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Development of lutein-loaded niosomes for topical delivery

Southamany Sisavengsouk[1], Thapakorn Charoenying[1,2], Praneet Opanasopit[1,2], and Supusson Pengnam[1,3]*

[1] Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[2] Department of Industrial Pharmacy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand
[3] Department of Biomedicine and Health Informatics, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand

***Corresponding author**: Supusson Pengnam
pengnam_s@su.ac.th*

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ABSTRACT

Lutein (Lut), a fat-soluble xanthophyll compound, is a derivative of carotenoids. It has several biological activities such as antioxidant, antimicrobial, and anti-inflammatory. In particular, the antioxidant activities of Lut are widely used as primary skin protection to alleviate oxidative injury caused by free radicals from sunlight. However, the structure of Lut is easy to decompose and is unstable to exposure by various stimuli such as pH, temperature, oxidation reaction, and light. Therefore, this study aimed to formulate niosomes to encapsulate Lut. Niosomes were prepared using the thin film hydration method. The droplet size, polydispersity index, zeta potential, encapsulation efficiency, and loading capacity were investigated. Moreover, photodegradation, the effect of limonene as a skin enhancer on skin permeation, and antioxidant activity were examined. The results revealed that the niosome formulation for Lut delivery contained Span20, cholesterol, and oleic acid at the ratio of 2:1:1. The niosomes with limonene exhibited the highest skin penetration compared to the niosomes without limonene and Lut suspension. The formulation with and without limonene as a component exhibited the highest antioxidant activity. In conclusion, encapsulating Lut in niosomes could facilitate its penetration into the skin as well as enhance antioxidant properties, which can be applied and further developed into cosmeceuticals.

Keywords: lutein; carotenoids; niosomes; antioxidant; topical delivery

1. INTRODUCTION

Lutein (Lut), a fat-soluble xanthophyll compound, is a derivative of carotenoids composed of the hydroxyl functional groups in its molecular structure. It is a natural substance that can be found in various fruits, vegetables, and medicinal herbs. It has garnered significant attention in recent years because it has several biological activities, such as antioxidant, antimicrobial, and anti-inflammatory effects (Madaan et al., 2017). With its potent antioxidant properties, Lut plays a crucial role in protecting cells from oxidative stress by scavenging free radicals and reducing oxidative damage to biomolecules such as lipids, proteins, and DNA. In addition to its antioxidant activity, Lut has also been recognized for its ability to protect the skin from various environmental stressors. Studies have shown that Lut exhibits skin-protective effects by reducing inflammation, inhibiting UV-induced damage, and promoting skin barrier function. These properties make Lut a promising candidate for skin formulations to maintain skin health and combat ageing-related skin issues (Zhang et al., 2022; Mitra et al., 2021). However, the structure of Lut is easy to decompose and is unstable on exposure to various stimuli such as pH, temperature, oxidation, and light. Additionally, its beneficial effects and the clinical efficacy of Lut in skincare products are often limited by its poor

skin permeability. Skin is the biggest organ in our body, and it is divided into 3 layers: the epidermis, dermis and subcutaneous. The skin presents a convenient pathway for the self-administration of medication, potentially enhancing patient compliance, but the barrier function of the skin poses a significant challenge for transdermal drug delivery. In particular, the stratum corneum, the outermost layer of the epidermis, provides a natural barrier that protects the body from harsh environments. The stratum corneum is assembled from corneocytes and the extracellular lipid matrix acts as a formidable barrier, restricting the penetration of many active ingredients with a molecular weight < 500 Da and log P value of 1–4 (Yu et al., 2021; Singpanna et al., 2023), including Lut. To overcome this challenge and enhance the skin permeation of Lut, various delivery systems have been explored, such as niosomes, liposomes, solid lipid nanoparticles, emulsion, microneedles, and polymeric nanoparticles. Among the delivery systems, niosomes have emerged as promising nanocarriers. Niosomes are lipid-based vesicles that are alternative carriers to liposomes. Their structures are composed of non-ionic surfactants, cholesterol, and charge-inducing agents, making them less susceptible to oxidation and decomposition compared to liposomes, which primarily consist of phospholipids (Bartelds et al., 2018). Moreover, they offer several advantages for drug delivery, including cost-effectiveness, biocompatibility, stability, permeability and ability to encapsulate diverse types of drugs or moieties such as hydrophilic, hydrophobic and amphiphilic compounds. Moreover, niosomes can enhance the penetration of active ingredients into the skin by overcoming the skin barrier and facilitating drug release at the target site (Ge et al., 2019). In addition, niosomes protect the active ingredients from the light because they can absorb and disperse UV rays, which enhances the stability and efficacy as well as the antioxidant activities of those active ingredients (Ge et al., 2019; Chaudhary et al., 2022; Moammeri et al., 2023). Therefore, this study aims to formulate niosomes to encapsulate Lut. Niosomes were prepared using the thin film hydration method, and their particle size and polydispersity index were evaluated using dynamic light scattering (DLS). In addition, the encapsulation efficiency and loading capacity were investigated. Moreover, photodegradation, the effect of limonene as a skin enhancer on skin permeation, and antioxidant activity were examined.

2. MATERIALS AND METHODS

2.1 Materials

Lutein (90%) was purchased from Acros Organics (Loughborough, UK). Cholesterol (Chol) was acquired from Carlo Erba Reagent (Cornaredo, MI, Italy). Span20 (Sp20) and oleic acid (OA) were purchased from Sigma Aldrich St. Louis (MO, USA). 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was purchased from Roche (Mannheim, Germany). Methanol was acquired from Honeywell International INC. (North Carolina, USA). Sodium phosphate and potassium persulfate were purchased from Fluka Analytical (Sigma-Aldrich Chemie GMbH, Germany). Naturally died neonatal pigs were obtained from Charnchai Farm, Ratchaburi, Thailand.

2.2 Preparation and characteristic particle size, zeta potential, and PDI of Lut-N

Lut-loaded niosomes (Lut-N) were prepared using the thin-film hydration technique, composed of Sp20, Chol, and OA at the ratio of 2:1:1 (total lipid 5 mM). The thin film was prepared by mixing Lut (2.157 mM, 1.23 mg/ml), Sp20, Chol and OA in a mixture of solvents (chloroform: methanol 2:1, V/V), then purged with N_2 gas to evaporate solvents and kept in a desiccator overnight. The thin film was hydrated with phosphate buffer saline (PBS) at pH 7.4 for Lut-N and at pH 7.4 with 1% of limonene for Lut with limonene, followed by probe sonication (ultrasonic processor model VCX134 and probe model CV244, SONICS VIBRA CELL) for 1 cycle (10,000 J). The niosomes were centrifuged at 2,000 rpm for 5 min and stored at 4°C.

All samples were diluted with deionized water at a ratio of 1:9 to evaluate the particle size, zeta potential and PDI by using dynamic light scattering at 25°C (Zetasizer Nano ZS, Malvern Instruments Worcestershire, UK).

2.3 Entrapment efficiency and drug loading capacity

Lut-N with and without limonene were mixed with methanol, then vigorously vortexed for 3 min. A mixture was assayed for lutein content by UV-spectrophotometer at 447 nm. A calibration curve was made with solutions of Lut at concentrations from 2 to 300 µg/mL. Each sample was assayed in triplicate. The percent entrapment efficiency (%EE) and drug loading capacity (LC) were calculated according to Equations (1) and (2), respectively.

$$\%EE = \frac{The\ remaning\ amount\ of\ drug\ (\mu M)}{The\ total\ amount\ of\ drug\ (\mu M)} \text{ x } 100\% \quad (1)$$

$$LC = \frac{The\ amount\ of\ drug\ in\ niosomes\ (\mu Mol)}{The\ total\ amount\ of\ niosomes\ (\mu Mol)} \quad (2)$$

2.4 Photodegradation of Lut

Lut suspension (in PBS 7.4) and Lut-N were exposed to a UV lamp at 356 nm wavelength and at a distance of approximately 10 cm, withdrawn at 0, 30, 60, and 120 minutes. Then, the amount of remaining Lut in each sample was measured by a spectrophotometer at 445 nm (VICTOR NIVO Multimode plate reader, PerkinElmer, USA).

2.5 In vitro skin permeation

Franz diffusion cells were employed to assess the permeability of Lut suspension, Lut-N, and Lut-N with limonene. Initially, subcutaneous fat was removed from neonatal pig skin and shaped into circular pieces using surgical scissors. The neonatal pig skin was placed in an acceptor chamber (Franz cells) that was filled with 50% v/v isopropanol (IPA). Then, a donor chamber was clamped on the acceptor chamber and connected to a thermostatic water circulation system at 32°C. The samples were added to a donor chamber and stirred at 500 rpm for 24 h. The skin was washed with PBS 2-3 times to remove the residue of Lut before collecting Lut in the epidermis layer by using a tape-stripping technique. The skin was soaked in methanol to further collect Lut in the deeper skin layer (dermis). The amount of Lut was measured by high-performance liquid chromatography (Agilent 1100 series HPLC Value System, ChemStation Program) at wavelength 445 nm using C18 4.6 mm × 250 mm × 5 µm the reverse phase column, methanol: ammonium acetate at a ratio 95:5 (v/v) as a mobile phase and flow rate 1.0 mL/min.

2.6 Antioxidant activities by ABTS assay

ABTS radical solution was prepared by mixing with 20 mM sodium phosphate and 4.95 mM potassium persulfate for 16 h in a dark place at room temperature. These substances could oxidize ABTS radicals to generate ABTS radical cation, which is a blue-green chromophore that ensures that the assay starts with a stable and consistent radical species. Then, the ABTS radical cation solution was diluted with PBS to obtain the absorbance at 734 nm. The ABTS radical solution was used to mix with the various concentrations of samples, and it was kept in a dark place for 15 min before being measured by a spectrophotometer. The antioxidant activities were calculated by using Equation (3).

$$\%\ Inhibition = \frac{(AC - AS)}{AC} \text{ x } 100\% \quad (3)$$

where AC is the absorbance of the control (ABTS radicals' cation alone) and AS is the absorbance of samples (Lut suspension, Lut-N or Lut-N with limonene).

2.7 Statistical analysis

All measurements were replicated thrice and expressed as the mean ± standard deviation (SD) by Excel 2019. The significant differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc test at p-value < 0.05 by SPSS software version 17.0.

3. RESULTS AND DISCUSSION

3.1 Preparation and characterization

Lut-N and Lut-N with limonene were formulated through thin-film hydration, utilizing SP20, Chol, and OA at the molar ratio of 2:1:1 as constituent component. Subsequently, particle size was reduced via sonication to obtain orange colloidal dispersion. The physicochemical properties of the samples were evaluated using DLS, with the niosome particle size and the PDI that indicate size distribution, as presented in Table 1. Additionally, the %EE and LC of Lut-N with limonene showed more than two-fold compared to Lut-N alone.

3.2 Photodegradation

The structure of Lut makes it easy to decompose and unstable upon exposure to light due to the conjugated bond, leading to rapid oxidation and degradation. The effects of UV light at 356 nm on Lut degradation of Lut-N and Lut suspension were examined over different time intervals. As depicted in Figure 1, The Lut suspension demonstrated a gradual decrease over successive time intervals, while the Lut-N formulation remained unchanged in the amount of Lut between the 30-min and 120-min intervals.

3.3 Skin permeation

Franz diffusion cells were utilized to investigate the skin permeation of samples on naturally died neonatal pigs. The cumulative amount of Lut in various samples across the epidermis and dermis layers is depicted in Figure 2. The findings revealed that Lut-N, which incorporates limonene as a component, exhibited the highest skin permeation rates, with 4.44±0.15 μg/cm² on the dermis and 0.77±0.03 μg/cm² on the epidermis. Conversely, Lut-N alone demonstrated lower permeation rates, measuring 1.28±0.20 μg/cm² on the dermis and 0.31±0.08 μg/cm² on the epidermis. Moreover, both formulations showed skin permeation enhancement values for Lut-N and Lut-N (limonene) approximately 2 and 8, respectively, which was Lut suspensions as a control as shown in Table 2.

Table 1. Particle size, PDI, encapsulation efficiency (%EE) and loading capacity (LC) of niosomes

Formulations	Particle size (nm)	PDI	Zeta potential (mV)	%EE	LC (μMol/μMol)
Lut suspension	5233.67±306.34	0.50±0.07	-30.63±2.67	-	-
Lut-N	508.23±15.49	0.48±0.08	-60.03±2.71	22.84±2.61	9.85±1.13
Lut-N (limonene)	290.33±1.36	0.40±0.05	-13.5±0.2	56.41±1.64	24.34±0.71

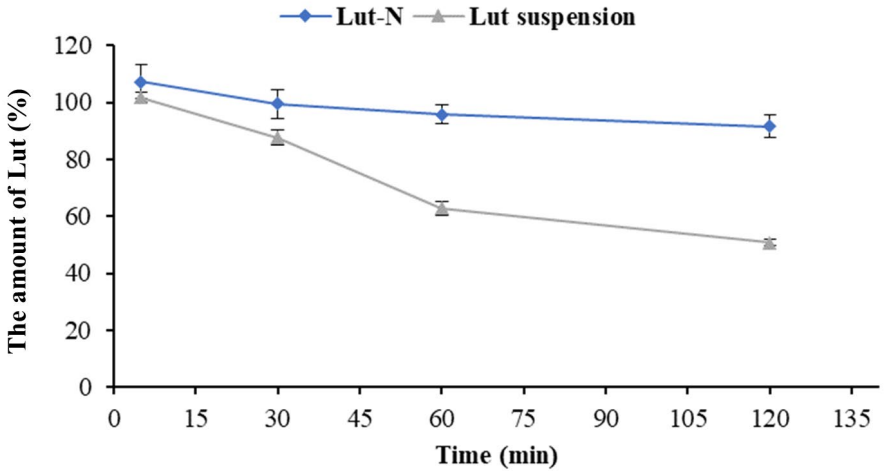

Figure 1. Photodegradation of Lut of samples at various intervals

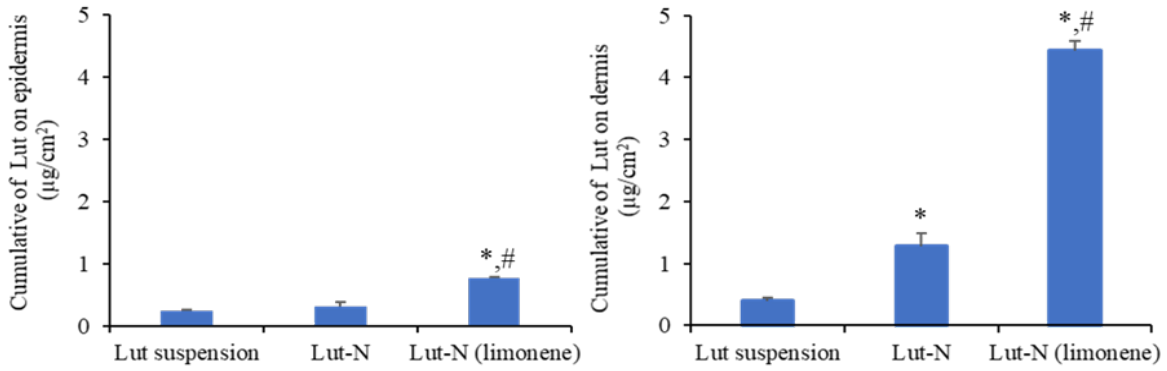

Figure 2. The cumulative of Lut suspension, Lut-N, and Lut-N (limonene) on (A) epidermis and (B) dermis
Note: * Significant difference from Lut suspension and from Lut-N at p-value 0.05.

Table 2. The skin permeation enhancement of niosomes

Formulation	Skin permeation enhancement
Lut suspension	-
Lut-N	2.64±0.43
Lut-N (limonene)	8.09±0.20

3.4 Antioxidant activities

ABTS radicals scavenging was used to investigate the antioxidant properties of samples. According to Figure 3, the SC50 for reaching 50% scavenging of ABTS radicals of Lut suspension was 0.068 mg/mL. The SC50 of Lut-N and Lut-N with limonene was lower than Lut suspension, which was 0.034 and 0.033 mg/mL, respectively. The results revealed that niosomes could enhance the antioxidant of Lut, which decreased SC50 by about 2 folds compared to Lut suspension.

4. DISCUSSION

In our study, niosomes were formulated as lipid vesicles to encapsulate Lut and protect it from several stimuli, especially light. By thin-film hydration, the Lut-N showed orange colloidal dispersions. The niosomes were prepared at a ratio of 2:1:1 of SP20, Chol, and OA, which self-assemble into surfactant bilayers that form vesicles, with the hydrophobic phase located inside the lipid bilayer. Particle size, represented in Table 1, ranged approximately from 200 to 500 nm. Lut was loaded between the hydrophobic layers of the niosomes. Moreover, as depicted in Figure 1, the niosomes effectively shielded Lut from degradation induced by light, which is attributed to the non-ionic and cholesterol components used in niosome formation because those components can absorb and disperse UV rays, reducing the likelihood of photodegradation, resulting in improved stability during storage, as discussed by Ge et al. (2019) and Moammeri et al. (2023). Ge et al. (2019) reported that insulin-loaded niosomes had the ability to protect insulin from degradation, while another study supported the protective role of niosome carriers in safeguarding oligonucleotides from degradation and enhancing cellular uptake of gene materials (Puras et al., 2014). In addition, a study conducted in 2015 demonstrated that diclofenac incorporated into topical niosomal formulations displayed improved photostability relative to conventional formulations when subjected to light exposure. This suggests that niosomes effectively shield diclofenac from photodegradation in topical products (Ioele et al., 2015).

Skin permeation routes are divided into transcellular, intercellular, and appendageal routes through hair follicles and sweat glands. Lut in its free form is a lipophilic compound with a high partition coefficient (log P) of 7.9, posing a challenge to reach the stratum corneum, the outermost layer of the skin, at adequate concentrations to exert their effects, as highlighted by Yu et al. (2021). The stratum corneum comprises corneocytes and an extracellular lipid matrix, which serves as a robust barrier, limiting the penetration of active ingredients with a molecular weight below 500 Da and a log P value ranging from 1 to 4 (Singpanna et al., 2023). Therefore, niosomes might facilitate Lut permeation through the roust barrier of the stratum corneum; however, there is currently no mechanism adequately explaining the ability of niosomes to augment drug transfer across the skin. (Muzzalupo and Tavano, 2015). As illustrated in Figure 2, the cumulative Lut content of Lut-N in the epidermis and dermis were higher than the Lut suspension, demonstrating that niosomes could enhance Lut absorption into the skin. Consistent with Junyaprasert et al. (2012), who developed ellagic acid-loaded niosomes, as well as Balakrishnan et al. (2009), who formulated minoxidil niosomes for enhanced skin delivery, these findings underscore the potential of niosomes in improving skin penetration and drug delivery. Additionally, limonene, a cyclic monoterpene, is a natural substance widely used in cosmeceuticals to enhance skin permeation. Formulated niosomal vesicles incorporating limonene as an enhancer for skin permeation, significantly improved the skin permeation of methotrexate compared to control formulations without limonene, as demonstrated through ex vivo and in vivo methods (Abdelbary and AbouGhaly, 2015). In addition, a study conducted in 2022 provided support for the concept that limonene, as a member of the terpene group, can enhance the permeation of both hydrophobic and hydrophilic drugs, even at low concentrations. This enhancement is achieved by influencing the lipids within the stratum corneum, particularly the intercellular lipids or hydrogen bond connections within the stratum corneum domain (Hmingthansanga et al., 2022).

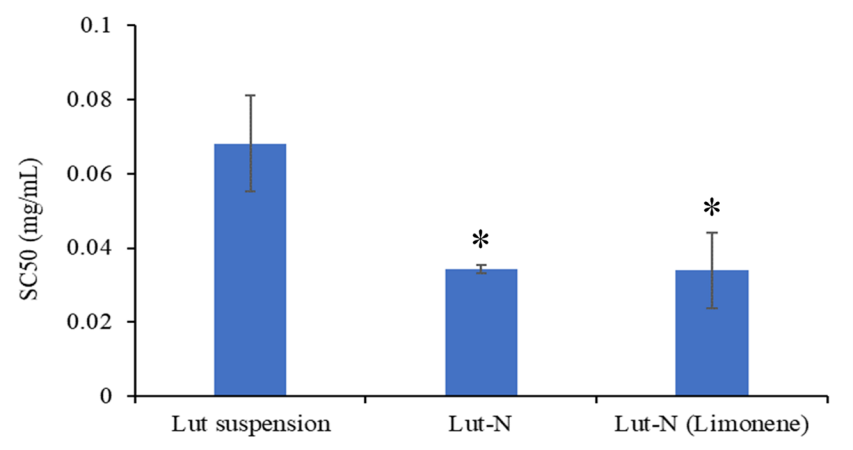

Figure 3. The SC50 of Lut-suspension, Lut-N, and Lut-N with limonene by ABTS assay
Note: * Significant difference from Lut suspension at p-value 0.05.

The antioxidant activity of Lut primarily involves free radical scavenging and stabilizing radicals by exchanging hydrogen bonds with antioxidant substances via the conjugated double bonds and hydroxyl groups that are present in the structure of Lut (Heinrich et al., 2003). In this study, the antioxidant properties of Lut encapsulated in niosomes were determined by using the ABTS radical scavenging method, in which the ABTS radical cation from dark blue-green color gradually changed to colorless after reacting with antioxidant substances. As depicted in Figure 3, the SC50 of Lut-N and Lut-N (limonene) decreased by 2 times compared to Lut suspension. Lut encapsulation in niosomes might improve Lut radical scavenging activity. This may be due to the improvement in its solubility and protection from degradation, which could preserve the antioxidant properties of Lut (Ge et al., 2019; Sguizzato et al., 2023). Furthermore, another research study provided the supporting idea that niosomes could enhance the solubility and reduce the photodegradation of quercetin, thereby boosting its antioxidant properties (Chaudhary et al., 2022). As mentioned by Jiao et al. (2018), Lut encapsulated in liposomes had higher ABTS and DPPH radical scavenging capacity compared to free Lut (Jiao et al., 2018). In the same way, Sguizzato et al. demonstrated that antioxidant molecules encapsulated in niosomes provided higher DPPH radical scavenging efficacy compared to those without encapsulation in topical application (Sguizzato et al., 2023).

5. CONCLUSION

In this study, Lut-N and Lut-N with limonene were successfully formulated using the thin-film hydration method. These niosomes exhibited favorable physicochemical properties and provided significant protection against light-induced degradation. Moreover, Lut-N and Lut-N with limonene enhanced skin permeation and improved antioxidant activity compared to Lut suspension, indicating their potential for topical application. Overall, the findings underscore the promising role of niosomes in enhancing the stability, skin permeation, and antioxidant properties of Lut for the development of an advanced cosmeceutical field as well as the enhanced therapeutic benefits. Nevertheless, additional research should be conducted to explore various aspects, such as pre-clinical and clinical safety assessments.

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