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Maria Eugenia Carlotti*, Simona Sapino, Elena Ugazio, Marina Gallarate

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Torino, Italy

Silvia Morel

Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Novara, Italy

*Corresponding author:

M.E. Carlotti, Dipartimento di Scienza e Tecnologia del Farmaco, via Giuria 9, 10125 Torino, Italy

e.mail: eugenia.carlotti@unito.it; Phone:+39-011-6707668; Fax:+39-011-6707686

Abstract

This report investigates the possibility of producing solid lipid nanoparticles as protective vehicle of resveratrol, an antioxidant characterised by a fast *trans-cis* isomerisation. SLN aqueous dispersions were produced by hot melt homogenisation technique and characterised. It was found that the presence of tetradecyl-γ-cyclodextrin in SLN formulation induced an improvement of nanoparticle characteristics. Moreover a significant reduction in resveratrol photodegradation was noted when the molecule was entrapped in SLN which became more pronounced in the presence of tetradecyl-γ-cyclodextrin. A notable *in vitro* porcine skin accumulation and an increased antioxidative efficacy were observed by entrapping resveratrol in nanoparticles.

Keywords: resveratrol, solid lipid nanoparticles, tetradecyl-γ-cyclodextrin, photostability, skin uptake

Introduction

Resveratrol (3,4',5- trihydroxystilbene, RV) was firstly detected by Langcake and Pryce [1] who found that it is a naturally occurring phytoalexin produced by some spermatophytes, such as grapevines, in response to injury, as fungal infection or exposure to ultraviolet light. As phenolic compound, resveratrol contributes to the antioxidant potential of red wine [2] and may play a role in the prevention of human cardiovascular diseases. The interest in compounds present in grapevines was stimulated when epidemiological studies showed an inverse correlation between red wine consumption and incidence of cardiovascular diseases, the so-called French paradox [3]. It has been suggested that constituents other than alcohol could have a protective effect and numerous studies provide support for the bioactivity of phenolic components which for a large part have a flavonoid structure [4-6]. Particularly, resveratrol has been shown to modulate the metabolism of lipids as

well as to inhibit the oxidation of low density lipoproteins and the aggregation of platelets [7-11]. It also possesses anti-inflammatory and anticancer properties [12, 13] and it was found to be a better radical scavenger than vitamin E and C, similar to the flavonoids epicatechin and quercetin [14]. However it must be taken into account that resveratrol exists in *trans* and *cis* stereo isomeric forms: due to differences in energetic state, the *trans* form is the more biological active and the more common in nature even if in wine the *cis* form is also present, resulting from photoisomerisation reactions easily induced by light exposure [15, 16]. In the literature a validated method to determine *trans*- and *cis*-resveratrol concentration in aqueous solutions has been described [17]. *Cis* isomer was obtained by exposing a *trans*-resveratrol aqueous solution to sunlight followed by HPLC separation and analysis by mass spectrometry (oxidation products were absent). Accurate values for UV absorbance in water were obtained allowing the authors to propose a simple and reliable UV-vis spectrophotometric method to assess the *trans*/*cis* resveratrol ratio. Summarizing, despite *trans*-resveratrol arouses interest in many field as chemopreventing agent, antibacterial, antifungine, antioxidant and antiradical, it is necessary to consider that this molecule presents physico-chemical instability phenomena related mainly to its fast *trans*-*cis* isomerisation and also to the reaction with oxygen, events that reduce its efficacy. One of the possibility to increase the stability of drug is the use of carrier system as SLN, liposomes, etc. [18, 19]. Besides cyclodextrins are well-known pharmaceutical excipient which can be used to improve the physico-chemical properties of guest drug (e.g solubility, stability and bioavailability) [20]. Some authors described the aggregation properties of cyclodextrins bearing hydrophobic substituents in aqueous media showing that they can form self-organized structures [21, 22]. Liposomes and SLN containing alkylcarbonates of γ-cyclodextrins have been recently proposed [23, 24] and results showed that the introduction of cyclodextrin-derivatives increased the drug loading and the stability of drugs compared to conventional carrier systems. Accordingly, the aim of the present work was to assess the possibility

of producing SLN that could protect resveratrol from its fast degradation. In formulating these colloidal systems the possibility of using tetradecyl-γ-cyclodextrin (C14CD) has been considered to enhance their protective ability and modulate resveratrol release. The UVA-induced photodegradation of both free and enclosed resveratrol was therefore investigated. Successively *in vitro* studies were carried out to evaluate the uptake and the anti-lipoperoxidative activity of resveratrol in porcine ear skin.

Experimentals

Materials

Sodium dodecyl sulfate (SDS), 1-tetradecanol, glycerol tridecanoate (tricaprin, TRIC) and polyoxyethylene sorbitan monostearate (Tween®60) were from Fluka (Milan, Italy). Phosphoric acid, hydroxylethylcellulose (Natrosol®MR, HEC), polyglyceryl-3-methyl glucose distearate (Tego Care®450) and octyl octanoate (Tegosoft®EE) were supplied by ACEF (Fiorenzuola d'Arda, Piacenza, Italy). 4,6-Dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid, TBA) was purchased from Merck (Darmstadt, Germany). Hexadecyl hexadecanoate (cetyl palmitate, CP), 1,1,3,3-tetraethoxypropane and 3,4',5-trihydroxy-*trans*-stilbene (*trans*-resveratrol, RV) were provided by Sigma (Milan, Italy). Potassium cetyl phosphate (Amphisol®K) was a product from Roche (Milan, Italy) while soybean lecithin (phosphatidylcholine content above 95%, Epikuron®200) was obtained from Lucas Meyer (Milan, Italy). Arachidyl alcohol/behenyl alcohol/arachidyl glucoside (Montanov®202) was a gift from Seppic (Paris, France) while C_{12-20} acid PEG-8-ester (Xalifin®15) was a gift from Vevy Europe (Genoa, Italy). C_{14}-alkylcarbonate-γ-cyclodextrin (tetradecyl-γ-cyclodextrin, C14CD) was synthesized in the laboratory of Macromolecular Chemistry according to the procedure reported in the literature [25]. Briefly, 1-tetradecanol was activated by reaction with an excess of carbonyldiimidazole in alcohol free chloroform. In the second step, the imidazoyl

derivative was allowed to react with anhydrous γ-cyclodextrin in anhydrous pyridine at 80 °C for 4 h. Once the reaction was over, the residual precipitate was filtered off and distilled water was added to the organic solution. The solid was recovered by filtration, washed many times with water and then freeze-dried. The mean degree of substitution was 3. All other reagents were analytical grade and obtained from Carlo Erba (Milan, Italy).

Resveratrol dosage

Trans-resveratrol concentration in the samples was determined at 306 nm by a UV-vis spectrophotometer (Perkin Elmer, Waltham, MA, USA) or using a HPLC apparatus (Shimadzu, Tokyo, Japan) employing as eluent a mixture of water/methanol/acetic acid (52/48/0.5) at a flow rate of 0.8 ml/min and a RP-C18 (150×4.6 mm, 5 μm) column. Diluted solutions of RV in methanol over the range 1.46×10^{-5} - 43.8×10^{-5} M were analyzed. The molar extinction coefficient of *trans*-resveratrol obtained spectrophotometrically was 40460 M^{-1} cm^{-1} (R^2 = 0.9979) while the equation of the calibration curve obtained by HPLC was y = 130057x -14599 (R^2 = 0.9973).

SLN preparation

SLN consist of a lipid core and an amphiphilic surfactant outer shell. In the present work a mixture of cetylpalmitate and tricaprin was chosen as the solid core. Moreover, since surfactants are of great importance in addition to the appropriate choice of the lipid material, different surfactants were tested in a preformulation step in order to select the most effective in terms of stability, particle size and entrapment efficiency.

SLN were prepared using a hot melt homogenization technique. Briefly, the melted lipid phase was added to the hot aqueous surfactant solution, pre-heated to 10 °C above the lipid's melting point, under homogenization by T25 basic Ultra-Turrax (IKA, Staufen, Germany) at 10,000 rpm for 5 min. The obtained O/W emulsion was then cooled in ice-bath to re-crystallize the lipid phase to the

solid state in the form of a SLN aqueous suspension. Drug-loaded SLN were prepared by dissolving RV in the melted lipid phase before addition to the aqueous phase. SLN with C14CD were prepared by dispersing the CD derivative in the hot aqueous phase before adding the lipid phase.

Size and zeta potential measurement

The mean particle size and the zeta potential (ZP) were obtained at 25 ± 0.1 °C by laser light scattering measurements using a 90 Plus Particle Size Analyzer (Brookhaven Instrument Corporation, Holtsville, NY, USA) at a fixed angle of 90° after appropriate dilutions. Each system was analyzed three times and for each of them five determinations were made. The polydispersity index (P.I.), which is a parameter for the width of the particle size distribution, was also determined.

DSC studies

Thermal analysis was performed by differential scanning calorimetry employing a DSC-7 instrument (Perkin Elmer, Waltham, MA, USA). The solid samples were placed in conventional aluminum pan and then heated from 25 to 300 °C at a scanning speed of 10 °C min^{-1}. The weight of each sample was around 5 mg.

Determination of RV entrapment efficiency

An aliquot (1.0 ml) of the SLN suspension was centrifuged for 30 min at 24,000 rpm. The sediment was washed with 30/70 methanol/water mixture to eliminate the absorbed RV. The solid residue was dissolved in methanol and analyzed by HPLC. The percentage of entrapment efficiency (% EE) was expressed as amount of RV in washed SLN vs total RV amount in 1 ml SLN suspension × 100.

Gel and O/W emulsions preparation

Gel was prepared by dispersing HEC in water at 90 °C and mechanically stirring by DLS stirrer (Velp Scientifica, Usmate, Italy) until room temperature was reached. Free RV or RV-loaded SLN were then dispersed in the final preparation under vigorous stirring.

The O/W emulsions (A and B) were prepared by dispersing the melted lipid phase (A: Montanov 202 and Tegosoft EE; B: Xalifin 15 and Tegosoft EE) in water at 70 °C under homogenization by Ultra-Turrax. Each emulsion was then cooled to room temperature under mechanical stirring by DLS stirrer. Free RV was pre-dispersed in the melted lipid phase while RV-loaded SLN were added to the final emulsion under vigorous stirring. The percentage compositions of these formulations are summarized in Table 1.

Photodegradation study

RV photodegradation trend was determined in different systems listed below. An aliquot (10 ml) of each sample was introduced in a Pyrex glass cell and placed, under magnetic stirring, at 20 cm from Actinic BLT 40W UVA lamp (Philips, Milan, Italy) with 2.8 W m^{-2} power emission of radiation. At fixed times of 5 min over 30 min of total irradiation an amount (100 µl) of each irradiated sample was withdrawn, properly diluted and HPLC analyzed after centrifugation to follow the kinetic of RV photodegradation.

The systems subjected to the photodegradation study were:

Free RV: RV (0.2 mM) dispersed in gel, in O/W emulsion A and in O/W emulsion B.

RV-loaded SLN aqueous suspensions: SLN without C14CD (RV 1.31 mM); SLN with C14CD (RV 1.31 mM); SLN with C14CD dispersed in gel, in O/W emulsion A or in O/W emulsion B (RV 0.2 mM final concentration).

***In vitro* skin uptake studies**

The RV skin uptake was determined *in vitro* using vertical Franz cell [26] and full-thickness pig ear skin. Firstly, the skin was pre-hydrated with normal saline added with 0.002% w/v sodium azide. The receptor chamber of the cell was filled with 6.0 ml of normal saline and magnetically stirred at 37 ± 0.1 °C. The tested formulations were: ethanol solutions of RV (0.2 and 1.31 mM) and RV-loaded SLN aqueous dispersion without or with C14CD (RV 1.31 mM). They were applied to the skin surface which had an available diffusion area around 1.5 cm^2. The amount of RV retained in the skin was determined 24 h after application as follows: the skin was washed with water/ethanol (50/50 v/v), cut into small pieces and magnetically stirred in methanol (3 ml) for 2 h at room temperature. After 5 min centrifugation at 12000 rpm the supernatant was assayed by HPLC and the skin uptake was expressed as μg cm^{-2} corresponding to amounts of RV vs skin diffusion area.

Anti-lipoperoxidative activity

The assay, currently used as an index of lipoperoxidation is based on the reactivity of malondialdehyde (MDA), a colourless end-product of degradation, with thiobarbituric acid (TBA) to produce a pink adduct (TBA-MDA-TBA) that absorbs at 535 nm. This adduct was detected spectophotometrically according to the method described by Bay et al. [27]. Firstly, skin slices were isolated by a surgical scissor from porcine ears freshly obtained from a local slaughterhouse and frozen at -18 °C for at least 24 h. Before the experiment the skin was equilibrated in 0.9% w/w normal saline added with sodium azide (0.002% w/v) at room temperature for 30 min. Pieces of skin slices (area around 1.5 cm^2) were allocated on Franz cells as previously described and on each of them a formulation (500 μL) was applied in the dark at room temperature for 24 h. The tested formulations were: *i)* O/W emulsion A without RV (reference); *ii)* O/W emulsion A containing free RV (0.2 mM); *iii)* O/W emulsion A containing RV-loaded SLN with C14CD (RV 0.2 mM final concentration). Therefore each skin piece was taken off, washed with normal saline and cut up in

small pieces that were dispersed in 10 ml normal saline to be irradiated in Pyrex cells for 3 h at 20 cm distance from the UVA light source. After irradiation, the skin pieces were dried under vacuum for 90 min and then magnetically stirred for 16 h in dichloromethane (10.0 mL) to extract MDA. The organic solvent was then evaporated by a RE 111 Rotavapor (Büchi, Flawil, Switzerland) and the residue was reconstituted with 3.0 mL of 8.1% w/w SDS. An aliquot (0.2 mL) of this dispersion was added with 0.1 mL of water, 0.2 mL of SDS (8.1% w/w), 1.5 mL of phosphoric acid (1.0% w/w) and 1.0 mL of TBA (0.6% w/w). The reaction mixture was heated for 45 min in water bath at 100 °C, then cooled in ice bath and finally added with 4.0 mL of 1-buthanol to extract the TBA-MDA-TBA adduct. After centrifugation the absorbance values were measured at 535 nm. The MDA concentration in the reaction medium was calculated from the calibration curve of 1,1,3,3-tetraethoxypropane, a MDA precursor that as MDA reacts with TBA to form a chromophore (ε = 7,098 M^{-1} cm^{-1}). The experiment was repeated thrice and the results were averaged.

RESULTS

In this study, the influence of formulation composition on the physico-chemical parameters of SLN was evaluated. It is known that the particle size distribution and the surface charge (zeta potential) are some of the most important characteristics for the evaluation of the stability of colloidal systems. Accordingly, nine different formulations have been developed, only two of them containing RV (Table 2); their physico-chemical parameters were investigated and compared. Cetyl palmitate and tricaprin were selected as solid lipid components of the SLN core, Tego Care 450 was employed as the main surfactant.

Since SLN prepared with combination of surfactants generally tend to have higher storage stability by preventing particle agglomeration more efficiently [28, 29] we tested different surfactant mixtures and investigated their effect on the size and zeta potential of resultant SLN.

As shown in Table 3, by replacing Amphisol K 0.6% w/w (SLN1) with C14CD 0.8% w/w (SLN2) the mean particle size decreased from 472.2 to 349.9; moreover by increasing the content of C14CD from 0.8% w/w (SLN2) to 1.6% w/w (SLN3) the diameter further decreased from 349.9 to 293.3. Probably, the interaction between lipid matter and C14CD might increase the surface curvature of the oil droplets leading to small size of SLN after cooling down.

The mean size of SLN 4 prepared with Amphisol K 0.4% w/w and C14CD 1.2% w/w resulted 334.4. Therefore the combination of Amphisol K and C14CD also contributed to the reduction in the size of the SLN. However as shown in Table 3, in the presence of Amphisol K the increase in C14CD content more than 1.2% w/w (SLN5 and SLN6) resulted in particle size, P.I. and zeta potential increment. It might be that Amphisol K markedly affected the arrangement of the increased C14CD molecules at the interface of the oil droplets. From these results the percentage of C14CD was fixed to lower value in the subsequent studies. The zeta potentials of SLN1, SLN2, SLN3 and SLN4 were similarly negative and around -35 mV (Table 3). Thus, the range of zeta potential obtained was high enough for a sufficient electrostatic stabilization. It can be also noted that the combination of Amphisol K and C14CD contributed to the increment in the negative values of zeta potential of SLN. The zeta potential of SLN4, SLN5 and SLN6 were -32.92, -42.69 and -52.68 mV, respectively. These data indicate that the mixture Amphisol K/C14CD could serve as electrostatic stabilizer in our SLN formulation.

The combination of Amphisol K and C14CD with lecithin (Epikuron 200) increased the particle size from 334.4 (SLN4) to 499.5 (SLN7). Moreover the P.I. of SLN with lecithin (SLN7) was about 1.4-fold higher (from 0.177 to 0.240) than those without lecithin (SLN4) suggesting the heterogeneous distribution of the nanoparticles prepared with phosphatidylcholine.

We also investigated the impact of RV loading on the physico-chemical characteristics of SLN in the absence and in the presence of C14CD. SLN8 and SLN9 were prepared with 1.2% w/w 1-

tetradecanol and 4.0% w/w Tween 60 in order to enhance the solubility of RV in the lipid phase. The obtained mean particle size of SLN prepared without C14CD (SLN8) was 586.1 while that of SLN prepared with C14CD (SLN9) was 379.5; their P.I. were 0.366 and 0.292 mV, respectively. These data indicate that the presence of C14CD in combination with Amphisol K may be desirable in getting stable RV-loaded SLN formulation as already observed with RV-free SLN formulation. By including C14CD in SLN their negative values of zeta potential increased from -42.95 mV of SLN8 to -53.55 mV of SLN9, in accordance with data above reported showing that the combination Amphisol K/C14CD contributes to the negative surface charge of SLN.

Thermal analysis (DSC) was performed to investigate the status of the inner phase in the nanoparticulate systems. Generally, the melting peak of lipid core in SLN is observed at lower temperature than that of bulk lipid due to the nanocrystalline size of lipids in SLN [28]. As shown in Figure 1, pure cetyl palmitate exhibited a main transition peak around 51 °C while tricaprin around 32 °C. The melting peak of the SLN was around 44 °C showing that an interaction occurs between the lipids and the other components of the nanoparticles. The thermogram of RV displayed a melting peak at 265.5 °C that was almost absent in the thermogram of RV-loaded SLN (data not shown) which indicates that RV was incorporated inside the lipid matrix of the SLN. Furthermore, comparing the thermogram of empty SLN with that of RV-loaded SLN it can be noted that incorporation of RV did not greatly change the melting point of SLN.

RV entrapment efficiency (% EE)

Results of physico-chemical characteristics, summarized in Table 3, indicated a favorable effect of the presence of C14CD in the SLN allowing an enhancement in their quality and stability. In order to investigate in depth the actual advantages derived from using C14CD in our SLN two different RV-loaded SLN have been developed: SLN8 and SLN9. The presence of C14CD contributed to slightly increase the entrapment efficiency. Particularly the % EE was 51.2% w/w in SLN8 (without

C14CD) and 53.3% w/w in SLN9 (with C14CD). Thus, an additional advantage resulted by the use of C14CD which contributed to slightly increase the RV loading in SLN.

Photodegradation study

As previously reported, RV isomerizes from the *trans* to the *cis* form under UV irradiation [17]. In our previous study [30] RV photodegradation was investigated and results indicated that the inclusion into a RV/HP-β-CD host-guest complex enhanced its photostability. We also found that RV entrapped in liposomes degraded slower than free RV. Accordingly, in the present work the increment of RV photostability by entrapment in SLN has been evaluated. Moreover it was investigated the possibility of further increase the protective ability of SLN by including C14CD in their formulation.

The curves of RV photodegradation followed a first order kinetic in all the media tested. Thus, they were linearised in a logarithmic form as follows:

$\ln[(C_t-C_{inf})/(C_0-C_{inf})] = k\ t$

where C_0 is the initial RV concentration, C_t the concentration of RV at time t, C_{inf} the concentration of RV at infinite time, k the degradation rate constant and t the irradiation time, in minutes.

SLN8 and SLN9 aqueous suspensions were firstly investigated. Interestingly, RV entrapped in SLN without C14CD (SLN8) degraded 2-fold faster (from 7.42×10^{-4} s^{-1} to 14.8×10^{-4} s^{-1}) than RV entrapped in SLN with C14CD (SLN9). These data indicate that the presence of C14CD in SLN formulation might contribute to protect RV from the photodegradation. From these results SLN9 was investigated in the subsequent studies of photodegradation. The kinetic constants of degradation rate of free RV were compared with those of RV entrapped in SLN9 as shown in Table 4.

A comparison of the obtained kinetic constants (Table 4) indicates that in all the tested media the entrapment of RV in SLN9 decreased the rate of photodegradation (from 2.39×10^{-3} to 1.63×10^{-3} s^{-1} in HEC gel, from 2.09×10^{-3} to 1.08×10^{-3} s^{-1} in emulsion A and from 2.11×10^{-3} to 1.53×10^{-3} s^{-1} in emulsion B). It can be also noted that the nature of the dispersion medium slightly affected the rate of RV photodegradation. Particularly, the degradation rate decreased following the sequence: HEC gel > emulsion B > emulsion A.

***In vitro* skin uptake studies**

RV is employed topically as antioxidant or as anticancer agent [31, 32], thus in the present study the ear porcine skin absorption of this molecule was *in vitro* investigated. Firstly, two ethanolic solutions of free RV, 0.20 mM and 1.31 mM, were tested as references. Next, the experiment was carried out on SLN8 and SLN9 aqueous suspensions. The results are presented in Table 5.

A linear relationship between RV concentration and skin accumulation was observed in ethanol solution. Particularly, as RV concentration increased from 0.20 mM to 1.31 mM the skin permeation varied from 2.08 µg cm^{-2} to 10.78 µg cm^{-2}. Secondly, the skin uptake data of RV from nanoparticulate systems were comparable to that observed from ethanol solution at the same concentration (1.31 mM). Considering that ethanol is one of the most efficacious enhancer of skin permeability [33], the percutaneous absorption of RV from our SLN can be considered notable. An occlusive effect [34] cannot be excluded to be of relevance for this remarkable uptake. Interestingly, addition of C14CD in SLN formulation slightly affected the skin uptake of RV that resulted 13.72 µg cm^{-2} from SLN8 and 11.01 µg cm^{-2} from SLN9. Our hypothesis is that the presence of C14CD might increase the affinity of RV towards the lipid core of the nanoparticles which would result in decreased release of RV from the vehicle and thereby reduce skin permeability.

Anti-lipoperoxidative activity

In this study the preventive effect of RV against the damages of UVA radiations to the porcine skin was investigated. MDA levels were monitored, through the TBA test, as markers of lipoperoxidation phenomena. As shown in Figure 2 the presence of RV was found to inhibit the skin lipid degradation. Surprisingly, MDA level detected in the skin treated with free RV was 5.6-fold higher than that observed in the skin treated with RV-loaded SLN9. This increased antioxidant property might be linked to the skin uptake of RV which, in accordance with previous finding, should be increased by entrapment in SLN.

Conclusions

In the present study nanoparticulate systems (SLN) were formulated to improve RV stability, whose *trans-cis* isomerisation has been a limiting factor in topical use. Firstly, the physico-chemical properties of RV-loaded SLN, including mean particle diameter and zeta potential, were modulated by changing the surfactant mixture. The loading of RV in optimized SLN formulation reduced the photodegradation rate of RV, compared with RV freely dispersed in the same medium. It was also found that the presence of C14CD improved the physico-chemical parameters of SLN formulation, enhanced the photostability of RV enclosed in SLN compared with that in SLN without C14CD and slightly increased the entrapment efficiency of RV in SLN. The skin uptake of RV from SLN formulation was comparable to that of RV freely dissolved in ethanol. Furthermore the anti-lipoperoxidative activity of RV could be improved by entrapping the molecule in SLN. Taken together these results suggest that loading of RV in SLN containing C14CD may provide an innovative formulation for dermatologic application.

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TABLE 1

Percentage compositions (% w/w) of gel and O/W emulsions

Components	Gel	Emulsion A	Emulsion B
HEC	2	-	-
Tegosoft EE	-	15	12
Montanov 202	-	6	-
Xalifin 15	-	-	8
Water	98	79	80

TABLE 2

Compositions (% w/w) of SLN tested in the preformulation step

Components	SLN1	SLN2	SLN3	SLN4	SLN5	SLN6	SLN7	SLN8	SLN9
Cetyl palmitate	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Tricaprin	2.4	2.4	2.4	2.4	2.4	2.4	2.4	1.2	1.2
Tego Care 450	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
Amphisol K	0.6	-	-	0.4	0.4	0.6	0.4	0.6	0.6
C14CD	-	0.8	1.6	1.2	1.6	1.6	1.2	-	0.6
1-Tetradecanol	-	-	-	-	-	-	-	1.2	1.2
Epikuron 200	-	-	-	-	-	-	0.4	-	-
Tween 60	-	-	-	-	-	-	-	4	4
RV	-	-	-	-	-	-	-	0.05	0.05
Water	92.5	92.3	91.5	91.5	91.1	90.9	91.1	88.5	87.9

TABLE 3

Particle size parameters (mean diameters and P.I.) and zeta potentials of SLN formulations

Sample	Mean size (nm)	P.I.	ZP (mV)
SLN1	472.2 ± 1.3	0.343 ± 0.008	-31.98 ± 0.11
SLN2	349.9 ± 1.1	0.234 ± 0.009	-37.71 ± 0.12
SLN3	293.3 ± 0.9	0.246 ± 0.007	-34.22 ± 0.21
SLN4	334.4 ± 1.0	0.177 ± 0.005	-32.92 ± 0.13
SLN5	483.6 ± 1.4	0.297 ± 0.007	-42.69 ± 0.24
SLN6	495.3 ± 1.2	0.365 ± 0.012	-52.68 ± 0.12
SLN7	499.5 ± 1.3	0.240 ± 0.006	-54.46 ± 0.26
SLN8	586.1 ± 1.4	0.366 ± 0.009	-42.95 ± 0.32
SLN9	379.5 ± 1.2	0.292 ± 0.011	-53.55 ± 0.21

TABLE 4

Kinetic constants (s^{-1}) of photodegradation rate of RV (0.2 mM), free or entrapped in SLN9, dispersed in HEC gel or in O/W emulsions

Medium	Free RV	RV in SLN9
HEC gel	$2.39 (\pm 0.09) \times 10^{-3}$	$1.63 (\pm 0.08) \times 10^{-3}$
Emulsion A	$2.09 (\pm 0.07) \times 10^{-3}$	$1.08 (\pm 0.08) \times 10^{-3}$
Emulsion B	$2.11 (\pm 0.05) \times 10^{-3}$	$1.53 (\pm 0.09) \times 10^{-3}$

TABLE 5

Uptake in ear porcine skin of RV, free or entrapped in SLN8 or SLN9

Samples	Uptake after 24 h (μg cm^{-2})
Free RV in ethanol (0.20 mM)	2.08 ± 0.9
Free RV in ethanol (1.31 mM)	10.78 ± 1.1
RV in SLN8 (1.31 mM)	13.72 ± 0.8
RV in SLN9 (1.31 mM)	11.01 ± 0.9

FIGURE CAPTIONS:

FIG.1. DSC thermograms of cetyl palmitate (CP), tricaprin (TRIC), cetilpalmitate/tricaprin physical mixture (CP/TRIC Phys mix) and SLN including (RV-SLN) or not including RV (empty SLN).

FIG. 2. MDA (nmol/mg) derived from porcine skin after 3 h of UVA irradiation, in the absence and in the presence of 0.2 mM RV (free or entrapped in SLN9) dispersed in emulsion A. Each bar represents the means ± SD obtained in three independent experiments (n=3).

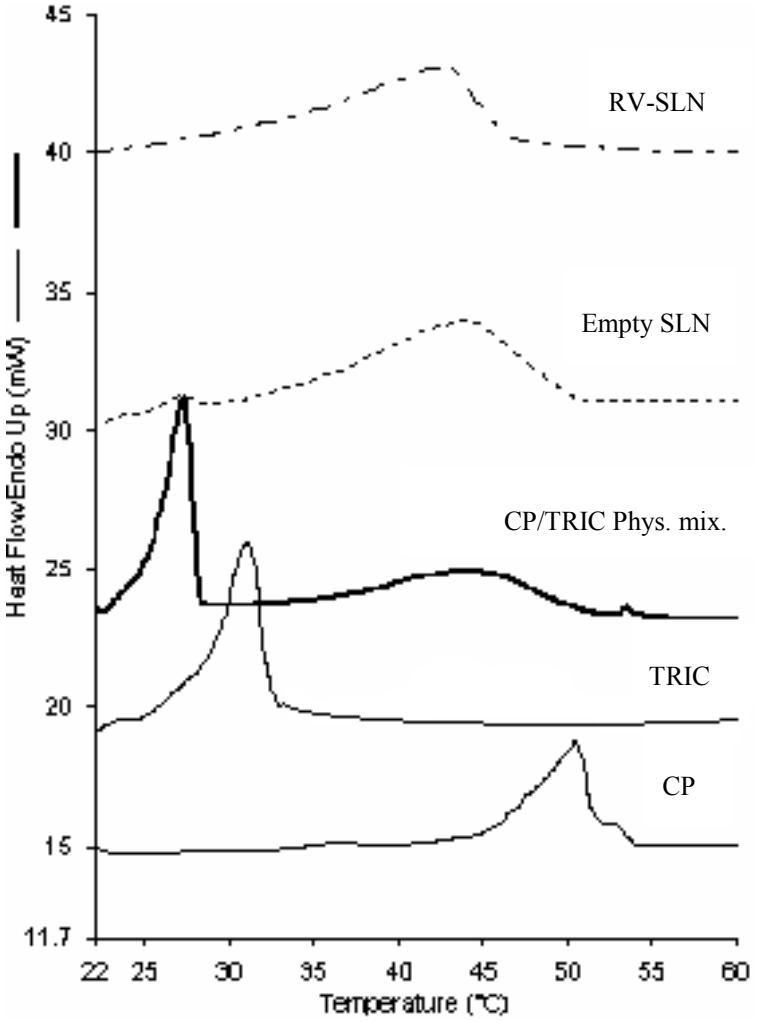
RV-SLN
Empty SLN
CP/TRIC Phys. mix.
TRIC
CP
Heat FlowEndo Up (mW)
Temperature (°C)
45
40
35
30
25
20
15
11.7
22
25
30
35
40
45
50
55
60

Figure 1

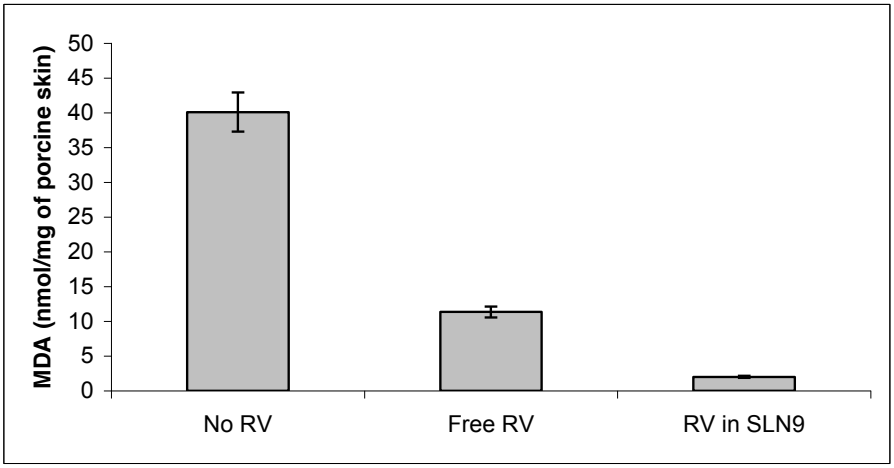
MDA (nmol/mg of porcine skin)
50
45
40
35
30
25
20
15
10
5
0
No RV
Free RV
RV in SLN9

Figure 2