ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 2 - Vol. 30
3/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

3B QUANG TRUNG - HOAN KIEM - HANOI - VIETNAM
TEL: (+84-24) 38247058 FAX: (+84-24) 39348740
Email: tapchiduoclieu@nimm.org.vn; tapchiduoclieu@gmail.com

CONTENTS VOL. 30(2), 2025
(Pages 65 - 144)

Page	SCIENTIFIC RESEARCH

Journal of Medicinal Materials, 2025, Vol. 30, No. 2 (pp. 132 - 137)

TRANSFERSOME FOR SKIN DELIVERY OF *CENTELLA ASIATICA* EXTRACT BY THIN FILM HYDRATION

Nguyen Hong Van[1,*], Nguyen Minh Chau[1], Doan Duc Ngoc Minh[1], Phan Thanh Thuy[2], Do Thi Thuy Linh[2], Nguyen Tuan Hiep[2]

[1]*Life Sciences Department, University of Science and Technology of Ha Noi, Hanoi, Vietnam*
[2]*Department of Extraction Technology, National Institute of Medicinal Materials (NIMM), Hanoi, 11022, Vietnam*

*Corresponding author: chodomthanyeu@gmail.com

Received March 14th, 2025 Accepted March 29th, 2025

Summary

Transfersome for Skin Delivery of *Centella asiatica* Extract by Thin Film Hydration

Centella asiatica is a traditional Vietnamese herb, which poses outstanding effectiveness in the treatment of wound healing, especially in infected wounds. However, the unpleasant physical properties of the bioactive chemicals in its extract, including asiaticoside, such as high molecular weight, low solubility, and permeability, limit the therapeutic applications. Among various nanocarriers, transfersome – a promising nanovesicle was prepared to improve the skin delivery of the extract from this herb. Asiaticoside transfersome consisted of soybean lecithin and different surfactants, like salts of fatty acid, hydrophilic emulsifiers, and hydrophobic emulsifiers, by thin film hydration method and ultrasonication. The obtained transfersomes had a size below 150 nm, homogenous distribution and the absolute value of zeta potential above 30 mV. The entrapment efficiency, based on asiaticoside quantity, was nearly 85%, and drug loading was above 30%. Sodium dodecyl sulfate was the optimum surfactant, promoting the highest drug permeability of *Centella* transfersomes through the dialysis membrane and mouse skin. Moreover, these vesicles remained stable until thirty days at 25°C. Therefore, asiaticoside transfersomes could be considered as a potential drug delivery system via skin.

Keywords: *Centella asiatica; Asiaticoside; Transfersomes.*

1. Introduction

Centella asiatica (L.) is a traditional herb for wound healing, diabetes, inflammation, and depression. *C. asiatica* (L.) extract has effective treatment on ulcerous skin abnormalities, even in diabetic rats/patients, burns-related wounds, duodenal and stomach ulcers [1]. These effects come from the presence of asiaticoside, a triterpene glycoside compound found in various plant species, most notably in this herb. However, asiaticoside is a pentacyclic triterpene with a high molecular weight of 959.12 g mol^{-1} and is difficult to ionise; its maximum solubility in water is 307.347 µg/mL and it has a short plasma half-life. The equilibrium oil-water partition coefficient is 2.24. Hence, the therapeutic use of asiaticoside is limited by its low solubility and poor penetration across the skin. Therefore, improving the loading and release management of asiaticoside would lead to better results for dermal medication.

Nanotechnology enables the delivery of drugs to the target site and increases its efficacy, stability, and safety. Among nanocarriers for skin delivery, transfersomes could be considered as one of the most innovative and potential drug delivery systems [2]. Along with certain advantages of nanosystems, this nanocarrier owns other superpriorities. Transfersomes are classified into nanovesicles, which form from phosphatidylcholine and single–chain surfactant (Fig. 1.). Compared to traditional liposomes, their bilayered structure allows for the encapsulation of hydrophilic, lipophilic, and amphiphilic drugs with improved skin penetration efficiencies. Transfersomes can penetrate the intact stratum corneum spontaneously along two routes in the intracellular lipid that differ in their bilayer properties. Because of their elastic nature, transfersomes can compress and deform through tiny pores that are much smaller than their actual size while still remaining an entire vesicle. Phosphatidylcholine remains the biocompatibility transfersomes with the cellular membrane. While single-chain surfactants increase the flexibility and deformation of these nanovesicles, resulting in dermal higher permeability, as well as drug loading capacity and stability.

Transfersomes were used to deliver many natural bioactive compounds through the skin, such as resveratrol [3], curcumin [4], etc. Transfersomes loading *Centella* extract was fabricated from lecithin, with Tween 80 or Span 80, sodium deoxycholate, by high pressure homogenization. The resulting transfersomes had a good size range and around 6% loading of asiatic acid [5]. Another study about *Centella* extract transfersomes had the highest entrapment efficiency of around 85% and D90 of 204.3 nm

with fixed Tween 80 as surfactant and various investigated loaded asiaticoside quantities [6]. In this study, transfersomes were prepared from soybean phosphatidylcholine and various surfactants, using the thin-film hydration and ultrasonication method (Fig. 1.). Many more surfactants were investigated in formation of this asiaticoside nanovesicles, including salts of fatty acid: sodium lactate, sodium caprylate, sodium oleate, hydrophilic surfactants: Tween 20, Tween 80 and sodium dodecyl sulfate (SDS), as well as hydrophobic surfactants: Span 60, Span 80 and sodium docusate. The obtained transfersomes had a significant drug loading of asiaticoside at more than 30% (w/w).

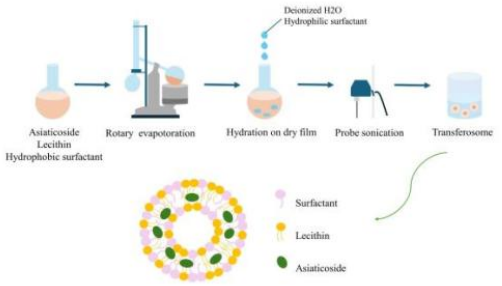

Fig. 1. Preparation of asiaticoside transfersomes

2. Materials and methods

2.1. Materials

Centella asiatica L. extract from above ground part (28% asiaticoside), phosphatidylcholine (soybean lecithin), sodium oleate, sodium caprylate, sodium dodecyl sulfate, sodium docusate from, and sodium lactate, Tween 80, Tween 20, Span 80, Span 60 from Shanghai Zhanyun Chemical. Absolute ethanol was from GHTECH, while chloroform was from Fisher. Distilled water was used in all samples.

2.2. Methods

2.2.1. Preparation of asiaticoside transfersomes:

Asiaticoside transfersomes were prepared following thin-film hydration (Fig. 1.). Firstly, asiaticoside and hydrophobic surfactants were dissolved in ethanol, while lecithin was dissolved in chloroform by IKA magnetic stirrer. Then, the obtained mixture was vapored by a rotational evaporator at 35^{0}C with 50 rpm and the pressure under 100 Pa (IKA rotary evaporator). Subsequently, the formed thin film was hydrated by water and hydrophilic surfactant at 38°C for 30 mins, at the rotary speed of 20 rpm. Lastly, the collected liquid was subjected to a probe sonicator with the parameters of 150W power in 30 seconds (VCX 500 Sonicator). The sample was stored in a glass bottle for further study.

2.2.2. Determination of transfersome particle size, polydispersity index, and zeta potential:

Size and polydispersity index (PDI) were measured using dynamic light scattering (DLS) with the Nano particle analyzer (Japan, Horiba, Model: SZ-100-Z2). Zeta potential was measured at the same dilution concentration. Each sample was carried out in triplicate.

2.2.3. Morphology:

The visualization of transfersome samples was investigated by transmission electron microscopy (TEM) (JEOL Inc, Jem – 1400).

2.2.4. Asiaticoside quantification:

The asiaticoside concentration was analyzed using the high performance liquid chromatography (HPLC) method. Column C18, 250*4.6 mm, 5 µm was used with detector at 205 nm. The mobile phase included acetonitrile (channel A) and H_3PO_4 0.15% (channel B) following a gradient program at the rate of 1.0 mL/min. Specifically, from 0-15 min, channel A: channel B at 21:79 then from 15-42 min, channel A went up from 21 to 36%, while channel B went down from 79 to 64%. The injection volume was 20 µL (Shimadzu SPD-20A).

2.2.5. Entrapment efficiency (EE) and loading capacity (LC):

The weight of the loaded *C. asiatica L.* extract was calculated based on asiaticoside in the transfersome by ultrafiltration. The sample was subjected to the filter (10000Da), at a centrifugation speed of 10000g in 10 mins (Eppendorf Centrifuge 5425 R). The asiaticoside in the filtrate was measured by the HPLC method. The quantification of asiaticoside in the transfersomes was calculated by multiplying the weight of detected asiaticoside in the filtrate ($m_{filtrate}$) from the initial input weight of asiaticoside ($m_{initial}$). The entrapment efficiency (%EE) and loading capacity (%LC) of prepared nanovesicles were determined by the following equations:

$$\%EE = (m_{initial} - m_{filtrate})/(m_{initial}) \times 100\%$$

$$\%LC = (m_{initial} - m_{filtrate})/(m_{initial} - m_{filtrate} + m_{excipient}) \times 100\%$$

2.2.6. *In - vitro* drug release:

The *in-vitro* release behavior of the transfersome was evaluated using a Franz diffusion cell apparatus. A dialysis membrane was positioned between the donor and receptor compartments. The phytosomal asiaticoside with the same quantify of 100 ug asiaticoside, was placed in the donor compartment, while the receptor compartment was filled with phosphate buffer saline. The system was maintained at a constant temperature of 37 ± 0.5°C to simulate physiological conditions. At predetermined time intervals, aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh media to maintain sink conditions. The samples were quantified using the HPLC method. The factor f_1, known as the dissimilarity factor, was used to measure the difference between the two profiles:

$$f_1 = 100.\frac{\sum_{i=1}^{n}\left|R_i^{Curve1} - R_i^{Curve2}\right|}{\sum_{i=1}^{n} R_i^{Curve1}}$$

where n is the number of time points, R_i^{Curve1} and R_i^{Curve2} were mean percentage of released bioactive chemicals from the first and the second transfersome formula at time point i with $R_{8(24h)}^{Curve1} > R_{8(24h)}^{Curve2}$.

2.2.7. Statistical analysis:

The results are reported as mean ± standard deviation. All analyses were carried out using the Excel software package (Microsoft Corp, Redmond, WA, USA) with built-in statistical tools. A significance level of $p < 0.05$ was considered for all tests, and each test was conducted in triplicate.

3. Results and discussions

3.1. Effect of the ratio between asiaticoside and lecithin on asiaticoside nanovesicles physical properties

The ratios between asiaticoside and lecithin were investigated in the range from 1:1 to 1:4 to prepare asiaticoside nanovesicles by thin film hydration (Table 1). The probe sonicator was employed to reduce particle size and achieve homogenization of the nanovesicles [7]. The higher the lecithin quantity, the larger the nanovesicles size, as the size gained from 109.10 ± 4.01 nm at the ratio between asiaticoside and lecithin of 1:1 up to 143.53 ±7.31 at the ratio of 1:4. The PDI gradually reduced and the absolute value of zeta potential increased when the lecithin amount rose. At the ratio 1:2 of asiaticoside: lecithin, the size of asiaticoside nanocarriers was below 120 nm and the PDI nearly 0.2, with EE at around 87% and the LC above 34%. Hence, the ratio 1:2 was chosen for further study.

Table 1. Asiaticoside transfersomes physical properties at various ratios of asiaticoside and lecithin (molar ratio)

Asiaticoside: lecithin	1:1	*1:2*	1:3	1:4
Size (nm)	109.10 ± 4.01	115.30 ± 1.55	125.30 ± 3.91	143.53 ±7.31
PDI	0.24 ± 0.04	0.19 ± 0.06	0.17 ± 0.03	0.15 ± 0.08
Zeta potential (mV)	-29.65 ±0.21	-35.75 ± 4.17	-35.45 ± 3.49	-36.8 ± 0.35
EE (%)	86.54 ± 0.74	87.72 ± 0.69	86.01 ± 0.82	85.91 ± 0.76
LC (%)	48.42 ± 0.24	34.07 ± 0.64	25.59 ± 0.32	20.71 ± 0.28

In order to fabricate asiaticoside transfersomes, different surfactants were added in the formulations, including salts of fatty acid – sodium lactate, sodium caprylate, sodium oleate, hydrophobic surfactants – Span 60, 80, sodium docusate, and hydrophilic surfactants – Tween 20, 80 and sodium dodecyl sulfate at the molar ratio of 1:2 in comparison with lecithin.

3.2. Effect of the salts of fatty acid on asiaticoside transfersome physical properties

Sodium lactate, sodium caprylate, and sodium oleate were the salts of low-chain fatty acid to medium and long–chain fatty acid, which are familiar edge activators in transfersome as well as usual skin penetrants [8]. The incorporation of these salts induced a higher absolute value of transfersomes, approximately 40 mV (Table 2), compared with the above asiaticoside nanovesicles (Table 1). Three tranferosomes had acceptable size, and the entrapment efficiency around 30%, while sodium lactate had the optimum PDI 0,16. Therefore, sodium lactate would be presented as a salt of fatty acid to prepare this nanovesicle for further study.

Table 2. Asiaticoside transfersomes physical properties with different salts of fatty acid

Salt of fatty acid	*Sodium lactate*	Sodium caprylate	Sodium oleate
Size (nm)	129.00 ± 0.96	121.87 ±1.21	144.67 ± 0.74
PDI	0.16 ± 0.01	0.49 ± 0.02	0.31 ± 0.01
Zeta potential (mV)	-42.50 ± 0.14	-38.60 ± 2.55	-37.80 ± 0.57
EE (%)	85.87 ± 4.30	86.66 ± 1.40	82.29 ± 1.44
LC (%)	31.32 ± 0.60	31.56 ± 0.51	28.49 ± 4.71

3.3. Effect of the hydrophobic surfactants on asiaticoside transfersome physical properties

Sodium docusate, Span 60, and Span 80 are hydrophobic surfactants, with hydrophilic - lipophilic balance exceeding 10. The transfersomes consisting of these surfactants had a good size of below 130 nm (Table 3). However, the homogeneity of these nanovesicles was not well obtained, as the PDI was 0.42. Only Span 80 generated an acceptable homogenous distribution of transfersomes, with EE at 85.31 ± 4.86 %. This result was in agreement with previous studies, in which Span 80 was presented in the best formulation with a vesicle size of 2.56 ± 0.21 µm and the highest drug entrapment efficiency of ketoconazole at 86.4 ± 0.61% [9]. Thus Span 80-containing transfersomes were chosen for further evaluation of asiaticoside delivery.

Table 3. Asiaticoside transfersomes physical properties at different hydrophobic surfactants

Hydrophobic surfactant	Sodium docusate	Span 60	*Span 80*
Size (nm)	107.47 ± 2.71	129.8 ± 1.40	108.15 ± 0.78
PDI	0.42 ± 0.03	0.38 ± 0.01	0.31 ± 0.02
Zeta potential (mV)	-62.8 ± 2.55	-46.65 ± 3.32	-41.25 ± 5.34
EE (%)	87.23 ± 2.60	80.96 ± 4.22	85.31 ± 4.86
LC (%)	31.99 ± 0.54	31.73 ± 0.21	32.07 ± 0.96

3.4. Effect of the hydrophilic surfactant on asiaticoside transfersome physical properties

Hydrophilic surfactants, like Tween 20, 80 also used to form transfersomes loading resveratrol [3], curcumin [4], etc. In comparison with hydrophobic surfactants, these formed asiaticoside with better size below 120 nm and narrower particle polydispersion (Table 4). However, the zeta potential values were lower than the counterparts, around 30 mV and more than 40 mV, respectively (Table 3). Additionally, although the EE is reasonable in comparison with transfersomes consisted salts of fatty acid or hydrophobic surfactants, the loading capacity of hydrophilic surfactant transfersomes was lower due to the considerably larger molecular weight of hydrophilic surfactants than others. Transfersomes made of Tween 80 were the best formulation for the delivery of declomethasone dipropionate to the lungs [10]. In this study, sodium dodecyl sulfate was chosen to fabricate asiaticoside transfersome for further investigation based on the homogeneity and highest loading capacity (Table 4). Sodium dodecyl sulfate improved the stability and skin permeability of salidroside nanovesicles in previous study [11].

Table 4. Asiaticoside transfersomes physical properties at different hydrophilic surfactants

Hydrophilic surfactant	Tween 80	Tween 20	*Sodium dodecyl sulfate*
Size (nm)	112.00 ± 2.26	101.55 ± 1.20	115.20 ± 1.56
PDI	0.39 ± 0.01	0.36 ± 0.01	0.25 ± 0.04
Zeta potential (mV)	-30.65 ± 5.18	-29.55 ± 5.87	-33.5 ± 3.96
EE (%)	93.06 ± 0.59	83.60 ± 1.80	83.76 ± 1.60
LC (%)	23.64 ± 0.01	21.73 ± 0.13	29.13 ± 0.55

To sum up, asiaticoside transfersomes were successfully prepared, with various surfactant types. Generally, the size of these nanovesicles was below 150 nm with acceptable polydispersion and quite great entrapment efficiency, about 85%, with a nearly round shape (Fig. 2.). All formulas, consisting of anionic surfactants – salts of fatty acid or non–ionic surfactants – Tween, Span, were negatively charged, with absolute value more than 30 mV, which in agreement with the previous study [12]. Among them, transfersomes with sodium lactate, Span 80, and sodium dodecyl sulfate were chosen for further investigation.

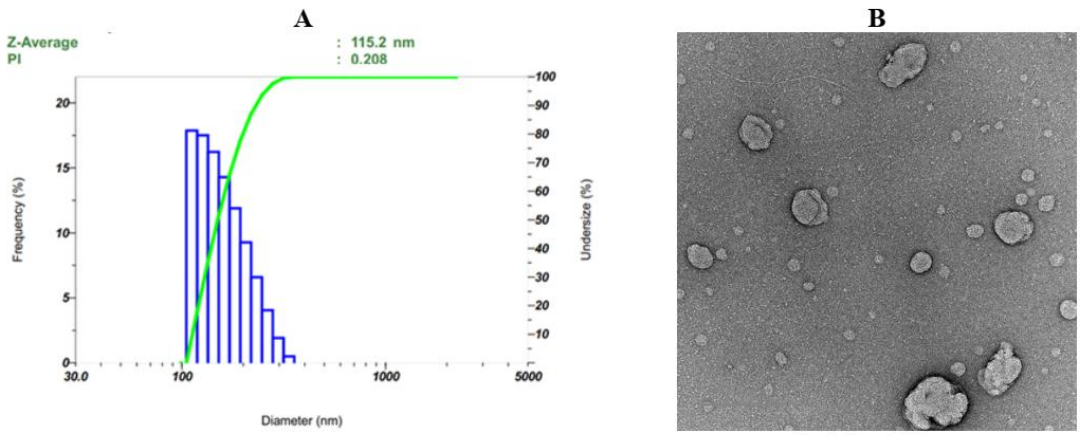

Fig. 2. Asiaticoside transfersome size distribution (A) and TEM image (B)

3.5. Short-term stability of asiaticoside transfersome

The changes in physical properties and entrapment efficiency were briefly evaluated in 30 days, at room condition (Table 5). All formulas gained the size, for example, sodium lactate transfersomes had the size increase from 129.00 ± 0.96 nm to 137.70 ± 5.91 nm. The entrapment efficiency was slightly reduced in all asiaticoside nanovesicles, except for sodium dodecyl sulfate transfersomes. This result confirmed the stabilized effect of this excipient for nanovesicles [11].

Table 5. Asiaticoside transfersomes physical properties at 30 days

	Sodium lactate	**Span 80**	**Sodium dodecyl sulfate**
Size (nm)	137.70 ± 5.91	125.50 ± 3.69	127.90 ± 6.67
PDI	0.29 ± 0.12	0.25 ± 0.05	0.26 ± 0.06
Zeta potential (mV)	-43.90 ± 5.04	-41.45 ± 4.74	-39.2 ± 3.82
EE (%)	82.32 ± 0.06	83.22 ± 1.93	83.79 ± 1.15
LC (%)	30.57 ± 1.19	27.56 ± 0.87	29.15 ± 0.11

3.6. Drug release of asiaticoside transfersome

The drug release of *C. asiatica* L. extract was evaluated following the Franz–cell model with a dialysis membrane and mouse skin. In both membranes, transfersomes improved the drug penetration compared to asiasicoside (Fig. 3.). Sodium dodecyl sulfate increased the asiaticoside release from transfersome the most, followed by sodium lactate and Span 80. These nanovesicles gained nearly 4 times at 24 hours through dialysis membrane and 3 times through mouse skin. The dissimilarity factors f_1 of transfersomes compared with asiaticoside were 66.27%, 103.91%, and 93.69% with sodium lactate, sodium dodecyl sulfate, and span 80, respectively. The values of f_1 were all far from the range of 1-15, indicating the significant improvement of asiaticoside release using transfersomes. Ivabradine HCl transfersomes also had the best formulation with sodium dodecyl sulfate as surfactant, at drug: phospholipid and surfactant: phospholipid ratio of 1:20 and 75:25 w/w, respectively. These transfersomes had 82% drug entrapment efficiency and 66.7% after 24 hours [13]. The cell survival of HaCaT and CCCESF, which were skin–model cells, was above 80% for optimum formulations containing sodium dodecyl sulfate. Moreover, these nanovesicles showed no skin irritation in rat skin with once-a-day topical application [11]. Additionally, there was unnoticeable irritation or allergic reaction between transfersomes consisting of sodium dodecyl sulfate with male rabbit skin [13]. Therefore, the drug release of asiaticoside transfersomes supported the effectiveness of sodium dodecyl sulfate in drug permeability enhancement.

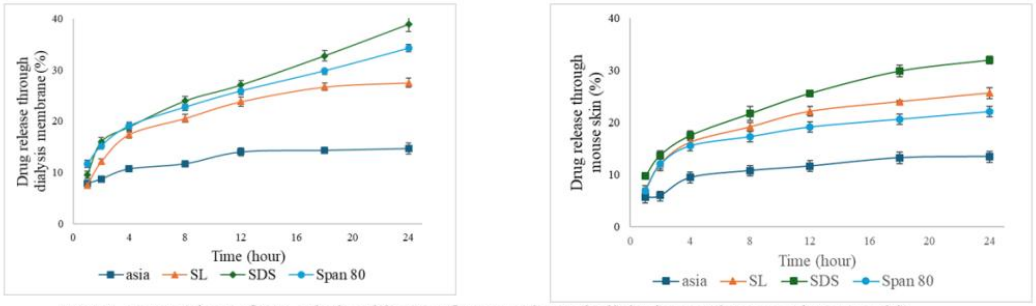

Fig. 3. Drug release from asiaticoside transfersome through dialysis membrane and mouse skin

4. Conclusions

Centella extract-loaded transfersomes were favorably fabricated with different surfactants, including salts of fatty acid, hydrophobic surfactants, and hydrophilic surfactants. All formulas had a good size, below 150 nm, and almost had homogenous polydispersion, with a high absolute value of zeta potential. The entrapment efficiency of asiaticoside transfersomes was above 80% and drug loading capacities were in the range of 25-35%. Among them, sodium dodecyl sulfate presented as the most stable transfersomes, with almost no change in drug loading capacity after 30 days. Moreover, this surfactant also enhanced the best drug release through *in–vitro* skin models with dialysis membrane and mouse skin. Therefore, transfersomes, especially with sodium dodecyl sulfate could be considered as the potential nanocarrier for skin delivery of *Centella asiatica L.* extract.

Conflicts of interest: *None.*

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