CO-ENCAPSULATION OF A MIXTURE OF ANTIOXIDANT AND SUNSCREEN AGENTS INTO SOLID LIPID NANOPARTICLES

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This paper aims to encapsulate into solid lipid nanoparticles a mixture of two bioactive compounds, a photoprotective agent and an antioxidant compound. The co-encapsulation effect of octocrylene sunscreen and flavonoid (luteolinon) has been studied on the specific properties like antioxidant activity, sun protection factor and photostability. Using the high preasure homogenization technique lipid nanoparticles with diameters between 109-132 nm and a polidispersity index between 0.18-0.3 have been obtained. The results obtained shown that adding a photoprotective agent along with the antioxidant compound does not diminish the antioxidant capacity. By measuring the sun protection properties, for a concentration of 1.25 % octocrylene encapsulated in solid lipid nanoparticles and formulated into a cream base, values of sun protection factor higher than 30 have been determined, this meaning that these materials assure good photoprotection. All the results obtained lead to conclusion that co-encapsulating the luteolin with octocrylene in the lipid nanoparticles has a result a higher photoprotection capacity, this fact provinging that solid lipid nanoparticles loaded with these two bioactive compounds may be used as systems for blocking the UV radiations in cosmetics with sunscreen action.

Keywords: solid lipid nanoparticles, sun protection factor, antioxidant activity, luteolin

1. Introduction

Nowadays an important role in the daily life is played by the cosmeceuticals. Creams, perfumes, antiperspirants are only few examples. Particularly skin creams are more and more used and the tendency is to find formulations with multifunctional actions. That is why these creams are seen as "multi activity" or in other words should have multidirectional complex effects. [1].

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Solid lipid nanoparticles (SLNs) are generally spherical particles with average diameters between 50 and 500 nm. SLNs have been introduced to the literature as a carrier system for poorly water soluble pharmaceutical drugs and cosmetic active ingredients. The main advantages of SLNs are given by the potentially wide application spectrum (dermal, oral, intravenous), the use of biodegradable physiological lipids or lipidic stabilisers which are Generally Recognised as Safe (GRAS), the production without organic solvents and the possibility of scaling up to industrial production level [2].

Solid lipids nanoparticles are proved to be very useful in dermal applications due to their specific properties like low toxicity and low citotoxicity (due to physiological and biodegradable lipids), skin hydration, burst release, sustained release, prolonged release, physical UV blocker, etc. [3].

Regarding the active compounds, in the past few years several studies have been performed on the encapsulation of different kinds of lipophil drugs with pharmaceutical applications and on the encapsulation of cosmetic ingredients into lipid nanoparticles which have as result improved anti-UV formulations. Remaining in the health domain, there is a wide range of bio-active compounds which appear in plants and vegetables and can exhibit therapeutic effects. Many of these are largely consumed and have received considerable attention for the treatment of various diseases [4, 5]. Among them, plant derived phenolic flavonoids might play a key role as dietary antioxidants due to their consumption in human diet, especially in fruits and vegetables.

Flavonoids are compounds found in fruits, vegetables, and certain beverages that have diverse beneficial biochemical and antioxidant effects. Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health (antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant) [6]. Luteolin is one of the most potent antioxidative plant polyphenols. Wolfle et al. showed that luteolin may protect human skin against UVB induced damage by a combination of UV-absorbing, DNA – protective, antioxidant, and anti – inflammatory properties [7]. It was also demonstrated that natural product based UV – absorbers show promise as alternatives to synthetic molecules and nanoparticles in sun screen products [8].

Due to the current ozone layer depletion in the stratosphere, radiation from the sun especially UVA and UVB can reach the earth in a higher extent leading to unwanted and harmful effects on human skin. The interest in developing of new sunscreens has been increasing in the last years due to the harmful effects of UV

radiation on the skin such as dryness, mottled pigment abnormalities, erythema, accelerated skin ageing (wrinkle, photoageing) and ultimately the induction of skin cancer. As a consequence, the formulation of safe sunscreen products with enhancement of efficient UV protective effects, is a topic of high importance to avoid exposure to harmful UV light and counteract the damage induced by UV photons in the skin, simultaneously with minimization of local side effects [9]. The UV absorbers are conjugated aromatic molecules with a structure that allow them to absorb high-energy UV rays and release the energy as lower-energy rays, thus preventing the skin damages by UV radiations. From the class of organic sunscreen, we selected octocrylene which is a liquid, oil-soluble UVB filter and offers additional absorption in the short-wave UVA spectrum. This oil-soluble UVB filter is ideal for formulation water-resistant sunscreen products [10].

In this paper a new method for synthesis of solid lipids nanoparticles is presented, in which a sunscreen agent (octocrylene) and a flavonoid (luteolin) have been co-encapsulated. Original results concerning antioxidant activity, encapsulation efficiency and sun protection activity of solid lipid nanoparticles obtained are presented and discussed.

2. Materials and Methods

SLNs loaded with octocrylene (OCT) as sun protection agent (2-Ethylhexyl-2-cyano-3,3-diphenylacrylate, Sigma Aldrich Chemie GmbH – for UV B domain) and Luteolin (Lu) as antioxidant compound (3,4,5,7 Tetrahydroxyflavone – Alfa Aesar GmbH) were obtained using a high pressure homogenisation technique which will be detailed in the next section. In order to encapsulate these materials for the SLN preparation were also used n-Hexadecyl Palmitate (CP), 95% Acros Organics, USA and Glyceril Stearate (GS) Cognis GmbH aslipid phase and Polyoxyethylenesorbitan monooleate (Tween 80), Synperonic F68 (block copolymer of polyethylene and polypropylene glycol), Lecithin Sigma Aldrich Chemie GmbH for aqueous phase.

Size analysis, measurement of the polydispersity index and zeta potential of the SLN dispersions were evaluated by the dynamic light scattering (DLS) technique on a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.), at a scattering angle of 90^{o} and 25^{o}C. All the samples have been diluted with deionised water to an adequate scattering intensity prior to the measurement. Each sample, was measured in triplicate. The particle size analysis data were evaluated using intensity distribution.

The encapsulation efficiency (E.E.) of the bioactive component was estimated using a UV-Vis spectrometer (Jasco V 670) by determining the concentration of the bioactive component remained in the solution outside lipide nanoparticles. Un appropriate amount of lyophylized Lu-OCT-SLN was

suspended into a minimum volume of ethanol (5 mL) in order to remove the potential luteolin not incorporated. The luteolin concentration in the six lyophylized lipid nanoparticle samples has been evaluated at λ_{max} = 254 nm (one of specific absorption maxima for luteolin), according to Lambert Beer law. The efficiency of incorporated Luteolin concentration has been calculated by using the calibration curve in the concentration range of 1 – 9 μg/mL (in ethanol), with a correlation coeficient of R = 0.998, n = 6 and the following relation:

$$EE\% = \frac{C_{TBC} - C_{UTB}}{C_{TBC}} \times 100 \quad (1)$$

where:

C_{TBC} is the theoretical (calculated) concentration of the bioactive component;

C_{UTB} is the bioactive component concentration unloaded in lipid nanoparticles.

The **antioxidant activity** of loaded SLN with active compounds has been estimated by chemiluminiscence technique using a Turner Desingn TD 20/20, USA chemiluminometer, a mixture of luminol and hydrogen peroxide as generator of reactive species in a TRIS – HCl buffer (pH = 8.6). The antioxidant activity was calculated as following:

$$\%AA = \frac{I_0 - I_s}{I_0} \cdot 100 \quad (2)$$

where:

I_0 = signal intensity of the reference at t = 5s;

I_s = signal intensity of the sample at t = 5s.

Sun Protection Factor (SPF) was estimated *in vitro* using Diffey and Robson theory [11]:

$$SPF = \frac{\sum_{(400-290)} E_\lambda \cdot B_\lambda}{\sum_{(400-290)} \frac{E_\lambda \cdot B_\lambda}{MPF_\lambda}} \quad (3)$$

where:

E – solar radiation extinction on the Earth (between 20 – 40 N latitude);

B – Relative extnction for every wavelength;

MPF – Monocromatic protection factor for the selected wavelength (the difference between sample spectra applied to the support and the support spectra).

For SPF evaluation, a Transpor^{TM} 3M artificial skin with a similar human derma structure was used as support. An amount of 2 mg/cm^2 cream is applied onto support and the sample spectrum is registered on 290-400 nm interval, by using a reference support – Transpore^{TM} 3M whitout cream.

The photostability of SLNs obtained was estimated by UVA and UVB irradiation. The irradiation energy was for both domains 19.5 J/cm^2, while the duration was 1h for UVA domain and 2h for UVB domain; the equipement used was BioSun, Vilber Lourmat, France.

3. SLN preparation

The SLN loaded with OCT sunscreen and luteolin were prepared by high shear homogenization and high preasure homogenization technique; according to the procedure presented in fig.1. The lipid mixture (1:1, w/w) formed by cetyl palmitate and glycerol stearate (10% from the total SLN dispersion) was melted at about 87^{o}C and an appropriate amount of OCT and luteolin was added (1%). An aqueous phase that contains a mixture of surfactants (3%), with a mass ratio of Tween 80: Synperonic F68: Lechitin = 2:0.5:0.5 was heated to the same temperature. After 30 minutes of individual stirring of the lipid and aqueous phases, respectively, the hot lipid phase was gradually poured into the hot aqueous-surfactants solution. Then, the resulted emulsion was stirred for 1h at 87^{o}C. This hot pre-emulsion was further processed by a high shear homogenization (using a Pro Scientific 200 homogenizer type; 0~30.000 rpm), by applying 16 000 rpm for 1 minutes. After this step the preemulsion was treated for 4 minutes at 800 barr by high pressure homogenization using a APV 1000 Invensys Homogenizer, Denmark.

The lipid dispersion was cooled at room temperature (while stirring), and solidified in order to obtain the aqueous SLN dispersions loaded with luteolin and OCT. In case of free-SLNs, they were produced in the same manner as loaded-SLNs, by replacing the bioactive components amount with deionised water. Using an Alpha 1-2 LD Freeze Dry System equipment (Germany) the excess water was removed by lyophilization in order to increase the SLN particle concentration. The SLN dispersions with 1% bioactive components were cast in glass dishes and freeze-dried in the following conditions: 1. freeze at -25^{o}C during 24h, 2.freeze at -55 ^{o}C during 72 h.

The liophylised SLNs contained an amount of 5-6,5 % sunscreen and 0.76-2 % antioxidant compound.

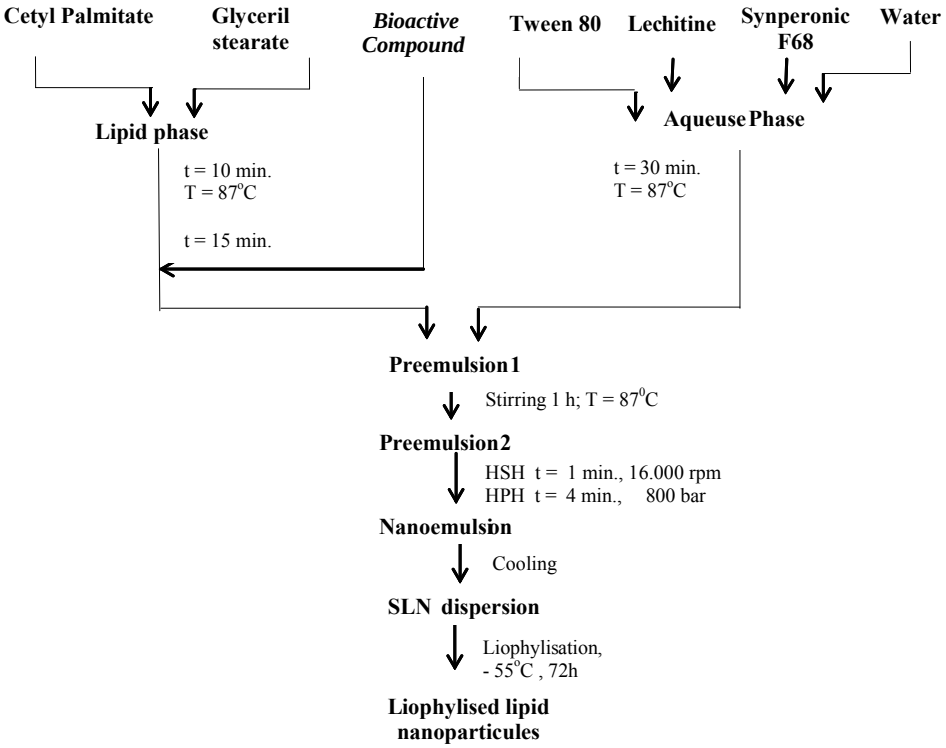

Fig.1. Synthesis procedure for the systems Lu-SLN, Lu-OCT-SLN

4. Results and discussion

4.1. DLS and Zeta potential measurements

The composition of the obtained formulations, the mean particle size and surface charges of the developed SLNs are shown in Table 1. One can be observed that the size distribution of optimized nanoparticles dispersions are in the nanometer range (100-130 nm) with a relatively narrow size distribution (PdI = 0.18-0.3), this indicating a high degree of homogeneity of all the samples.

Table 1

Composition and physical characterization of lipid nanoparticle obtained

	Composition (m/m)				Characterisation		
	OCT (%)	Lu (%)	Lipid mixture (%)	Surfactants mixture (%)	Z ave (nm)	PdI	ξ (mV)

Lu1-SLN	-	0.1	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	110.4	0.19	-41.2
Lu2-SLN	-	0.2	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	115.3	0.29	-40.2
Lu3-SLN	-	0.28	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	128.5	0.30	-41.6
Lu 1-OCT-SLN	0.9	0.1	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	112.9	0.18	-32.6
Lu 2-OCT-SLN	0.8	0.2	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	132.0	0.24	-43.5
Lu 3-OCT-SLN	0.72	0.28	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	127.2	0.31	-49.6
OCT-SLN	1	-	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	112.3	0.19	-33.2
SLN Matrix	-	-	10 CP:GS (1:1)	3 Tween 80:Synperonic F68:Lechitine	109.5	0.20	-32.1

Fig. 2 presents the results concerning the SLN size evaluation.

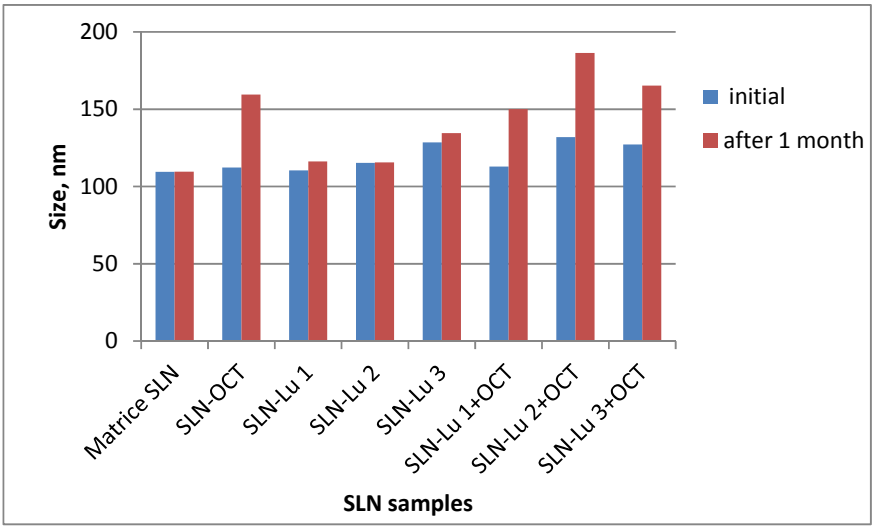

Fig. 2. Size of obtained SLNs

One can observe that increasing the concentration of bioactive compound, luteolin, encapsulated leads to a slight increase in the nanoparticles size, while the co-encapsulation with protective agent OCT does not result in significant changes of the SLN size.

The values of Zeta potential presented in table 2 ranging between -32 and -50 mV show that adding luteolin to the SLN leads to more stable compounds and this stability is maintained after 1 month, all the values being lower as compared to free SLNs. All the SLNs encapsulated with Lu manifest good stability in time after 1 month of storage, while in the presence of sunscreen an increasing size tendency is noticed up to 40% as referring to Lu-SLNs wihthout OCT.

Table 2

Zeta potential values for the obtained SLNs

Sample	Initial	After 2 weeks	After 1 month
	ξ (mV)	ξ (mV)	ξ (mV)
SLN matrix	-32.1	-32.5	-32.3
SLN - OCT 1%	-33.2	-36.4	-37.4
SLN - Lu 0.1%	-41.2	-39.6	-40.3
SLN - Lu 0.2%	-40.2	-42.1	-43.3
SLN - Lu 0.3 %	-41.6	-41.3	-41.5
SLN - Lu 0.1% + OCT 0.9%	-32.6	-38.0	-38.5
SLN - Lu 0.2 % + OCT 0.8%	-43.5	-44.3	-45.3
SLN - Lu 0.28 % + OCT 0.72%	-49.6	-45.9	-46.9

4.2. Antioxidant activity (AA)

In order to assess the effect of encapsulation into lipidic matrix of the bioactive flavonoid, the antioxidant activity of solutions containing 1.35, 3.17 and 3.93 mg/L of pure luteolin in ethanol has been compared with that of corresponding SLNs loaded with the same amounts of luteolin. Surprisingly, in all cases a higher antioxidant activity has been registred for Lu-SLNs (by 3-7 units). As can be seen from Fig. 3, the encapsulation of luteolin into SLN matrix results in important enhancement of AA up to 0.2% Lu content, a higher concentration of Lu having no further AA increase. As referency to the effect of OCT co-encapsulation it has no significant decrease on AA of the samples.

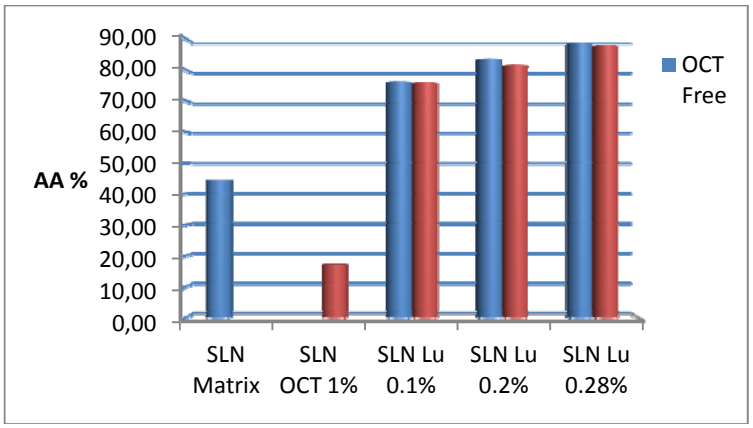

Fig. 3. Antioxidant activity of Lu-SLNs samples with and without OCT

4.3.Encapsulation efficiency

Table 3 presents the results concerning the encapsulation efficiency obtained for the samples prepared. The best efficiency for encapsulation of luteolin was obtained for the samples of SLN loaded with 0.1 % Lu, at higher luteolin content the loading capacity being exceeding, some of flavonoid remaining outside lipidic core. By adding octocrylene these efficiencies are increased, having again the highest value for the sample with 0.1 % luteolin. This means that the nanoparticles formed with octocrylene favours the encapsulation of luteolin due to the change of composition of lipidic core, with a higher contribution of OCT as liquid lipid compound.

Table 3

Efficiency encapsulation results for the nanoparticles synthesized

No.	Sample	Theoretical concentration of Luteolin, g/mL	Measured concentration of Luteolin, g/mL	EE%
1	Lu 0.1% - SLN	8.10	5.61	30.74
2	Lu 0.2% - SLN	7.88	6.07	22.97
3	Lu 0.28% - SLN	8.70	7.57	12.99
4	Lu 0.1% - OCT 0.9 % - SLN	7.57	3.84	49.27
5	Lu 0.2% - OCT 0.8% - SLN	7.42	4.15	44.07
6	Lu 0.28% - OCT 0.72 % - SLN	8.20	5.72	30.24

4.4. Sun Protection Factor

Sun protection factor (SPF) was calculated using a base cream without any sun protection factor and the protective agent was of 2.5 % in all the samples. Fig.

4 summarises the results obtained for quantifying the sun protection efficiency of these formulations. Firstly, the effect of OCT encapsulation into SLN matrix results in doubling SPF value as compared with not encapsulated sunscreen OCT, wheras loading of Lu in the SLNs does not improve the SPF. Secondly, co-encapsulation of Lu and OCT leads to increasing SPF, the maximum value of 12.5 being obtained for the optimum composition of Lu 0.2% - OCT 0.8% - SLN.

The UV stability of these samples has been also tested at two irradiation steps, each of them consisting in 1 h irradiation on UV A and 2 h irradiation on UVB domains. An unexpected enhancement of SPF values is registerd in all the samples, with maximum of 36.7 value obtained for the optimized sample Lu 0.2% - OCT 0.8% - SLN after the second irradiation step.

This behaviour could be explained by the mechanism of photoprotection process involving reaction products with significant absorbance on both UVA and UVB domains.

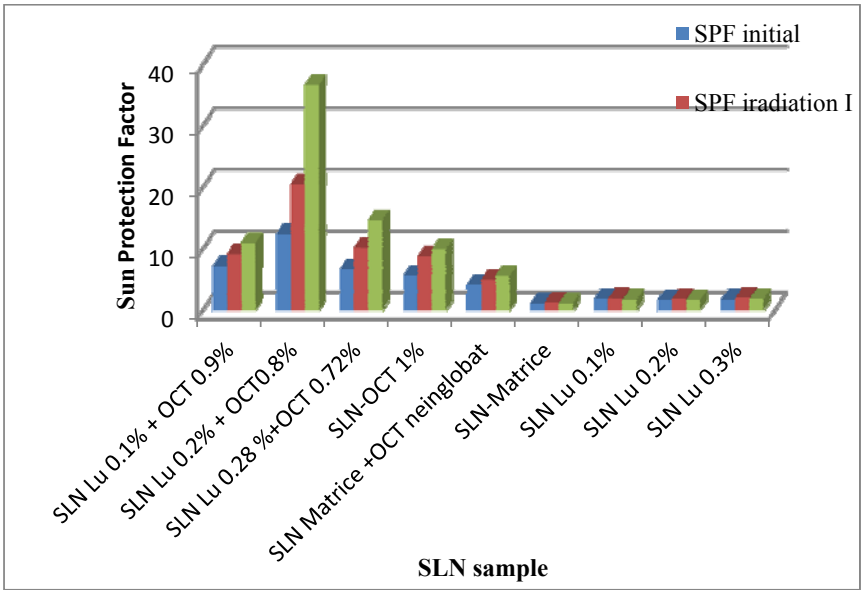

Fig. 4. Sun Protection Factor for lipid nanoparticles tested before and after irradiation

5. Conclusion

This paper reports results obtained for a series of solid lipid nanoparticles in which a protective agent - octocrylene and an antioxidant compound - luteolin have been co-encapsulated.

A synergistic effect has been observed during encapsulation of the two compounds resulting in a significant enhancement of both antioxidant and sunscreen efficacies.

Determination of photoprotection factor has lead to high SPF ratings, with values of 36.7 for a base cream with a content of 1.25% molecular sunscreen (OCT) after two irradiation steps on both UV A and UV B domains, which means a protection capacity higher than 90%. At the same time the octocrylene content is ten times lower than the maximum concentration admited by the FDA - Food and Drug Administration for OCT (10%).

By corroborating all these results one can conclude that formulations of SLN loaded with Lu 0.2% - OCT 0.8% represents the optimum composition able to confer synergistic antioxidant and sun protection activities of these nanostructured materials.

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R E F E R E N C E S

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