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Lipid Nanosystems in Topical PUVA Therapy

Luigi Battaglia, Elena Peira, Simona Sapino e Marina Gallarate

Abstract: 8-Methoxsalen was vehicled in nanoemulsion and in solid lipid nanoparticles (SLN) prepared by the hot homogenization technique in order to be used in topical psoralen UVA (PUVA) therapy. Drug entrapment efficiency in nanoparticles was improved by choosing the appropriate lipid matrix. The use of α -tocopherol in the lipid phase reduces 8-methoxsalen induced photooxidation of porcine skin, which was evaluated in vitro by a malondialdehyde (MDA) test: This result is promising to reduce in vivo human skin irritation after PUVA therapy, which can be attributed to skin photooxidation.

INTRODUCTION

Psoralen and ultraviolet A radiation (PUVA) therapy is used for severe psoriasis treatment only in patients who are not responsive to other pharmacological therapies because of its toxicological issues. PUVA therapy is based on the administration of drugs belonging to the psoralen family, followed by UVA-body irradiation; this therapy has an anti-proliferative effect on the skin through a mechanism that remains uncertain: once it was attributed to photo-induced cross-linking of psoralen to DNA, but recently it has been demonstrated that a specific UVA-mediated bond of psoralen to EGF (epidermal growth factor) receptor is involved.^[1] However the chemical linking of psoralen to biological molecules after UVA activation is essential for its activity.

Psoralen administration can be oral (oral-PUVA), topical (topical-PUVA), or by immersion in a psoralen water diluted solution (bath-PUVA).^[2] While oral-PUVA is considered obsolete, due to its toxic effect on liver and eye, bath-PUVA and topical-PUVA are useful, respectively for total body extent therapy and for the treatment of specific body parts.

Many formulations for topical-PUVA have been developed in last years, in order to increase drug accumulation into the skin;^[3 , 4] but while efficacy of topical-PUVA therapy can be related to drug accumulation within the skin, no formulation has been developed in order to limit its toxicity, which is due mainly to the increase of skin cancer risk after high exposure to cross-linking agents,^[5] and to psoralen induced photooxidation of the skin,^[6] which leads to skin irritation after treatment.^[7]

Therefore, in this work, safe lipid-based vehicles, like SLN and nanoemulsion, were chosen to deliver 8-methoxsalen (8-MOP), the most widespread compound of the psoralen family. In order to minimize skin oxidation, α -tocopherol was added as antioxidant in formulations: oxidation of porcine skin, assumed as model of human skin, was evaluated in vitro by MDA test, after exposure to 8-MOP and UVA.

EXPERIMENTAL

Materials

8-Methoxsalen (8-MOP) 1,1,3,3 tetraetoxypropane (Malondialdehyde; MDA), polyoxyethylene-polyoxipropylene copolymer (Pluronic F68) and cetylpalmitate were from Sigma (Dorset, UK), phosphoric acid, α -tocopherol, octyloctanoate and cetostearyl alcohol were from A.C.E.F. (Fiorenzuola d'Arda, Italy), thiobarbituric acid (TBA), arabic gum, trimyristin, and stearyl alcohol were from Merck (Darmstadt, Germany), Mygliol 818 (linoleic-capric acid triglyceride) was from Hüls AG (Witten, Germany), polyoxyethylene sorbitan monooleate (Tween 80) and sodium dodecyl sulfate (SDS) were from Fluka (Buchs, Switzerland), butanol was from Riedel de Haen (Seelze, Germany), dichloromethane, methanol were from Carlo

Erba (Milano, Italy). Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals were analytical grade and used without any further purification.

Methods

8-MOP HPLC Analysis

HPLC system was constituted by a LC-9A pump, a SPD-2 UV lamp, a C-R5A integrator (Shimadzu, Kyoto, Japan); column was an Allsphere ODS-2 5 μm 150 \times 4.6 mm, mobile phase was 50:50 water-methanol; 8-MOP was detected at 300 nm and retention time was 8 minutes.

8-MOP Solubility and Apparent Partition Coefficient (P_{app})

8-MOP water solubility at room temperature was measured by dispersing 5 mg drug in 1 ml water at 25°C: after 5 minute-magnetic stirring, the sample was centrifuged at 14000 rpm with 5417 Centrifuge (Eppendorf AG, Hamburg, Germany) and the supernatant was injected to HPLC.

8-MOP apparent partition coefficient between water and Mygliol 818 (chosen as model lipid phase) was determined, to predict if drug partitioned in the inner lipid phase during nanoemulsion preparation. Briefly: 5 ml of a 8-MOP solution were shaken for 10 minutes with 5 ml of Mygliol 818, then the phases were allowed to separate; 8-MOP concentration in water before and after partition was determined by HPLC to calculate apparent partition coefficient, according to the following formula.

$$P_{app} = \frac{[]_{oil}}{[]_{water}} = \frac{[]_{water \text{ before partition}} - []_{water \text{ after partition}}}{[]_{water \text{ after partition}}}$$

Nanoemulsion Preparation

Nanoemulsion was prepared by the homogenization method: 8-MOP was dissolved in the lipid phase at room temperature by vortexing; water and surfactants were added and emulsified with UltraTurrax (IKA, Staufen, Germany) high shear homogenizer at 15000 rpm for 1 minute: this primary emulsion was homogenized with Emusiflex high pressure homogenizer (Avestin, Ottawa, Canada) at 5000 psi for 5 minutes at 25°C.

SLN Preparation

SLN were prepared by the hot homogenization method[8]: 8-MOP was dissolved in melted lipid at 70°C by vortexing; water and surfactants were added at the same temperature and emulsified with UltraTurrax (IKA, Staufen, Germany) high shear homogenizer at 15000 rpm for 1 minute: this primary emulsion was homogenized with Emusiflex high pressure homogenizer (Avestin, Ottawa, ON, Canada) at 5000 psi for 5 minutes; obtained nanoemulsion was slowly cooled to room temperature by stirring.

SLN and Nanoemulsion Characterization

SLN and nanoemulsion were analysed for size distribution using a 90 Plus laser light scattering (LLS – Brookhaven Instruments, New York, NY, USA). SLN phase transitions were detected with a Perkin Elmer differential scanning calorimeter (DSC–Norwalk, CT, USA) directly on nanoparticles suspension.

Entrapment efficiency (EE%) was measured as the ratio between the lipid matrix-encapsulated drug and the total drug used for SLN preparation: 8-MOP loaded SLN were diluted in water up to obtain a drug concentration in the suspension two-fold lower than its water solubility. In this condition, the drug adsorbed on SLN surface diffuses towards the external phase according to the so called “burst release.”[8] This suspension was centrifuged at 26000 rpm with an Allegra 64 R centrifuge (Beckman Coulter, Brea, CA, USA) and the supernatant was directly injected to HPLC. Precipitate was dried under vacuum overnight and dissolved in a known amount of methanol, by mild heating when necessary; an equal amount of water was then added to precipitate the lipid matrix; centrifugation of the obtained suspension was followed by injection of the supernatant to HPLC to determine 8-MOP entrapped in the lipid matrix.

SLN Freeze-Drying

A known amount of SLN was diluted up to 2.5% lipid concentration; then, 5% arabic gum was added as cryoprotectant to suspension, which was freeze-dried for 24 hours by using a Modulyo freeze-drier (Edwards Alto Vuoto, Milano, Italy). Conditions were as follows:

- 1 freezing: -40°C, 2 hours,
- 2 primary drying: at 1.0 mbar, -30°C, 12 hours,
- 3 secondary drying: 0.1 mbar, 30°C, 3 hours.

The obtained powder was then milled in a mortar and slowly added to various volumes of water to allow the dispersion: each sample was then analysed by LLS. Rheograms of the suspension before and after freeze drying were measured using a LVDV DV-III rotational viscometer (Brookfield Co., Middleboro, MA., USA) equipped with a small camber adapter at 25°C.

MDA Assay

MDA is a marker of lipid peroxidation, used for biomedical application, too[9 , 10]: in this study a method was developed for determination of skin lipid peroxidation by a MDA assay.

Full-thickness pig ear skin was used for the experiment: ears were preliminarily washed with cold water and then dried by pressing them between filter papers. The hair of the outer skin surface was removed with dissecting scissors brought as close as possible to the skin without damaging it. The skin was carefully dissected with scalpel and forceps, in order to isolate stratum corneum. Skin was frozen until use, when it was cut in very small pieces, and rinsed with normal saline at 25°C; next, nearly 1 g chopped skin was put in radiation cells together with 10 ml of each formulation and irradiated under UVA lamp (Philips TL K05 40 W) for 3 hours, 10 cm far from lamp. Declared power for this lamp is 40 W between 320 and 380 nm and the radiation intensity of the lamp, measured by a multimeter (CO.FO.ME.GRA, Milan, Italy) was 8.9×10^{-4} W/cm².

Finally, skin was withdrawn and washed with water, before being dried under vacuum for 3 hours; dried samples were left in 5 ml dichloromethane overnight to extract MDA. Dichloromethane was evaporated in a rotovapor and the residue in the flask was collected with 3 ml of an 8.1% SDS solution.

This solution was analysed for MDA: briefly, 0.2 ml sample were added of 0.3 ml distilled water, 1.5 ml 1% phosphoric acid and 1 ml 0.6% TBA, and heated at 95°C for 45 minutes; after cooling in ice bath, MDA was then extracted by shaking with 4 ml butanol: the organic phase was analysed at 533 nm with a Lambda 2 spectrophotometer (Perkin Elmer, Norwalk, CT, USA). A calibration curve for MDA was prepared by using standards of analytical purity.

RESULTS AND DISCUSSION

8-MOP water solubility at 25°C is 58 µg/ml, and it can increase by adding methanol. LogPapp of 8-MOP between Mygliol 818 and water is 2,07. All these results agree with literature data[11] and suggest drug partitioning in the lipid phase of nanoemulsions and consequently a possible drug entrapment into SLN produced through the hot homogenization technique.

First, nanoemulsion was prepared, whose composition, mean diameter, and polydispersity of inner phase droplets (LLS) are reported in Table 1. Five percent α -tocopherol was incorporated in the emulsion as antioxidant.

	Nanoemulsion
octyloctanoate	10 %
α -tocopherol	5 %
8-MOP	0.03 %
Mygliol 818	
Pluronic F68	2.5 %
Distilled water	q. s. 100 %
Mean diameter (nm)	224 \pm 21
Polydispersity	0.051

TABLE 1 Nanoemulsion composition, mean diameters and polydispersity

SLN then were prepared by hot homogenization technique, by substituting the oil phase of the nanoemulsion with a solid lipid, like trimyristin (a triglyceride) and cetyl palmitate (a long chain ester), but 8-MOP EE% was negligible (<2%) for all the formulations (data not shown). It is reported in literature[12] that the substitution of a part of the solid lipid with a fluid lipid can enhance drug EE% within nanoparticles, but, in this case, the use of 5% Mygliol 818 as fluid lipid did not affect 8-MOP EE% (data not shown).

Therefore, stearyl alcohol-SLN were prepared according to a formulation already reported in literature[13]: an amount of the lipid matrix was substituted with two fluid lipids, Mygliol 818 and α -tocopherol, which has antioxidant properties, too. SLN composition is shown in Table 2, together with mean diameters, polydispersity and EE%.

	Stearyl alcohol (SLN 1)	Stearyl alcohol Mygliol 818 (SLN 2)	Stearyl alcohol α -tocopherol (SLN 3)	Cetostearyl alcohol α -tocopherol (SLN 4)
Stearyl alcohol	15%	10%	10%	10%
8-MOP	0.03%	0.03%	0.03%	0.03%
Mygliol 818		5%		
α -tocopherol			5%	5%
Tween 80	1%	1%	1%	1%
Distilled water	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%
Mean diameter (nm)	402 \pm 39	406 \pm 27	505 \pm 49	418 \pm 35
Polydispersity	0.160	0.177	0.265	0.089
Mean diameter after 1 month storage (nm)	N.D.	N.D.	N.D.	423
Polydispersity after 1 month storage	N.D.	N.D.	N.D.	0.127
Mean diameter after lyophilization (nm)	N.D.	N.D.	N.D.	465
Polydispersity after lyophilization	N.D.	N.D.	N.D.	0.261
EE%	1.5%	33.2%	32.1%	45.6%
All percentages are w/w.				
N.D.: not detectable.				
All percentages are w/w. N.D.: not detectable.				

TABLE 2 Fatty alcohols-SLN composition mean diameters, polydispersion and EE%

Stearyl alcohol SLN showed an unsatisfactory EE%, but the addition presence of a fluid lipid, both Mygliol 818 and α -tocopherol determined a noteworthy increase of EE%. Although α -tocopherol-containing SLN showed a broader size distribution and larger mean diameter, α -tocopherol can be useful since it is also an effective antioxidant on the skin, widely used in cosmetic and pharmaceutical products. SLN 1, 2, 3, all containing stearyl alcohol, were not stable and gave coalescence within 1 day storage at room temperature: this is due to fatty alcohols polymorphism, as discussed below.

To avoid these stability problems, as shown in Table 1, stearyl alcohol was substituted by cetostearyl alcohol, which is a mixture of stearyl and cetyl alcohol and does not show the polymorphism of pure fatty alcohols.[14] Substitution of stearyl alcohol with cetostearyl alcohol led to smaller nanoparticles with a narrow size distribution, which were stable within a month; moreover, also EE% increased compared to that obtained in stearyl alcohol-based formulations.

DSC measurements of fatty alcohols nanoparticles are reported in Figure 1.

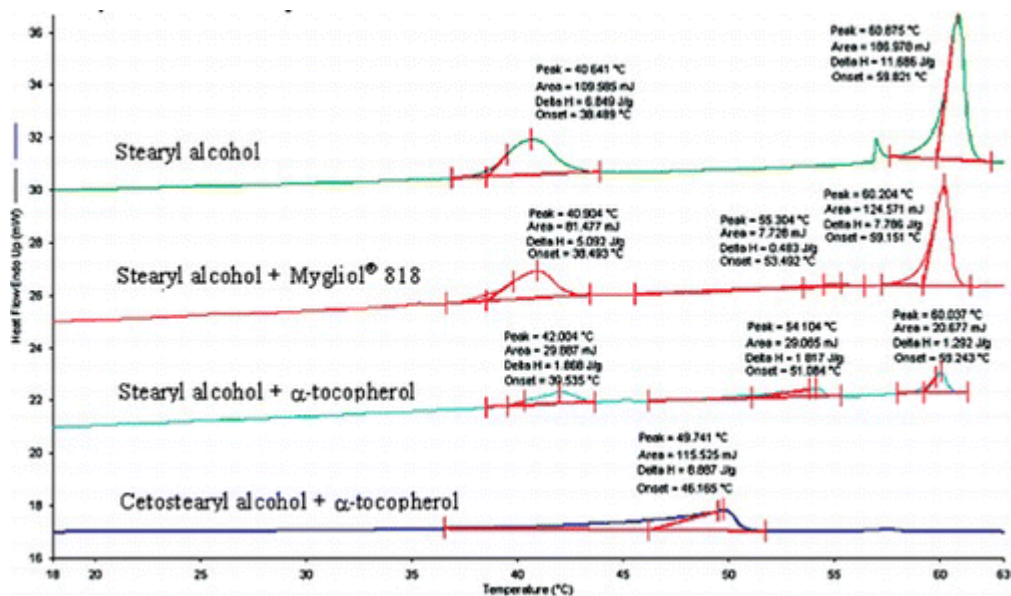


FIG. 1 DSC of fatty alcohols nanoparticles.

Fatty alcohols polymorphism was already described in literature:[14] fatty alcohols can exist in two different melting crystalline forms, and show a time dependent shift from a high melting form to a low melting one. This phenomenon can be present also in raw materials, but it is enhanced in aqueous systems, like emulsions or ternary mixtures. Generally, it causes phase separation of these systems, because of the instability at the water-lipid interface.

In stearyl alcohol-SLN, polymorphism was noted too, which can be identified by more than one peak in DSC thermograms; transition enthalpies and temperatures depend on the presence and the type of fluid lipid: generally, fluid lipids lead to the decrease in the enthalpy

of the two main transitions of stearyl alcohol, and to the appearance of an intermediate peak. Cetostearyl alcohol lacks crystalline purity of stearyl alcohol,[14] therefore, the polymorphism is no more present, and SLN 4 show only one eutectic transition, with increase in stability of the system.

SLN 4 mean particle size was preserved after 1 month-storage at room temperature, with a slight increase in polydispersity. To obtain a more stable product, SLN 4 were freeze-dried with the addition of arabic gum as cryoprotectant to avoid particle aggregation.

SLN were diluted to 2.5% lipid concentration with 5% arabic gum and freeze-dried; obtained powder was resuspended in the same volume of water under simple stirring. Although size distribution was broader after freeze-drying, mean diameter was maintained, as shown in Table 2.

Resuspended SLN were more viscous than 2.5% SLN with 5% arabic gum, showing a pseudoplastic behavior and a small tixotropy, which can be desirable for topical administration. The volume of dispersing water can be reduced, owing to the desired drug concentration, with consequent increase of viscosity and tixotropy area. In Figure 2, rheograms of freeze-dried and resuspended SLN are shown.

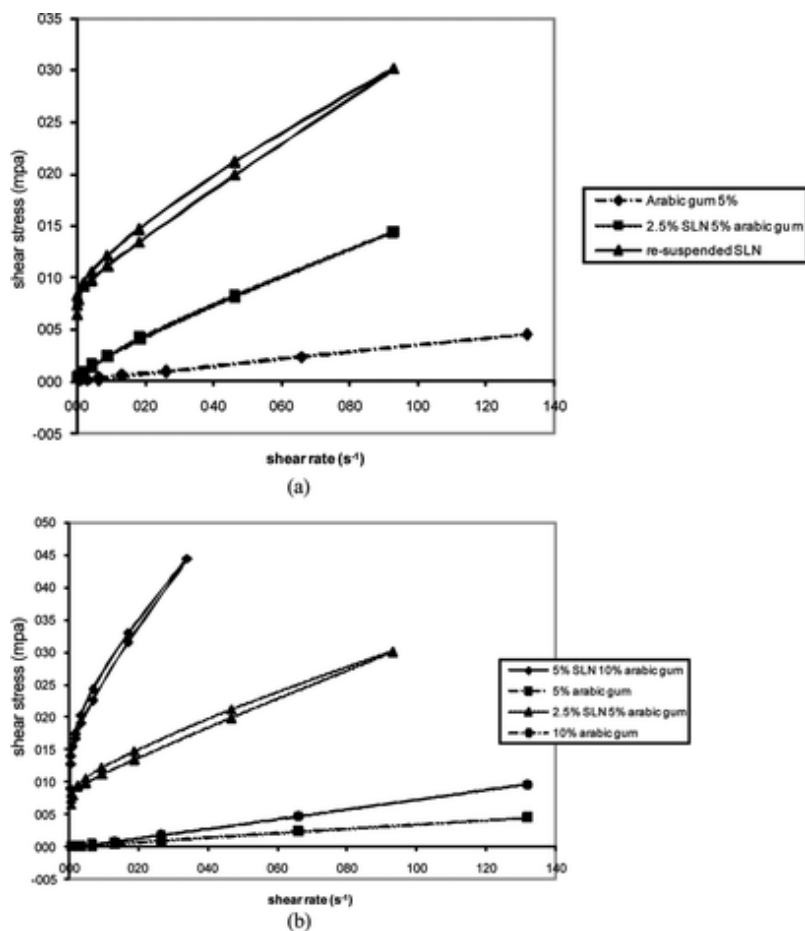


FIG. 2 Rheograms of freeze-dried and resuspended SLN. (A) comparison between SLN before and after freeze-drying and resuspension; (B) comparison between freeze-dried SLN resuspended in different volumes of water.

Finally, nanoemulsion and SLN 4 were tested on pig ear skin during UVA irradiation. 0.03% 8-MOP solution in water/methanol 50:50 was used as reference, while blank was made by incubating the skin with water/methanol 50:50 without UVA irradiation.

MDA present in the skin was then evaluated as lipid oxidation marker and results are reported in Figure 3.

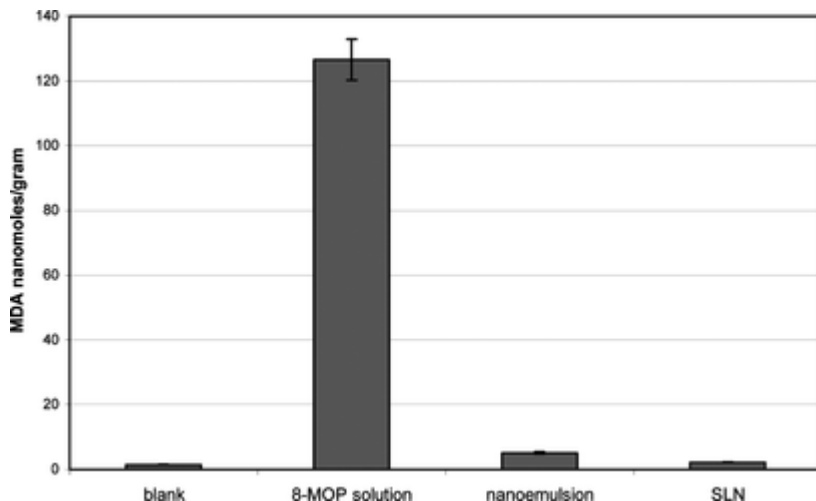


FIG. 3 MDA in pig ear skin after UVA irradiation.

In blank sample no significant amount of MDA was revealed, whereas 8-MOP solution caused skin photooxidation, which can be reduced by adding α -tocopherol; α -tocopherol is effective as antioxidant both in nanoemulsion and in SLN.

CONCLUSIONS

8-MOP loading nanoemulsion and SLN were prepared: a significant percentage of drug was encapsulated in the lipid core of SLN 4, due to the use of fluid lipids. SLN were stable, as to particle size, within a month, and after freeze-drying a redispersible powder was obtained which showed interesting rheological properties for topical delivery. α -tocopherol worked as a fluid lipid enhancing 8-MOP EE% within SLN, and was also effective as antioxidant on skin during UVA irradiation: this could be an interesting perspective to reduce photooxidation based skin irritation, which is a common side effect in topical PUVA.

Notes

All percentages are w/w.

N.D.: not detectable.

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