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Safranal-loaded solid lipid nanoparticles: evaluation of sunscreen and moisturizing potential for topical applications

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ABSTRACT

Objective(s): In the current study, sunscreen and moisturizing properties of solid lipid nanoparticle (SLN)-safranal formulations were evaluated.
Materials and Methods: Series of SLN were prepared using glyceryl monostearate, Tween 80 and different amounts of safranal by high shear homogenization, and ultrasound and high-pressure homogenization (HPH) methods. SLN formulations were characterized for size, zeta potential, morphology, thermal properties, and encapsulation efficacy. The Sun Protection Factor (SPF) of the products was determined *in vitro* using transpore tape. The moisturizing activity of the products was also evaluated by corneometer.
Results: The SPF of SLN-safranal formulations was increased when the amount of safranal increased. Mean particle size for all formulas was approximately 106 nm by probe sonication and 233 nm using HPH method. The encapsulation efficiency of safranal was around 70% for all SLN-safranal formulations.
Conclusion: The results conclude that SLN-safranal formulations were found to be effective for topical delivery of safranal and succeeded in providing appropriate sunscreen properties.

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Introduction

Preparing novel types of sunscreen products is an approach to reduce the adverse effects of sunlight and enhance anti-solar activity (1). Natural compounds such as polyphenols attract a great deal of attention in cosmetic formulations as they can protect the skin against exogenous and endogenous damaging agents such as UVB and UVA radiations (2, 3). Most commonly used products in herbal sunscreen preparations include aloe vera, basil, green tea, almond, olive, jojoba, and cucumber (4). Safranal, as a natural substance, has a wide range of pharmacological activities. Recently, the sunscreen properties of safranal and similar derivatives have been investigated, showing that this substance could exert sunscreen activity (5, 6).

For UV-blocker molecules, limited penetration to the viable layer of skin and presence on the top layers of horny layer are the two most important properties. It is well established that using a novel carrier offers the ability to modify this feature (7). Over the last decade, groups of researchers have focused on solid lipid nanoparticle (SLN) formulations as a novel drug delivery system (8-10). SLN has pronounced advantages over conventional drug delivery systems including novel dosage forms such as polymeric micro/nanoparticles, lipid emulsions, and liposomes. SLN can also reduce the definite limitations of such systems (11). SLN has recently been used as a transdermal drug carrier because of its permeability-enhancing properties (12); it increases skin water content, block UV lights, and exhibits occlusive properties (13, 14). Thanks to these specific properties, SLN could be used as a potential physical sunscreen (14, 15). Due to these unique characteristics, SLN might be a promising vehicle for formulating sunscreen agents and improving the efficacy.

Incorporation of safranal into SLN (SLN-safranal) could be a desirable means to enhance the efficacy of safranal. In this study, the skin hydration of prepared SLN-safranal was evaluated using corneometer *in vivo* method. UV protection properties of SLN-safranal were also investigated by transpore tape 3M *in vitro* method.

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Materials and Methods

Materials

Glyceryl monostearate (GMS) was gifted by Gattefossé (France). Tween 80 and Safranal were purchased from Sigma Aldrich (Germany). All of the original samples were used on arrival. Only double-distilled water was used throughout.

Methods

Preparation of SLN

High-shear homogenization and ultrasound methods were used to prepare SLN formulations. GMS (10%) was melted by heating at 70˚C, and then different amounts of safranal (0%, 1%, 2%, and 4% w/v) were added to the lipid phase at the end of the melting process. The aqueous phase was prepared by dissolving Tween 80 (5%) in double-distilled water up to 10 ml, then heated up to the melting point temperature of the lipid phase. Hot aqueous and molten lipid phases were mixed together and homogenized by a Diax 900 homogenizer (Heidolph, Germany) for 2 min at 11,000 rpm. The temperature was kept at 5˚C above the melting point of the lipid. The obtained emulsion was ultrasonicated by a probe sonicator (Bransonic, USA). The sonication was performed at 6 cycles with 30 sec of sonication separated by intervals of 15 sec. The obtained nanoemulsion was cooled to room temperature. SLN-safranal was also prepared by high-pressure homogenization method (HPH). SLN was provided using GMS (10% w/v) as the lipid phase and Tween 80 (5%w/v) as the surfactant, and then different amounts of safranal (0%, 1%, 2% and 4% w/v) were added to the lipid phase at the end of the melting process. The melted lipid was added to the hot water containing Tween 80. An emulsion was then prepared using T 25 Ultra Turrax (Janke und Kunkel GmbH and Co KG Staufen, Germany) for 2 min at 20,500 rpm. The pre-emulsion was processed at 1,000 bar for 3 cycles using a high pressure homogenizer (EmulsiFlex-C5® (Avestin Inc., Canada). Samples were then cooled to room temperature and SLN were obtained.

Characterization of SLN

Particle size and zeta potentials

The mean particle size, polydispersity index and zeta potential of the SLN formulations were assessed by dynamic light scattering (DLS) (ZetaSizer Nano-ZS; Malvern Instruments Ltd., United Kingdom) method.

Transmission electron microscopy (TEM)

The morphology properties of SLN formulations was characterized with TEM assessment as previously described (TEM; CEM 902A; Zeiss, Oberkochen, Germany). Briefly, the SLN were diluted 50 fold with water and placed on a carbon-coated copper grid for 30 sec and the excess water was wiped off with filter paper. Then 20 µl of 2% uranyl acetate in water was placed on SLN and after 30 sec were wiped off by another filter paper. The grid was dried at room temperature and assessed by TEM (16).

Differential scanning calorimetry (DSC)

DSC studies were performed using Mettler DSC 821e (Mettler Toledo, Gießen, Germany). An empty aluminum pan was used as reference. Samples were scanned from 25˚C to 100˚C at a rate of 5˚C/min under nitrogen atmosphere (20 ml/min). The melting point of SLN formulations was compared to the bulk lipid. Prior to the DSC measurements, the bulk lipids were heated up to 75˚C and cooled to room temperature to imitate the production conditions. Analysis was performed under nitrogen purge.

Entrapment efficiency (EE)

The entrapment efficiency (%) was determined by measuring the concentration of entrapped safranal after purification. To purify the SLN-safranal, 500 µl of the SLN dispersion was transferred to the upper chamber of an ultrafilter (Amicon Ultra-15, PLHK Ultracel-PL Membrane, 100 kDa, Millipore). Amicon tubes were centrifuged at 10,000 rpm for 30 min. The filtrate was analyzed for encapsulated safranal at 310 nm using a valid UV-spectrophotometric method after suitable dilution with ethanol (17). Then the percent of entrapment was determined using the following formula:

$$EE\ (\%) = \frac{Safranal\ concentration\ after\ purification}{Safranal\ concentration\ before\ purification} \times 100$$

Moisture content measurement of the skin using corneometer

After application of different SLN formulations, the hydration level of the skin was determined by a corneometer (Courage, Khazaka, Cologne, Germany).

After informed consent, six healthy Caucasian volunteers (age range, 20–35 years) with normal skin at room temperature participated in the study. Prior to the test, participants were given time to adapt to room conditions without obstructing the measuring area. On the day of examination, no other substance was applied to the skin surface, nor was washing performed. Subjects were aware that they could not apply any preparation to the site to be examined one week before investigation. The humidity level of the stratum corneum was determined with a corneometer CM 820 that measures electrical capacitance. Each experiment was conducted in triplicate. The measurements were tested exactly at the same sites. The measuring place was in the middle of the forearm. In the first instant,

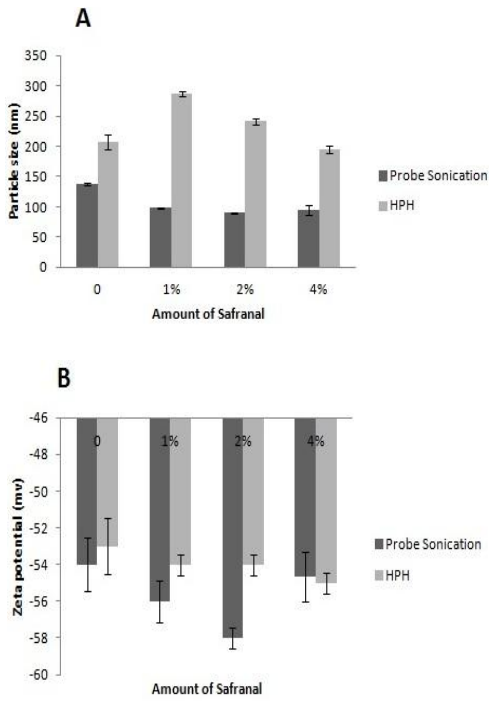

Figure 1. A) Particle size of different SLN formulations for both probe sonication and HPH methods. B) Zeta potential of different SLN formulations for both probe sonication and HPH methods

the humidity level of the skin was measured without any application of the product which is named as control; subsequently the measurements were performed after 30 min, 1, 3, and 5 h following application of the sunscreens (5, 6 and 15).

SPF determination of the formulations using transpore tape in vitro method

The principle of this method is based on the assessment of the spectral transmittance of UVR through a transpore tape with and without the sunscreen. Diffey was the first to introduce and apply this type of method. According to this method, different formulations and sunscreen standard (Homosalate 8%) were applied on the surface of the transpore TM tape at 2 mg/cm^2. After 15 min, the transmission values were recorded from 290 nm to 400 nm at intervals of 5 nm at five distinct points. The predicted SPF value was obtained according to the following equation (Eq. 1) (3, 5, 18).

$$SPF = \frac{\int_{290}^{400} E(\lambda).S(\lambda).d\lambda}{\int_{290}^{400} E(\lambda).S(\lambda).T(\lambda).d\lambda}$$

In this equation according to CIE (Commission Internationale de l'Eclairage): E(λ): Relative Erythemal Spectral Effectiveness, S(λ): Solar Spectral Irradiance (Wm-2nm-1), T(λ): Spectral Transmittance of the sample (as measured on the UV-1000S). Solar spectrum irradiance and action spectrometer erythema in human skin used to calculate Sun Protection Factors has been mentioned (18).

Results were presented as mean SPF and relative standard deviation as percentage of the mean SPF.

Statistical analysis

All tests were performed at least in triplicate. A one-way analysis of variance (ANOVA) was used for analyzing differences. Differences between means were deemed statistically significant if the *P*-values were less than 0.05.

Results

Characterization of SLN

Mean diameters of SLN formulations were determined by DLS for 2 preparation methods (Figure 1A). The differences between the sizes were significant based on the preparation method (P<0.0001). The mean diameters of SLN formulations were drastically increased upon applying the HPH method, and the smallest SLN was obtained using 4% safranal. There were no differences observed between zeta potential of formulations (P>0.05) (Figure 1B). The size of SLN formulations after 1, 2, and 3 months storage at 4˚C did not show significant differences in comparison with the size of SLN measured at the day of preparation (P>0.05). The zeta potentials of nanoparticles were approximately −50 mV. The encapsulation efficacy of safranal for SLN-safranal 1%, 2%, and 4% were 72.24%, 82.46%, and 85.26%, respectively.

Transmission electron microscopy (TEM)

The TEM studies were performed to get more information about the morphology of the SLN. TEM photomicrographs of SLN-safranal formulations (Figure 2) revealed almost spherical shape of these formulations with the same size and shape. The particle size as given by TEM was in line with that found using DLS.

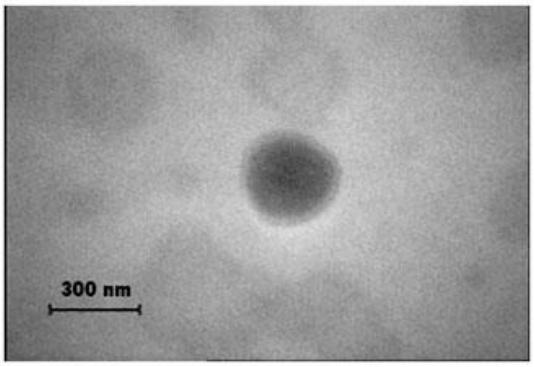

Figure 2. The TEM imaging of SLN-Safranal 1% formulation prepared by probe sonication method

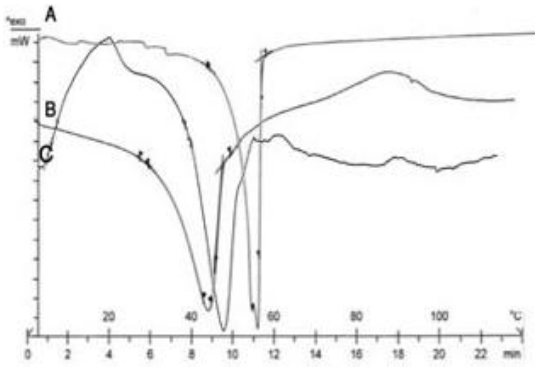

Figure 3. DSC curve of GMS bulk (A), safranal SLN-Safranal 1% formulation prepared by probe sonication method (B) and empty SLN (C)

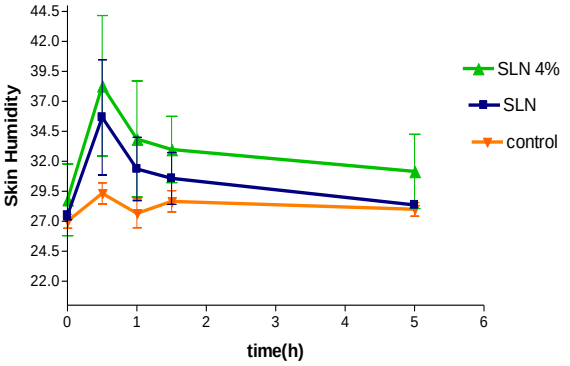

Figure 4. Percentage of increase in skin hydration for SLN-Safranal formulation, bare SLN and control groups

DSC analyses

The thermogram showed that the melting peak of the lipid core of the SLN was a lower temperature than bulk lipid (Figure 3). The DSC curve of GMS bulk was characterized by a main peak centered at about 58˚C, which was related to the compound melting point. However, in all SLN formulations the melting point was lower, around 43 and 44˚C for probe sonication and HPH methods, respectively.

Skin hydration measurement

Moisture content measurement of the skin was evaluated by measuring skin hydration values using a corneometer. The skin hydration results were illustrated in Figure 4. The skin hydration increased after application of SLN-safranl and free-SLN formulations. The SLN-safranal formulations further enhanced the skin hydration properties as evident from the properties of free-SLN formulations.

SPF determination of the formulations

Figure 5 (A) showed the SPF of SLN and homosalate reference formulations by *in vitro* method. According to the statistical analysis, significant differences were observed between the SPF values of homosalate reference and SLN-safranal formulations. Among all SLN formulations, the one containing 4% safranal showed higher SPF values in comparison with other formulations. The SPF of SLN-safranal 1% and empty SLN were significantly lower than 8% homosalate reference. There were no significant differences between SLN-safranal 2% and 8% homosalate reference. According to the transmittance method, the SLN-safranal could absorb UV light and the absorption of UV increased by increasing the safranal content (Figure 5B).

Discussion

Protection against UV light is an important issue throughout the human life. Applying herbal preparations and novel drug delivery systems are two major approaches for enhancing UV protection. The results of a previous study indicated that safranal could be used as a natural UV-blocking agent (5). SLN can also be considered an interesting and promising carrier system for molecular UV-blockers, as they have UV protection by UV reflecting and scattering abilities like other physical sunscreens (12).

The aim of the present study was to investigate the skin hydration and UV protection properties of SLN-safranal formulation.

The particles with nano-sized diameter showed sufficient occlusive and skin hydration properties (19-21). According to DLS results, the particle sizes of SLN were mainly between 50 and 300 nm (Figure 1A). The effect of safranal incorporation on particle size was also investigated. The formulations containing 4% safranal were the smallest ones

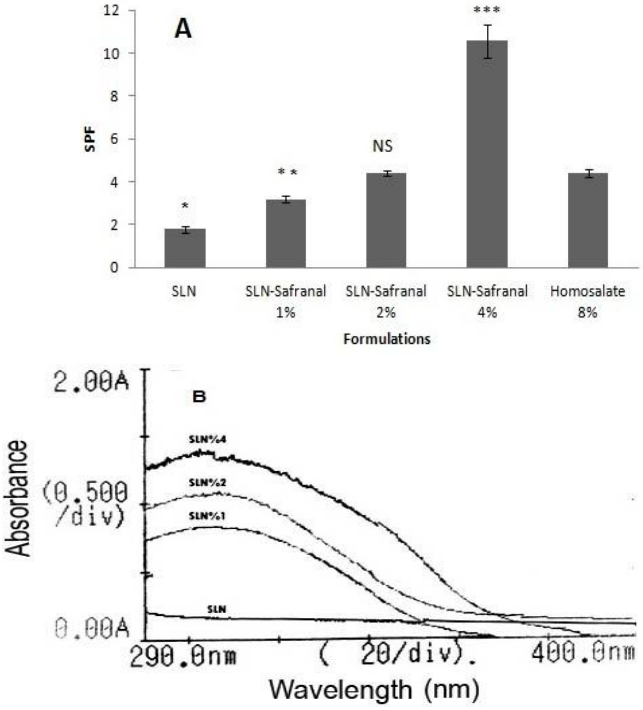

Figure 5. A) The SPF values of SLN-Safranal (1, 2 and 4%) and homosalate reference determined by Transpore tape method. Values are mean±SD, n= 3; ns $P>0.05$, *$0.01 <P< 0.05$, **$P< 0.01$. B) Absorbance curves of the formulations from 200 to 400 nm

in HPH method. It can be assumed that with enhancing the content of safranal, the particle sizes became smaller due to more solubility of safranal in the solid lipids using this method of preparation.

The SLN with zeta potential higher than ± 30 mV are normally considered physically stable (22). The zeta potential of particles for both preparations was negative. The surface charge of prepared SLN was −40 mv (Figure 1B). This was probably due to OH groups in the lipid phase. Taking into account both zeta potential and size of the particles, SLN formulations prepared with either method were suitable candidates for topical delivery systems.

The high encapsulation efficacy of safranal was seen for all prepared formulations (more than 70%). It was known that safranal is a lipid soluble molecule that could disperse in lipid mixture easily (23-24). This feature of safranal played an important role in high encapsulation efficiency. Since safranal is very volatile, after the preparation of SLN free safranal will be evaporated and separated during centrifuging on amicon filter.

The size obtained by electron microscopic images was in line with DLS results (Figure 2). A spherical shape of SLN was observed in TEM images.

The DSC studies were performed to assess the extent of crystallinity (Figure 3). As previously reported in the literature, DSC analysis is a useful method to determine the physical properties of the core lipid in SLN (25). The sharp melting endothermic peak of bulk lipid around 58˚C, indicated that the initial material was crystalline. Consistent with previous finding, the melting peak of the lipid core of SLN was observed at a lower temperature than that of bulk lipid. This circumstance might be due to the nanocrystalline size of the lipids in SLN, high specific surface area and the presence of the surfactant (23). DSC curves showed that the melting peak of lipid in SLN was decreased when the safranal was loaded in SLN. It was also observed that the DSC pick of safranal is not visible in SLN-safranal. These results, together with the absence of safranal flashing point pick, could suggest the drug incorporation and dissolution to the lipid matrix. It seems that SLN-safranal prevent the evaporation of safranal. This result was also observed in Qian *et al* research in 2012 (26). Blending tripalmitin with low melting point lipids led to a considerable alteration in the SLN phase behavior and stability. DSC measurements indicated that the presence of the carrier oils reduced the crystallization temperature, melting temperature, and melting enthalpy of tripalmitin as a solid lipid (27-28).

Various methods have been explained by Fluhr *et al* for measuring the hydration of the stratum corneum. Among these methods, the corneometer instrument is more commonly used (29).

Skin hydration properties of SLN were demonstrated previously. These features might be attributed to the film formation after application to skin, and consequently increasing the hydration of stratum corneum (30). According to Figure 4, skin hydration increased by application of SLN to skin. SLN-safranal formulations were slightly better than free formulations for improving skin hydration, however, there were no significant differences between loaded and unloaded SLN. Corresponding to these results, skin hydration properties were only related to SLN and were not related to safranal. Notably, the lack of hydration effect of safranal was proved previously (5). The occlusive features of SLN are related to the film formation after application to skin (31). These properties of SLN depend on various factors, e.g., particles size, lipid, and lipid concentration. In previous studies, the effects of size and composition of SLN were investigated, showing that the size of SLN and crystallinity index of the lipid in SLN could change the occlusion factor (5, 32). The results demonstrated that the crystallinity and occlusive factor of solid lipid in addition to other factors can influence skin hydration (5).

The anti-solar activity of SLN-safranal was assessed by determination of SPF. Due to these results, SPF values of SLN formulation were increased upon increasing the amount of safranal. As can be seen in Fig. 5A, among all of the formulations, the SLN containing 4% safranal had higher SPF values and was significantly higher than 8% homosalate reference. It shows that there were no significant differences between SLN-safranal 2% and 8% homosalate reference. In the previous study, the SPF of liposomes containing 8% safranal (Lip-safranal 8%) was significantly higher than 8% homosalate reference. These data indicate that safranal was an anti-solar agent. The potential UV-blocking effect of safranal was suggested earlier (5). The ability of SLN-safranal in blocking UV irradiation can be concluded from Figure 5B. These findings indicate that SLN formulation of safranal provides a more efficient UV blocking property than liposomes due the solid state of the lipids in SLN.

Conclusion

These results indicate that SLN-safranal formulations could be a promising carrier for topical delivery of safranal as an herbal UV blocking agent. SLN containing 4% safranal showed higher SPF values in comparison with other SLN formulatios and 8% homosalate reference. There were no significant differences between SLN-safranal 2% and 8% homosalate reference. The SLN-safranal formulations enhanced the skin hydration properties due to the properties of free-SLN formulations. The effects of SLN-safranal formulations as a sunscreen needs further research on human skin.

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