



Research paper

Development and evaluation of resveratrol, Vitamin E, and epigallocatechin gallate loaded lipid nanoparticles for skin care applications



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ARTICLE INFO

Article history:

Received 11 April 2016

Revised 30 November 2016

Accepted in revised form 10 April 2017

Available online 12 April 2017

Chemical compounds studied in this article:

Resveratrol (PubChem CID: 445154)

Vitamin E (PubChem CID: 14985)

Epigallocatechin Gallate (PubChem CID: 65064)

Keywords:

Solid lipid nanoparticles

Skin penetration

Resveratrol

Vitamin E

Epigallocatechin gallate

Nanostructured lipid carriers

UV induced degradation

Liquid chromatography

Release profile

ABSTRACT

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have been studied as potential carriers for both dermal and transdermal drug delivery. SLN contain lipid droplets that are fully crystallized and have a highly-ordered crystalline structure. NLC are modified SLN in which the lipid phase contains both solid and liquid lipids at room temperature. SLN and NLC are thought to combine the advantages of polymeric particles, liposomes and emulsions. Therefore they provide high encapsulation percentages, better protection for incorporated actives and allow for control of desired release profile. In this work, Resveratrol, Vitamin E (VE), and Epigallocatechin Gallate (EGCG) all potent antioxidants known to provide protection to the skin, were formulated into lipid nanoparticles. Several different formulations were successfully developed and demonstrated high uniformity and stability. Both resveratrol and VE lipid nanoparticles provided effective protection of actives against UV induced degradation. However, lipid nanoparticles did not show protection from UV degradation for EGCG in this work. An active release study exhibited a sustained release of resveratrol over 70% after 24 h. Skin penetration studies showed that lipid nanoparticles directionally improved the penetration of resveratrol through the stratum corneum. Our findings suggest that lipid nanoparticles are promising viable carriers for the delivery of resveratrol and VE to provide longlasting antioxidant benefits to the skin.

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1. Introduction

As the population demographic ages we see a growing need to develop solutions to improve skin strength and elasticity. The sizable revenue and growth potential in anti-aging skin care products was approximately \$1.2B in 2015. Actives such as Resveratrol (Res), Epigallocatechin Gallate (EGCG), and Vitamin E (VE) are examples of skin benefiting actives [1–3]. However, there are issues associated with their delivery to target areas, namely skin irritation if delivered in too large a dose, difficulty to penetrate the stratum corneum, and active instability upon exposure to light

and air. One way to solve these limitations is through the use of a delivery vehicle [4,5].

In traditional delivery systems, actives are delivered in a burst release, meaning the entire dose of active is delivered at once or in a very short time span. In many applications this method is ineffective. Delivering too much of the active at once can cause irritation, whereas too little of the active will render it ineffective. To compensate for this, consumers of these products are forced to use the product several times a day. This is not convenient or economical to the consumer and can often times lead to negative side effects. It is thought that by tailoring the release profile, the above-mentioned sensitivity of the actives could be overcome.

Solid lipid nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) were proposed to address the active delivery needs for

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Table 1
Actives selected for study with respective classification and challenge.

| Active compounds | Resveratrol | VE | EGCG |
|-------------------------|--|--|--|
| Classification & source | <ul style="list-style-type: none"> • Plant antioxidant, flavonoid • Skin of grapes, berries | <ul style="list-style-type: none"> • Fat-soluble antioxidant • Plant oil, sunflower, soybean | <ul style="list-style-type: none"> • Plant antioxidant, polyphenol • Green tea |
| Delivery challenges | <ul style="list-style-type: none"> • Limited water solubility • Easily oxidized • Photo degradation • Limited permeability | <ul style="list-style-type: none"> • Lipid soluble, incompatible with water • Limited permeability | <ul style="list-style-type: none"> • Limited water solubility • Easily oxidized • Photo degradation • Limited permeability |

the issue of aging skin. Solid lipid nanoparticles have been studied as carriers for both dermal and transdermal drug delivery. NLC are modified SLN in which the lipid phase contains both solid and liquid lipids at room temperature. Their structure is thought to provide better protection for incorporated actives as well as to enhance skin penetration. SLN and NLC were originally developed as an alternative active carrier to emulsion, liposomes, and polymeric nanoparticles. They are composed of solid lipids, unlike traditional flexible vesicle carriers such as liposomes [6]. SLN and NLC are made by replacing liquid lipid (oil) of an o/w (oil/water) emulsion with a solid lipid with or without oil blend, so that the lipid particle is solid both at room and body temperatures. It is reported in the literature that SLN and NLC are advantageous over traditional nanoemulsions and liposomes for improved active stability and reduced skin irritation [7,8]. It is also documented that SLN and NLC have better safety profiles than synthetic nanoparticles, since they are based on non-irritation and non-toxic, often generally regarded as safe (GRAS) status, lipid; therefore well suited for dermal applications [4,9,10]. SLN and NLC are thought to have high encapsulation percentages with hydrophobic actives, as well as having superior stability to other carrier systems. In addition, it is reported that solid lipid nanoparticles form a film when applied to the skin which promotes skin hydration and active penetration [11].

In this paper, SLN and NLC are studied for their potential ability to carry, protect, control the release, and deliver skin health actives. The objective of this work was to assess the effectiveness of SLN as a potential carrier for skin health actives by determining their ability to protect, release, and deliver the actives to their intended location. The actives that were studied were EGCG, Resveratrol, and VE (including VE and VE acetate). As stated above, there are delivery challenges associated with the actives including limited water solubility, photo degradation, and limited skin permeability (Table 1). The hypothesis was that lipid nanoparticle carriers would efficiently deliver the active compounds to the targeted skin location, addressing the delivery challenges. VE acetate was first used as the model active compound for SLN synthesis. Throughout the text SLN and NLC are referred to as lipid nanoparticles.

2. Materials

2.1. Lipid nanoparticles

Distilled water was obtained from the house distillation system. Other ingredients included: Tween 80 as a surfactant, Cetyl Palmitate for the solid matrix, VE acetate and VE as the active (BASF, Florham Park, NJ), Phospholipon 80 as the solid matrix (a gift from Lipoid, LLC, Newark, NJ), Compritol® 888 as the solid matrix (Gattefossé, Paramus, NJ), organic sesame oil as the oil matrix (MAPI Inc., Fairfield, IA) and Resveratrol and EGCG as the active (DSM, Augusta, GA).

2.2. Liquid chromatography

All reagents were purchased from commercial suppliers and used without further purification. Isopropyl alcohol, ACS reagent grade, was purchased from Avantor (Center Valley, PA) and the ethanol, ACS reagent 200 proof, and acetic acid, ≤99% reagent plus, were purchased from Sigma Aldrich (St. Louis, MO). Milli Q water was obtained in-house. OmniSolv® Methanol, acetonitrile, and tetrahydrofuran were purchased from EMD Millipore (Billerica, MA).

3. Methods

3.1. SLN synthesis & formulation

Lipid nanoparticle formulation included varying amounts of actives, solid lipid, sesame oil as liquid lipid, Tween 80 as surfactant, and water. Three different types of solid lipid were selected, cetyl palmitate (CP), compritol® 888, and phospholipon 80. Three model compounds, resveratrol, VE, and Epigallocatechin Gallate (EGCG) were used to evaluate the technology efficacy. Lipid nanoparticles with a range of the above mentioned active compounds were made and tested. VE Acetate, a relatively stable compound, was first used as the model active compound for lipid nanoparticle synthesis and characterization. Fig. 1 illustrates the experimental matrix for this study.

A summary of the formulations is located in Table 2. It is interesting to note that formulation containing mixed actives were successfully prepared, including Resveratrol (Res) + EGCG, VE + EGCG. The latter is a combination of hydrophobic and hydrophilic actives in one formulation.

3.2. Microfluidizer process

Lipid nanoparticles were synthesized using the M-110Y Microfluidizer Materials Processor (Microfluidics, New Town, MA) with F12Y-H30Z or F20Y-H30Z IXC configurations at a pressure of 20,000–30,000 psi. M-110Y is a portable, small-footprint, high-pressure pneumatic lab homogenizer, with an air-powered intensifier pump that can maintain a steady pressure to achieve tight particle size distributions. In a beaker, distilled water was heated to 70 °C, and surfactant was added to the heated water with constant stirring to make the water phase. In another beaker, solid lipid was melted and heated to 70 °C. Sesame oil and the hydrophobic active (s) were added into the molten lipid to make the lipid phase. In the case of EGCG, the active was added to the water phase due to poor solubility in the oil phase. While maintaining the temperature, the lipid phase was added to the water phase and mixed with a homogenizer for 5 min to make the emulsion. The microfluidizer instrument was warmed up to 70 °C by processing 70 °C hot distilled water through M-110Y system several times. After emptying distilled water from the microfluidizer reaction reservoir, heated

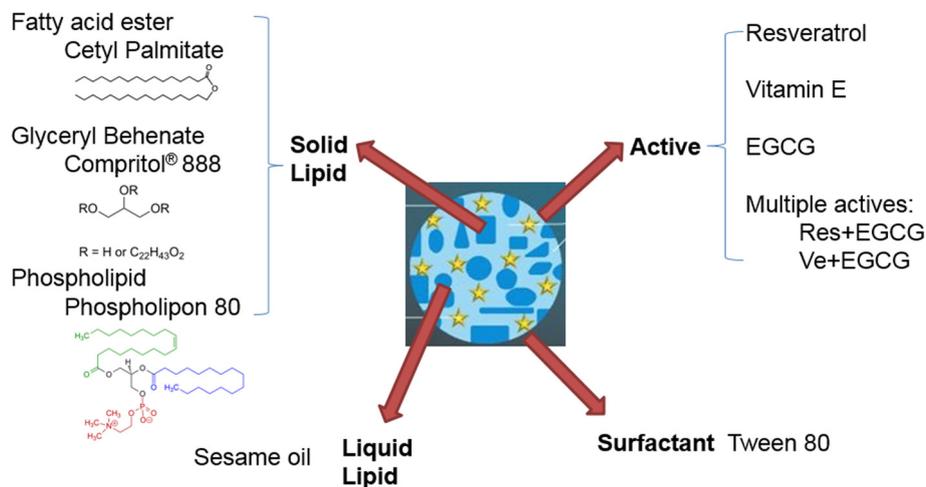


Fig. 1. Lipid nanoparticle formulation.

Table 2

Formulation matrix showing varying quantities of active, solid lipid, and oil described in ratio by weight%.

| Formulation | Active (g) | Solid lipid (g) | Liquid lipid Sesame oil (g) | Surfactant Tween 80 (g) | Water (g) |
|-------------|----------------------------|--------------------|-----------------------------|-------------------------|-----------|
| 1 | VE acetate (5) | CP (10) | 5 | 4 | 76 |
| 2 | Resveratrol (0.2) | CP (10) | 5 | 4 | 80.8 |
| 3 | Resveratrol (0.2) | Phospholipon80 (1) | 9 | 2 | 87.8 |
| 4 | Resveratrol (0.2) | Compritol (5) | 3.5 | 2.5 | 88.8 |
| 5 | VE (5) | CP (10) | 5 | 4 | 76 |
| 6 | VE (5) | Phospholipon80 (1) | 9 | 2 | 83 |
| 7 | EGCG (0.5) | CP (8) | 2 | 3 | 86.5 |
| 8 | EGCG (0.5) | Phospholipon80 (1) | 9 | 2 | 87.5 |
| 9 | Resveratrol;EGCG (0.2;0.5) | CP (8) | 2 | 3 | 86.3 |
| 10 | VE; EGCG (0.5;0.5) | CP (8) | 2 | 3 | 86 |

emulsion was added and high pressure microfluidization was started. Samples were collected at the end of the process line, and re-fed into the reaction reservoir for desired number of passes.

3.3. Zeta potential and particle size distribution

Zeta potential and particle size distribution analysis was carried out using a Malvern Instruments NanoZS (Westborough, MA). The samples were diluted by 0.1 mL in 40 mL of deionized water. This solution is inserted using a syringe into a U-shape zeta cell, being careful not to produce bubbles. The parameters that were used were the following: refractive index for the lipid bi-layer as 1.430, and the refractive index for the dispersant (water) of 1.330, a dispersant viscosity of 0.8872 cP, temperature of 25 °C. To obtain the size, 5 measurements were taken. To obtain the zeta potential, 3 measurements were taken.

3.4. Transmission Electron Microscopy (TEM)

A 5 µL lipid nanoparticle suspension was placed onto a 400 mesh carbon coated copper grid that had been made hydrophilic by glow discharge treatment, blotted with filter paper after 2 min. For negative staining, 5 µL 1% aqueous phosphotungstic acid (PTA) (pH 6.5) was applied onto grid immediately after suspension removal, and then removed as described above after 30 s. Grid was let completely dry before viewing on a JEOL JEM-1400 transmission electron microscope (Peabody, MA).

3.5. VE acetate loading and encapsulation efficiency characterization

Liquid Chromatography (Thermo finnegan, Surveyor system, San Jose, CA) was used to determine the loading and encapsulation

efficiency of VE acetate lipid nanoparticle. VE acetate was separated on a Luna C8 column (50 × 3.0 mm, 3 µm) (Phenomenex, Torrance, CA) with a mobile phase of 95% acetonitrile/5% water. A UV-Vis detector at 285 nm or an ion trap mass spectrometer (Thermo finnegan LTQ, San Jose, CA) equipped with an APCI source in the positive mode was used for detection. An autosampler set at 15 °C stabilized the VE acetate solutions during the run. To determine the total VE acetate in the SLN, THF was used as it dissolves both CP and VE acetate. To determine the “free + outside” VE acetate, acetonitrile was used as it dissolves VE acetate but not CP. Both solutions were further diluted with 90/10 (acetonitrile/water) and centrifuged prior to analysis.

3.6. Resveratrol, VE, and EGCG loading and encapsulation efficiency characterization

Loading and encapsulation efficiency characterization for Resveratrol, VE, and EGCG lipid nanoparticles were also determined using liquid chromatography. An Agilent 1100 series liquid chromatograph (Agilent Technologies, Santa Clara, CA) was used to quantify Resveratrol, VE, and EGCG in various samples. The mobile phases consisted of a mixture of isopropanol, water and acetic acid and the columns used were Hypersil BDS-C18 (150 mm × 4.6 mm, 5 µm) (Supelco, Bellefonte, PA) and Synchronis Phenyl (150 mm × 4.6, 3 µm) (Thermo Scientific, Waltham, MA). UV absorption was monitored at 310 nm, 292 nm, and 275 nm for resveratrol, VE, and EGCG, respectively.

Filtrate samples were analyzed “as is”. Concentrate samples were analyzed by the following procedure. The centrifuge cap top was removed and the tube was placed into a 40 mL vial. Ethanol (30 mL) was added to the vial contents, the vial was capped, and the vial contents were sonicated until all tube contents were

dissolved or dispersed. (Note: some intermittent shaking was required to remove solid remaining in the centrifuge tube). Filtering was required, where 0.45 μm nylon syringe filters were utilized.

3.7. Resveratrol, VE, and EGCG UV degradation

UV degradation was determined by placing glass vials of the formulations under a UVL-28 EL Series UV lamp, 365 nm (UVP, Upland, CA) and sampling at varying time points. Liquid chromatography (Agilent Technologies, Santa Clara, CA) was used to quantify Resveratrol, VE, and EGCG in various samples as previously described in Section 3.6. Standards and samples were prepared in (90:10) (isopropyl alcohol: water).

3.8. Resveratrol release profile

A simple and efficient release method was developed in order to monitor the release of resveratrol from solid lipid nanoparticles and other carriers. The release test employed an Agilent Dissolution Bath and a UV–Vis spectrophotometer (Cary, NC). Using UV–Vis spectrophotometry at 308 nm, a calibration curve was generated prior to testing to determine the amount of release at each time point. Lipid nanoparticle samples were placed in dialysis bags to help simulate release and blank samples were run for interference determination.

3.9. Stratum corneum penetration

Abdominal skin from female donor, age 45 or older regardless of race, was used. Skin with visible stretch marks was excluded from this study. Human cadaver skin was obtained from accredited US human tissue banks, including Community Tissue Services (Kettering, OH) and ZenBio (Research Triangle Park, NC). All skin was delivered as frozen and stored in -80°C freezer until the day of the experiment. Skin was trimmed into rectangular shape (1.5×1.5 cm) sufficient to cover the effective diffusion area of the receptor chamber. Dermis was removed from epidermis by treatment with 5 M sodium bromide (NaBr; Sigma, St. Louis, MO) solution overnight at 37°C . Stratum corneum layer was separated from the epidermis by vortexing in a 0.5% Trypsin solution. Integrity of the stratum corneum was verified using trans-epidermal water loss (TEWL) measurement following equilibration in the Franz diffusion cells. Specimens with TEWL value of 15 (grams of water loss per square meter of skin over 1 h) or higher were excluded from this study. Water-jacketed Franz diffusion cells and stirrer station were purchased from PermeGear (Hellertown, PA). Stratum corneum sheet was mounted on the Franz diffusion cell filled with deionized water in its receptor chamber. The donor compartment was filled with 0.7 mL of formulation 2 (Table 2) or un-encapsulated resveratrol solution. All openings including donor top and receptor compartment sampling arm were sealed with plastic wrap to prevent water evaporation. The resveratrol level in the receptor compartment was measured at 2, 4, 6, 8, and 24 h via liquid chromatography.

3.10. Resveratrol permeation quantification

Liquid chromatography was used to determine resveratrol permeation. An Agilent 1100 series liquid chromatograph (Agilent Technologies, Santa Clara, CA) was used and the mobile phase was composed of (30:70) Isopropyl Alcohol (IPA):water with 0.2% acetic acid overall. The column used was a Hypersil BDS-C18 ($150\text{ mm} \times 4.6\text{ mm}$, $5\ \mu\text{m}$) (Supelco, Bellefonte, PA) and UV absorption was monitored at 310 nm. The standards were prepared in phosphate buffered saline (PBS) and the resveratrol limit

of detection (signal to noise ratio 3:1) was 1.7 ng/mL. Permeation samples were analyzed “as is”.

4. Results & discussion

4.1. Lipid particle characterization

Highly uniform & stable VE acetate containing lipid particles were synthesized. Results indicate the particle size and stability are highly dependent on the lipid ratio and surfactant concentration. A higher lipid ratio resulted a larger particle size. A higher surfactant concentration resulted a smaller particle size with higher stability. In addition, increased number of microfluidization passes and pressure reduced particle size. From these VE acetate studies, the optimum processing conditions (chamber type, pressure, number of pass, etc.) and formulation ranges (LP:Oil:Surfactant) to yield 100–300 nm particles with long lasting stabilities were identified. Similarly, resveratrol, VE, and EGCG containing lipid nanoparticle were synthesized with high uniformity and high stability. Table 3 summarizes lipid nanoparticle characterization results. Lipid Nanoparticles enable higher concentration of these hydrophobic actives in aqueous based formulation for potentially enhanced skin benefit. Fig. 2 shows a Transmission Electron Micrograph of resveratrol loaded lipid nanoparticles.

4.2. UV induced degradation study

Lipid nanoparticle solutions containing either resveratrol, VE, or EGCG were evaluated for their photostability under UVA radiation. Three 0.2% resveratrol containing lipid nanoparticles with different types of solid lipid, cetyl palmitate, phospholipon, and Compritol, sample 2, 3, 4, were evaluated against UV degradation. The photodegradation profile of all the three lipid nanoparticles showed a slow degradation over 24 h time period, while the control sample which is resveratrol solution in mixed ethanol and water gave a steep degradation for the first 8 h, then remained constant over the remaining 16 h. At the end of 24 h, the remaining resveratrol amount in phospholipon, Compritol, cetyl palmitate based lipid nanoparticles, and control solution were 51%, 43%, 41%, and 35% respectively. Over the first 4–6 h of UV exposure, which is a normal time frame wearing a skin care product outdoors, lipid nanoparticle formulation showed much better protection of the active against UV radiation compared to the control. Phospholipon based formulation was able to retain 92% and 83% resveratrol at 4 h and 6 h, whereas in the free resveratrol solution, only about 28% resveratrol remains. See Fig. 3.

Two VE containing lipid nanoparticles, formulations 5 and 6, with cetyl palmitate or phospholipon as solid lipid were also studied for their UV degradation profile. After 120 h, remaining VE in lipid nanoparticles 5 and 6 is over 20% higher than control. The remaining VE amount in formulations 5, 6, and control are 88%, 82%, and 62%, respectively, as shown in Fig. 4.

Photodegradation of EGCG containing lipid nanoparticles was also studied with cetyl palmitate and phospholipon based NLC, formulations 7 and 8. After 168 h, 30% EGCG and 21% EGCG remained in Formulations 7 and 8, respectively, whereas the control sample contained 39% EGCG. This data indicates lipid nanoparticles didn't give protection against UV radiation over control for EGCG. Data is not shown.

Photodegradation of nanoparticles containing mixed actives produced interesting results. Degradation profile of sample 9 which contains 0.2% Resveratrol and 0.5% EGCG showed 39% resveratrol remained after 24 h, slightly higher than control with 35% resveratrol. After 168 h UV exposure, sample 9 contained 43% EGCG, whereas 39% EGCG remained in control. Data is not shown.

Table 3
Resveratrol, VE, and EGCG characterization, including size, particle distribution, Zeta potential, loading percent, and encapsulation efficiency.

| Actives | Size (nm) | Zeta potential (mV) | Loading (%) | Encapsulation efficiency (%) |
|-------------|-----------|-----------------------|-------------|------------------------------|
| Resveratrol | 102–311 | 25–49 | 1.0–1.7 | 99 |
| VE | 86–169 | 41–53 | 11–25 | 99 |
| VE acetate | 125–155 | 25–35 | 47 | 94 |
| EGCG | 126–167 | 38–49 | 2.7–3.6 | 99 |

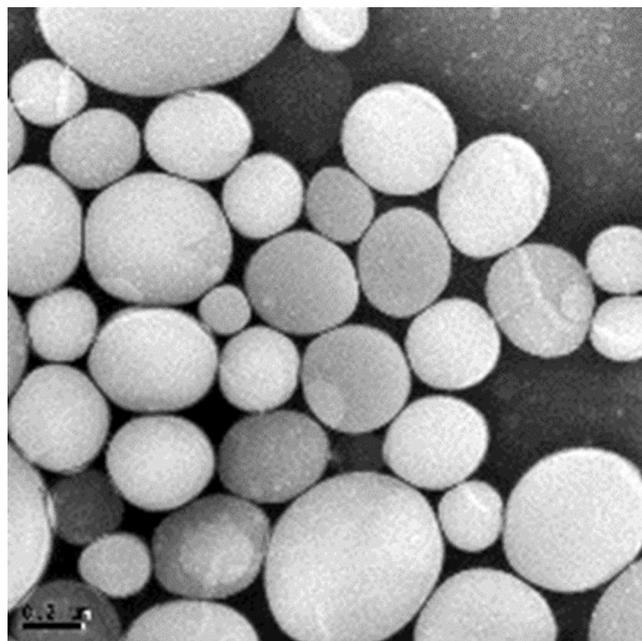


Fig. 2. TEM image of resveratrol loaded lipid nanoparticles.

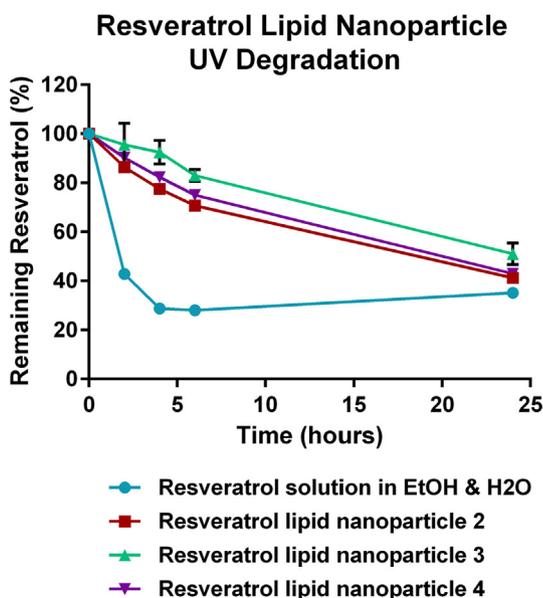


Fig. 3. Resveratrol lipid nanoparticle UV induced degradation profile.

UV degradation profile of formulation 10, which encapsulated a mixture of 0.5% VE and 0.5% EGCG demonstrated great protection of VE compared to the control. After 120 h, 75% VE remained in the lipid nanoparticle formulation, while all the VE was consumed in control. In this same formulation, after 169 h of UV exposure only 11% EGCG remained in the formulation, where as 39% EGCG

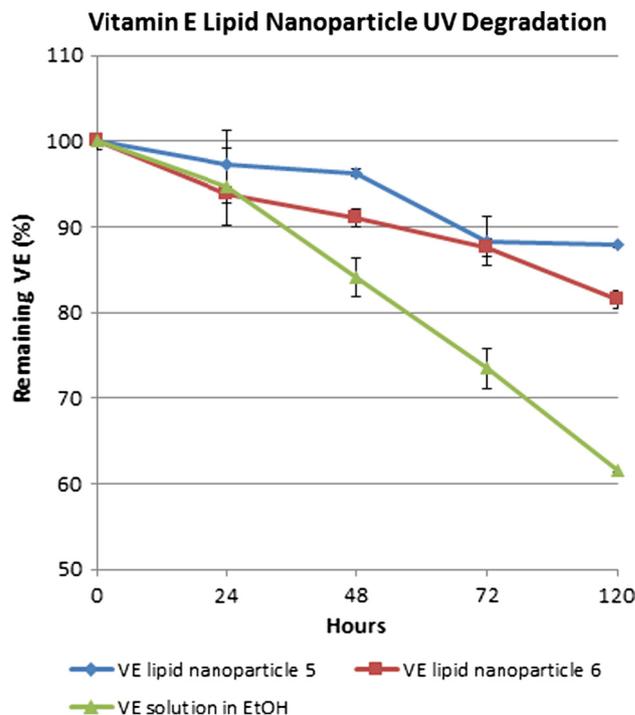


Fig. 4. Vitamin E lipid nanoparticle UV induced degradation profile.

was left in control solution. This data suggests EGCG is sacrificed to protect VE in the formulation. Data is not shown.

While the characterization of particles and UV degradation study was carried out for all three actives, Resveratrol was selected as a representative compound to investigate the release profile and skin penetration (Sections 4.3 and 4.4).

4.3. Lipid nanoparticle enables sustained release of resveratrol

The release study of Formulation 2 using a dissolution system showed sustained release for the resveratrol loaded lipid nanoparticle,

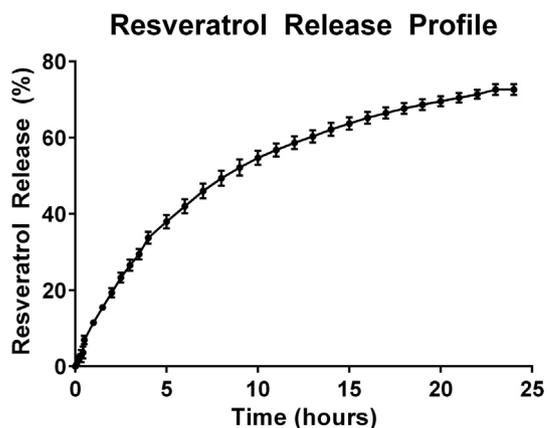


Fig. 5. Resveratrol release from lipid nanoparticles.

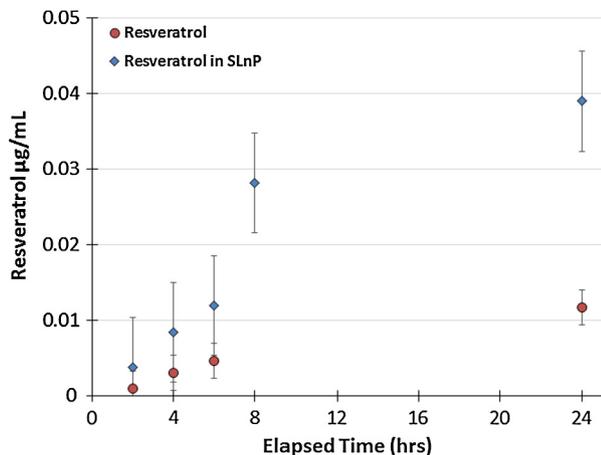


Fig. 6. Resveratrol penetration through stratum corneum.

where 50% of the loaded active released after 7.5 h and 70% released after 24 h (Fig. 5). Release profiles were not carried out for the other actives.

4.4. Resveratrol penetration through stratum corneum

As shown in Fig. 6, when resveratrol was dissolved in EtOH:PG (Ethanol and Propylene Glycol) co-solvent as a control, a very small amount of resveratrol penetrated through the stratum corneum. After 24 h static diffusion test, the concentration of resveratrol in the recipient chamber was 0.012 µg/mL. Lipid nanoparticles (Formulation 2) directionally improved the penetration of resveratrol through the stratum corneum. After 2 h of diffusion using lipid nanoparticles, the resveratrol concentration in the recipient chamber reached 0.003 µg/mL. It increased linearly to 0.03 µg/mL after 8 h. After 24 h of diffusion, the resveratrol concentration in the recipient chamber reached 0.04 ± 0.01 µg/mL. Skin penetration was not carried out for the other actives.

5. Conclusion

The objective of this work was to assess the effectiveness of lipid nanoparticles as potential carriers for skin health actives by evaluating their ability to protect, release, and deliver the actives to their intended location. EGCG, resveratrol and VE containing lipid nanoparticles were produced and tested. VE acetate was first used as the model compound for SLN synthesis. These different formulations were successfully developed and demonstrated high uniformity and stability. The technology was proven compatible with various actives and active mixtures, including resveratrol and EGCG, VE and EGCG, which suggests that lipid nanoparticles

are a potential method to deliver both hydrophobic and hydrophilic actives in one formulation. Both resveratrol and VE lipid nanoparticles provided effective protection of actives against UV induced degradation. Lipid nanoparticles did not show protection from UV degradation for EGCG in this work. A release study exhibited a sustained release of resveratrol over 70% after 24 h. Skin penetration studies showed that lipid nanoparticles directionally improved the penetration of resveratrol through the stratum corneum. Our findings suggest that lipid nanoparticles are promising viable carriers for the delivery of resveratrol and VE to provide long-lasting antioxidant benefits to the skin.

Acknowledgements

All of the funding for this work was provided by Kimberly-Clark Corporation. The authors would like to acknowledge Bruce Baretz from Lipoid LLC for the gift of Phospholipon 80, and Hong Li for producing the TEM images at Emory University. We also like to thank Jeremy Brunette and Debbie Ngai for editing the graphs. We acknowledge Peter Elliker, Ben Minerath, Joy Bauman, Mark Mleziva, Rick Wehrle, Laura Dellaripa, Cathy Scott, Sylvia Little, Stephen Quirk, Susan Tarr, and Pete Dulcamara for their support on this work. In addition, we thank Karien Rodriguez, Clay Bunyard, and Xuedong Song for the insightful discussion.

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