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Development of Curcumin Loaded-Nanovesicles for Antiaging Cosmetics: Liposomes, Transfersomes, Ethosomes and Niosomes

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Introduction

Transfersomes®, first introduced in the early 1990s, could deliver drugs into deep skin region[(1)]. They have elastic properties which make them easily squeeze themselves easily through the outer most of skin barrier, the stratum corneum. The possible mechanism of transfersomesis a generation of the osmotic gradient as the transpiration of skin moisture. Several researches reveal that the incorporation of an edge activator in the vesicle bilayer can modify the elastic properties of conventional liposomes. The essential compositions of these vesicles were phospholipid and/or cholesterol as membrane stabilizerand a small amount of edge activator as penetration enhancer (PE). During the last three decades, numerous types of vesicle formulations are considered to add the PE in formulations because the intrinsic properties of vesicles are able to change with the incorporation of various PEs in vesicle bilayers. A single-chain surfactant, nonionic surfactant, ethanol, terpenes, menthol, fatty acids (as PE) have been incorporated into new generation of liposomes such as transfersomes,[(2, 3)] noisomes,[(4)] ethosomes,[(5, 6)] flexosomes,[(7, 8)] invasomes,[(9)] menthosomes,[(10)] transethosomes,[(11)] transinvasomes.[(12)] However, the effectiveness of nano-vesicles on the dermal delivery remains a much-debated question and has to be demonstrated on a case-by-case basis. There are several skin models and conditions to investigate the influences of different PE incorporation into liposomes. It causes a problem to compare the effect of liposome formulations incorporating different PE. To date, the comparison of the effects of individual PE on the intrinsic properties of liposomes has not yet been established. In this study, four types of nano-vesicles, e.g. conventional liposomes (CLP), transfersomes (TFS), ethosomes (ETS) and niosomes (NS) were prepared as dermal delivery carriers of curcumin (CUR). The liposomes composition ratio was obtained from the optimization process and the experimental design. The influences of the PE on the physicochemical characteristics (e.g., vesicle size, size distribution, zeta potential and entrapment efficiency), *in vitro* skin permeation, antioxidant activity and stability of the nano-vesicles (CLP, TFS, ETS and NS) were investigated.

Methods

Materials

Phosphatidylcholine (Phospholipon 90G, Lipoid; PC) was sponsored as a special gift from LIPOID GmbH (Cologne, Germany). Cholesterol (CHOL) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Curcumin (CUR) and oleic acid (OA) were purchased from Sigma-Aldrich (St. Louis, MO). Polysorbate-20 (Tween 20®, T20) was purchased from the NOF Corporation (Osaka, Japan).

Nano-vesicle preparation

The CLP, TFS, ETS and NS were prepared by thin-film hydration method (Table 1). The CLP and TFS, ETS composed of a constant amount 10 mM PC, 1 mM of CHOL and various amount of 1 mM oleic acid (as PE). Whilst, the NS composed of 5 mM Tween® 20 (T20), 5 mM CHOL. CUR was simultaneously added in lipid composition to prepare the lipid thin film of CUR loaded nano-vesicles. The dried lipid thin film loaded CUR was hydrated with phosphate buffer solution (PBS; pH 7.4) for CLP, TFS and NS; and 10% ethanol in PBS for ETS. All nano-vesicles were subsequently sonicated for two cycles using a bath- and probe-type sonicator (5510J-DTH Branson Ultrasonics,

Danbury, U.S.A.). The CUR loaded vesicle formulations were freshly prepared and stored in airtight containers at 4°C prior to use.

Physicochemical characterization: Vesicle size, size distribution, zeta potential and CUR content

Average vesicle size, size distribution and zeta potential of the nano-vesicles were determined by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, U.K.). All measurements were conducted at room temperature (25 °C), after diluting the nano-vesicle formulations. Twenty microliters the sample formulations were diluted with 1480 µL of deionized water. At least three independent measurements were performed, and the vesicle size, size distribution and zeta potential were measured at least three times.

The concentration of CUR in the formulation at initial was determined after vesicle disruption with Triton® X-100 (0.1% w/v) at a 1:1 volume ratio and appropriate dilution with phosphate buffer solution (pH 7.4). The vesicles/Triton® X-100 solution was centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was filtered through a 0.45 µm nylon syringe filter, and then analyzed by the HPLC.

HPLC analysis

The concentration of CUR in all samples was analyzed using a HPLC (Thermo Scientific™ UltiMate 3000 UHPLC System). A C18 reversed-phase column (Symmetry®, VertiSep™, Vertical, Thailand) with dimensions of 5 µm, 4.6×150 mm was utilized. The mixture of acetonitrile and 0.01% phosphoric acid (65:35) was used as the mobile phase for CUR. A UV-VIS detector was set at 425 nm for CUR detection at 40 °C. The flow rate was 1.5 ml/min and the injection volume was 20 µL.The calibration curve for CUR was in the range of 20-200 µg/ml with a correlation coefficient of 0.999.

In vitro skin permeation

A Franz diffusion cell with an available diffusion area of 2.01 cm^2 was conducted at least triplicate. The shed snake skin of Siamese cobra (*Naja kaouthia*) was used as a model membrane for the skin permeation study because of its similarity to human skin in lipid content and permeability.[13] The donor and receiver chamber was filled with 1 mL of nano-vesicle formulation and 15 mL of ethanol : PBS pH 7.4 (1:1 volume ratio), respectively. The water jacket was set at 32°C and stirred at 150 rpm. At time intervals; 0, 2, 4, 6 and 8 h, 0.5 mL of the receiver fluid was withdrawn, and the same volume of fresh ethanol : PBS pH 7.4 was replaced. The concentration of CUR was determined using HPLC as described previously.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of CUR loaded nano-vesicles was determined according to the previous study[14] with slight modification. Briefly, individual nano-vesicles (40 µL) was added to 160 µL of 0.2 mM DPPH radials. After reaction time of 30 min, absorbance was measured at 517 nm. The DPPH radical-scavenging ability was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ was the absorbance of the control (containing all reagents except the sample extract), and A_{sample} was the absorbance of the CUR loaded nano-vesicles. Trolox was used as positive controls.

Stability study

The physicochemical stabilities of the CLP, TFS, ETS and NS formulations were evaluated by monitoring the formulations for at least 150 days after their initial preparation. Various vesicle formulations were kept in glass bottles with plastic plugs at 4±1°C for 150 days to determine the stability of the formulations. The physicochemical stabilities of vesicle formulations were evaluated by visual inspection for sedimentation. The vesicle size, size distribution and zeta potential were measured by PCS.

Data analysis

The data are reported as the means ± standard deviation (SD) (n=3). A *p*-value of less than 0.05 was considered to be significant.

Results and Discussion

An approach to success the development of CUR loaded nano-vesicle as anti-aging cosmetics was to understand the factors affecting the safety, the efficacy and stability of cosmetic product. Thus, this study focused on the physicochemical characterization, skin permeation, antioxidant activity and stability of the nano-vesicle formulation as anti-aging cosmetics.

Physicochemical characterization: Vesicle size, size distribution, zeta potentialand CUR content

The physicochemical characteristics such as vesicle size, size distribution, zeta potential and CUR content in the formulation are shown in Figure1. The CLP was defined as the control of all nano-vesicles in this study. The vesicle size of all CUR loaded nano-vesicle formulations was smaller than 200 nm (Figure1A) with size distribution less than 0.4 (Figure1B). The vesicle size of ETS and NS was significantly greater that of CLP and TFS, respectively. Verma D.D. and coworkers reported that nano-vesicles size less than 120 nm can permeate through skin layer.[15] It

is agreed that the vesicle of TFS and CLP was an important first criterion in allowing to deliver CUR into the skin. The zeta potential of all nano-vesicles was negative charge (Figure1C). The net surface charge of nano-vesicle was defined by the intrinsic properties of their compositions. Under the experimental pH, the pH of the formulation (pH 7.4) was higher than the isoelectric point (IP) of PC (IP = 6). Therefore, PC carried a negative charge with this pH. Moreover, the increasing of cholesterol amount in a phospholipid membrane may decreases surface charge nano-vesicles.[16] On the other hand, the pKa of CUR may be a factor affecting the net charge of nano-vesicles. CUR has three pKa values of pKa_1 = 7.8, pKa_2 = 8.5 and pKa_3 = 9. CUR loaded nano-vesicles were also carried a positive charge as the pH was slightly lower than its pKa.[17] Therefore, the net charge was defined by the total intrinsic properties of formulation component. The CUR content of TFS was significantly higher than that of CLP, NS and ETS, respectively (Figure1D). The nano-vesicles not only deliver the entrapped compound, but also the non-entrapped compound into the deep skin region.[18] It could be concluded that the compositions in the formulation were the major factor affecting the vesicle size, surface charge and CUR content of the nano-vesicles.

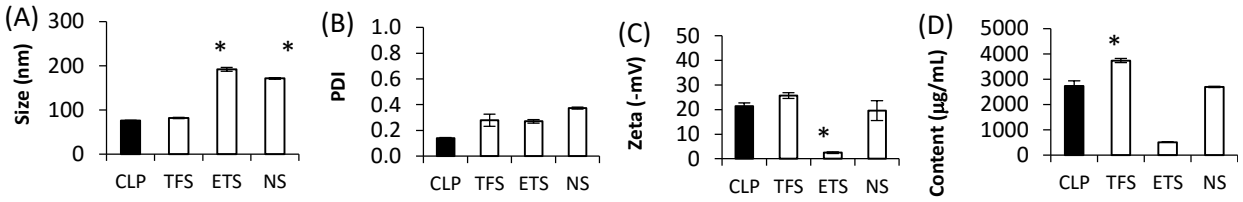

Figure 1(A) vesicle size, (C) size distribution (C) zeta potential and (D) CUR content of different curcumin loaded nano-vesicles at the initial

In vitro skin permeation

The efficacy of CUR loaded nano-vesicle as anti-aging cosmetics was determined using the skin permeation as a parameter. The skin permeation profile and skin permeation flux of CUR loaded nano-vesicles are shown in Figure 2A and 2B. The skin permeation flux represented the CUR amount in each nano-vesicles permeated the skin per area in one hour. The skin permeation flux of TFS was significantly higher than that of CLP, ETS and NS, respectively. This result coincided well with the previous study that the skin permeation of tetanus toxoid loaded TFS was significantly higher than that of CLP and NS, respectively.[19] The finding seems to indicate that the osmotic gradient generation may a primary possible mechanism of TFS to permeate the skin. The high elasticity of new generation vesicles (TFS) might the second or third possible mechanism, as it can squeeze themselves and permeate the intercellular lipid membrane.[1, 20] The result was due to the elasticity index. In previous study, the elasticity index of TFS was found to be maximum 124.4±4.2 while the elasticity index of CLP and NS was 29.3 ± 2.1 and 21.7 ± 1.9, respectively.[19] Thus, the definite mechanism of nano-vesicles should be confirmed in further study.

DPPH radical-scavenging activity

The antioxidant activity of different CUR loaded nano-vesicles are shown in Figure 2C. The finding clearly indicated that the % inhibition of TFS and CLP was significantly higher than that of NS, ETS and control (CUR in PBS). This result suggested that CUR loaded TFS and CLP nano-vesicles should be promoted as anti-aging cosmetic products.

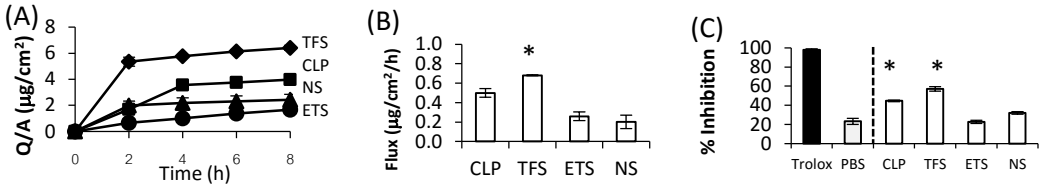

Figure 2(A) the skin permeation profile and (B) the skin permeation flux and (C) the antioxidant activity of CUR loaded nano-vesicles

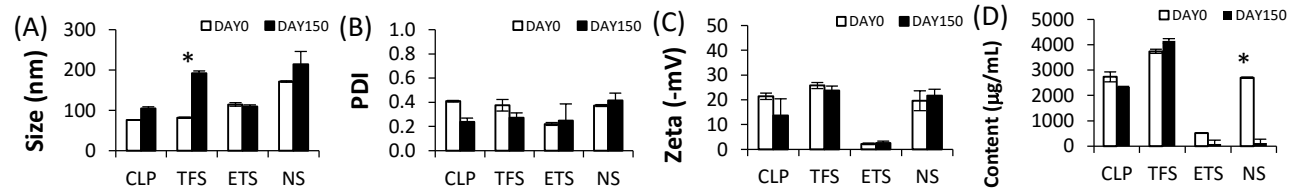

Figure 3 The stability study: (A) vesicle size, (C) size distribution (C) zeta potential and (D) CUR content of different curcumin loaded nano-vesicles at the initial (day 0) and day 150

Stability study

The stability of the formulation is an ideal factor that is used to assess expiration dating and storage conditions forall products. The stability of products related to the establishment and guarantee of safety, quality and efficacy of the product. The stability study can be summarized that the vesicle size of most CUR loaded nano-vesicles was a slightly increased, although the vesicle size of TFS at the initial was significantly different compare to day 150 (Figure 3). However, all nano-vesicles were in nano-size range under 250 nm with size distribution around 0.4. A decrease in zeta potential was likely to cause a rise in instability. The zeta potential of greater than ±30 mV has a beneficial role to prevent the aggregation of nano-vesicle.[21, 22] The result indicated that the instability may occur in ETS as its low zeta potential. The CUR content remaining in CLP, TFS and ETS was slightly decreased in day 150. While the CUR content in NS was 10% remaining. The remaining of CUR in the formulation after the incubation period was directly attributable to their major composition. A critical packing parameter of CLP, TFS and ETS may sufficient to satisfy the principle of opposing forces that was needed for stable membrane equilibrium. The polar head group of PC may responsible for the stability of the bilayer membrane as a self-assembled nanostructure more than nonionic surfactant.[23]

Conclusion

According to our results, we suggested that CUR loaded TFS and CLP should be promoted as antiaging cosmetic products.

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References

1. Cevc G, Schatzlein A, Blume G. Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. J Control Release. 1995;36:3-16.
2. van Kuijk-Meuwissen MEMJ, Junginger HE, Bouwstra JA. Interactions between liposomes and human skin in vitro, a confocal laser scanning microscopy study. Biochim Biophys Acta. 1998;1371(1):31-9.
3. Benson HA. Transfersomes for transdermal drug delivery. Expert Opin Drug Deliv. 2006;3(6):727-37.
4. Ghanbarzadeh S, Khorrami A, Arami S. Nonionic surfactant-based vesicular system for transdermal drug delivery. Drug Deliv. 2015;22(8):1071–7.
5. Ghanbarzadeh S, Arami S. Enhanced Transdermal Delivery of Diclofenac Sodium via Conventional Liposomes, Ethosomes, and Transfersomes. Biomed Res Int. 2013;2013:616810.
6. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praça FG, et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. Int J Nanomedicine. 2015;10:5837-51.
7. Song YK, Hyun SY, Kim HT, Kim CK, Oh JM. Transdermal delivery of low molecular weight heparin loaded in flexible liposomes with bioavailability enhancement: comparison with ethosomes. J Microencapsul. 2011;28(3):151-8.
8. Song YK, Kim CK. Topical delivery of low-molecular-weight heparin with surface-charged flexible liposomes. Biomaterials. 2006;27(2):271-80.
9. SureewanDuangjit, TassananNimcharoenwan, NutchaChomya, NatthpornLocharoenrat, TanasaitNgawhirunpat. Design and development of optimal invasomes for transdermal drug delivery using computer program. AJPS. 2016;11(1):52-3.
10. Duangjit S, Obata Y, Sano H, Kikuchi S, Onuki Y, Opanasopit P, et al. Menthosomes, novel ultradeformable vesicles for transdermal drug delivery: optimization and characterization. Biol Pharm Bull. 2012;35(10):1720-8.
11. Chen ZX, Li B, Liu T, Wang X, Zhu Y, Wang L, et al. Evaluation of paeonol-loaded transethosomes as transdermal delivery carriers. Eur J Pharm Sci. 2017;99:240-5.
12. Duangjit S, Nimcharoenwan T, Chomya N, Locharoenrat N, Ngawhirunpat T. Computational design strategy: an approach to enhancing the transdermal delivery of optimal capsaicin-loaded transinvasomes. Drug Dev Ind Pharm. 2017;43(1):98-107.
13. Rigg PC, Barry BW. Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies. J Invest Dermatol. 1990;94(2):235-40.
14. Zeng PY, Wu JG, Liao LM, Chen TQ, Wu JZ, Wong KH. In vitro antioxidant activities of endophytic fungi isolated from the liverwort Scapania verrucosa. Genet Mol Res. 2011;10(4):3169-79.
15. Verma DD, Verma S, Blume G, Fahr A. Particle size of liposomes influences dermal delivery of substances into skin. Int J Pharm. 2003;258(1-2):141-51.
16. Magarkar A, Dhawan V, Kallinteri P, Viitala T, Elmowafy M, Róg T, et al. Cholesterol level affects surface charge of lipid membranes in saline solution. Sci Rep. 2014;4:5005.
17. Priyadarsini KI. The chemistry of curcumin: from extraction to therapeutic agent. Molecules. 2014;19(12):20091-112.
18. Verma DD, Verma S, Blume G, Fahr A. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm. 2003;55(3):271-7.
19. Gupta PN, Mishra V, Rawat A, Dubey P, Mahor S, Jain S, et al. Non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study. Int J Pharm. 2005;293(1-2):73-82.
20. Schatzlein A, Cevc G. Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br J Dermatol. 1998;138(4):583-92.
21. Rakesh R, Anoop KR. Formulation and optimization of nano-sized ethosomes for enhanced transdermal delivery of cromolyn sodium. J Pharm Bioall Sci. 2012;4(4):333-40.
22. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Control Release. 2007;123(2):148-54.
23. Marsh D. Thermodynamics of Phospholipid Self-Assembly. Biophysical Journal. 2012;102(5):1079-87.