffusion chamber, and the experiment was initiated. The temperature of the system was maintained at 37°C. The experimental duration was determined to align with the in vitro release study. At the end of the experiment, the amount of baicalein that permeated from the apical to the basolateral compartment was quantified using a UV spectrophotometer (n=3) [37].

4.8. Antioxidant activity

4.8.1. DPPH● Radical Scavenging Activity

DPPH● radical scavenging effects of the samples (the amount of baicalein that permeated through L929 cells from the formulations) were determined using the method proposed by Gyamfi and colleagues [38]. The samples and Tris-HCl buffer (50 nM, pH 7.4) were mixed and a DPPH solution (0.1 mM) was formed in methanol. Absorbance was measured at 517 nm via UV spectrophotometer, after 30 min incubation period in darkness at room temperature (n=3).

4.8.2. ABTS●+ Radical Scavenging Activity

ABTS●+ radical scavenging effects of the samples (the amount of baicalein that permeated through L929 cells from the formulations) were determined using the method proposed by Re and colleagues [39]. An ABTS+ radical solution (7 mM) with the absorbance adjusted to 0.700 (±0.030) at 734 nm was obtained for analysis. The reaction kinetics were monitored and documented at 734 nm in 1 min intervals for 30 min, utilizing 990 µL of the freshly prepared radical solution and 10 µL of the sample solutions (n=3).

4.9. Statistical analyses

Statistical analyses were performed using SPSS version 27.0 for Windows. One-way ANOVA was employed to assess statistical significance, with a p-value of less than 0.05 considered statistically significant. Data are presented as mean ± standard deviation (SD).

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REFERENCES

[1] Chmiel M, Stompor-Goracy M. Promising Role of the *Scutellaria baicalensis* Root Hydroxyflavone-Baicalein in the Prevention and Treatment of Human Diseases. Int J Mol Sci. 2023;24(5):4732. https://doi.org/10.3390/ijms24054732
[2] Lei C, Yu Y, Zhu Y, Li Y, Ma C, Ding L, Zhang H. The most recent progress of baicalein in its anti-neoplastic effects and mechanisms. Biomed Pharmacother. 2024;176:116862. https://doi.org/10.1016/j.biopha.2024.116862
[3] Shang X, He X, Li M, Zhang R, Fan P, Zhang Q, Jia Z. The genus *Scutellaria*: an ethnopharmacological and phytochemical review. J Ethnopharmacol. 2010;128(2):279-313. https://doi.org/10.1016/j.jep.2010.01.006
[4] Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021;20(3):200-216. https://doi.org/10.1038/s41573-020-00114-z
[5] Li W, Pi J, Zhang Y, Ma X, Zhang B, Wang S, Qui D, Li N, Guo P, Liu Z. A strategy to improve the oral availability of baicalein: The baicalein-theophylline cocrystal. Fitoterapia. 2018;129:85-93. https://doi.org/10.1016/j.fitote.2018.06.018

[6] Rahman HS, Othman HH, Hammadi NI, Yeap SK, Amin KM, Abdul Samad N, Alitheen NB. Novel drug delivery systems for loading of natural plant extracts and their biomedical applications. Int J Nanomedicine. 2020;15:2439-2483. https://doi.org/10.2147/IJN.S227805
[7] Zang J, Zhang L, Guo R, Kong L, Yu Y, Li S, Liu M, Wang J, Zhang Z, Li X, Liu Y. Baicalein loaded liposome with hyaluronic acid and polyhexamethylene guanidine modification for anti methicillin-resistant *Staphylococcus aureus* infection. Int J Biol Macromol. 2024;276(Pt 1):133432. https://doi.org/10.1016/j.ijbiomac.2024.133432
[8] Markowski A, Zaremba-Czogalla M, Jaromin A, Olczak E, Zygmunt A, Etezadi H, Boyd BJ, Gubernator J. Novel liposomal formulation of baicalein for the treatment of pancreatic ductal adenocarcinoma: design, characterization, and evaluation. Pharmaceutics. 2023;15(1):179. https://doi.org/10.3390/pharmaceutics15010179
[9] Kim AR, Lee NH, Park YM, Park SN. Preparation and characterization of novel pseudo ceramide liposomes for the transdermal delivery of baicalein. J Drug Deliv Sci Technol. 2019;52:150-156. https://doi.org/10.1016/j.jddst.2019.04.009
[10] Liga S, Paul C, Moaca EA, Peter F. Niosomes: composition, formulation techniques, and recent progress as delivery systems in cancer therapy. Pharmaceutics. 2024;16:223. https://doi.org/10.3390/pharmaceutics16020223
[11] Kaur P, Muskan, Kriplani P. Quality by design for niosome-based nanocarriers to improve transdermal drug delivery from lab to industry. Int J Pharm. 2024;666:124747. https://doi.org/10.1016/j.ijpharm.2024.124747
[12] Muzzalupo R, Tavano L. Niosomal drug delivery for transdermal targeting: recent advances. Res Rep Transdermal Drug Deliv. 2015;4:23-33. https://doi.org/ 10.2147/rrtd.S64773
[13] Yucel C, Seker Karatoprak G, Degim IT. Anti-aging formulation of rosmarinic acid-loaded ethosomes and liposomes. J Microencapsul. 2019;36(2):180-191. https://doi.org/10.1080/02652048.2019.1617363
[14] Yang J, Luo J, Tian X, Zhao Y, Li Y, Wu X. Progress in understanding oxidative stress, aging, and aging-related diseases. Antioxidants. 2024;13(4):394. https://doi.org/10.3390/antiox13040394
[15] Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-772. https://doi.org/10.2147/CIA.S158513
[16] Tumilaar SG, Hardianto A, Dohi H, Kurnia D, Ahmed M. A comprehensive review of free radicals, oxidative stress, and antioxidants: overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. J Chem. 2024;2024: 5594386. https://doi.org/10.1155/2024/5594386
[17] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, Khorasani S, Mozafari MR. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018;10(2):57. https://doi.org/ 10.3390/pharmaceutics10020057
[18] U.S. Food and Drug Administration (FDA). Liposome drug products chemistry, manufacturing, and controls; human pharmacokinetics and bioavailability; and labeling documentation. 2018. http://www.fda.gov (accessed May 3, 2025).
[19] Mawazi SM, Ge Y, Widodo RT. Niosome preparation techniques and structure—an illustrated review. Pharmaceutics. 2025;17(1):67. https://doi.org/10.3390/pharmaceutics17010067
[20] Kutsyk A, Ilchenko O, Pilhun Y, Nikonova V, Obukhovsky V. Complex formation in methanol-chloroform solutions: vibrational spectroscopy and quantum cluster equilibrium study. J Mol Liq. 2022;367:120499. https://doi.org/10.1016/j.molliq.2022.120499
[21] Lee WL, Loei C, Widjaja E, Loo SC. Altering the drug release profiles of double-layered ternary-phase microparticles. J Control Release. 2011;151(3):229-238. https://doi.org/10.1016/j.jconrel.2011.02.012
[22] Nemeth Z, Csoka I, Semnani Jazani R, Sipos B, Haspel H, Kozma G, Konya Z, Dobo DG. Quality by design-driven zeta potential optimisation study of liposomes with charge imparting membrane additives. Pharmaceutics. 2022;14:1798. https://doi.org/ 10.3390/pharmaceutics14091798
[23] Eren Boncu T, Yucel C, Ceylan A, Cadir M, Sahmetlioglu E. Formulation, characterization and evaluation of minocycline hydrochloride loaded polyurethane/collagen nanofibers via electrospinning as wound dressings. Drug Dev Ind Pharm. 2024;50(10):892-906. https://doi.org/ 10.1080/03639045.2024.2426581
[24] Obeid MA, Khadra I, Aljabali AAA, Amawi H, Ferro VA. Characterisation of niosome nanoparticles prepared by microfluidic mixing for drug delivery. Int J Pharm X. 2022;4:100137. https://doi.org/10.1016/j.ijpx.2022.100137
[25] Shiri S, Gharanjig K, Tahghighi A, Hosseinnezhad M, Etezad M. Formulation and characterization of BBR loaded niosomes using saponin as a nonionic biosurfactant investigating synergistic effects to enhance antibacterial activity. Sci Rep. 2025;15(1):5231. https://doi.org/10.1038/s41598-025-87950-4
[26] Gupta S, Rather SR, Sen P. Dynamics of solvent response in methanol-chloroform binary solvent mixture: a case of synergistic solvation. J Phys Chem B. 2015;119(7):3135-3141. https://doi.org/ 10.1021/jp5120338
[27] Eren Böncü T, Yücel Ç. Development and characterization of ascorbic acid loaded polyelectrolyte chitosan-gelatin hydrogels. Ankara Univ Ecz Fak Derg. 2023;47(3):719-730. https://doi.org/10.33483/jfpau.1269790
[28] Asghar H, Riaz T, Mannan HA, Khan SM, Butt OM. Rheology and modeling insights into dye-sensitized solar cells (DSSCs) material: bridging the gap to solar energy advancements. Renew Sustain Energy Rev. 2024;193:114298. https://doi.org/10.1016/j.rser.2024.114298
[29] Yücel Ç, Şeker Karatoprak G, Ilbasmis-Tamer S, Değim İT. Ferulic acid-loaded aspasomes: a new approach to enhance the skin permeation, anti-aging and antioxidant effects. J Drug Deliv Sci Technol. 2023;86:104748. https://doi.org/10.1016/j.jddst.2023.104748

[30] Chen L, Wang F, Hu X, Li N, Gao Y, Xue F, et al. Transdermal delivery of baicalin based on bio-vesicles and its efficacy in antiaging of the skin. J Cosmet Dermatol. 2025;24(2):e70024. https://doi.org/10.1111/jocd.70024
[31] Yen Thi Hai T, Giang Ngoc T, Anh Lan H, Giang Thi Thu V. Niosomes loaded with diclofenac for transdermal administration: physico-chemical characterization, ex vivo and in vivo skin permeation studies. J Appl Pharm Sci. 2020;10(12):53-61. https://doi.org/ 10.7324/japs.2020.101207
[32] Soni S, Baghel K, Soni ML, Kashaw SK, Soni V. Size-dependent effects of niosomes on the penetration of methotrexate in skin layers. Future J Pharm Sci. 2024;10(1):48. https://doi.org/10.1186/s43094-024-00624-2
[33] Solanki A, Parikh JR, Parikh RH, Patel MR. Evaluation of different compositions of niosomes to optimize aceclofenac transdermal delivery. Asian J Pharm Sci. 2010;5(3):87-95.
[34] Hozan F, Uslu EN, Karatoprak GŞ, Yücel Ç. Baicalein-loaded aspasomal formulations: development, characterization and evaluation of antioxidant and anti-inflammatory effects. J Pharm Innov. 2025;20(2):872-882. https://doi.org/10.1007/s12247-025-09979-2
[35] Wolosiak R, Druzynska B, Derewiaka D, Piecyk M, Majewska E, Ciecierska M, Worobiej E, Pakosz P. Verification of the conditions for determination of antioxidant activity by ABTS and DPPH assays—a practical approach. Molecules.2021;27(1):50. https://doi.org/10.3390/molecules27010050
[36] Ergin AD, Oltulu C, Turker NP, Demirbolat GM. In vitro hepatotoxicity evaluation of methotrexate-loaded niosome formulation: fabrication, characterization and cell culture studies. Turk J Med Sci. 2023;53(4):872-882. https://doi.org/ 10.55730/1300-0144.5651
[37] Yucel C, Seker Karatoprak G, Yalcintas S, Eren Boncu T. Ethosomal (-)-epigallocatechin-3-gallate as a novel approach to enhance antioxidant, anti-collagenase and anti-elastase effects. Beilstein J Nanotechnol. 2022;13:491-502. https://doi.org/ 10.3762/bjnano.13.41
[38] Gyamfi M, Yonamine M, Aniya Y. Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. Gen Pharmacol. 1999;32:661-667.
[39] Re R, Pellegrini NP, Proteggente A. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9–10):1231-1237.