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LIPID NANOPARTICLES FOR MODULATION OF UV RADIATION: INFLUENCE OF COMPOSITION AND PRODUCTION TECHNOLOGY

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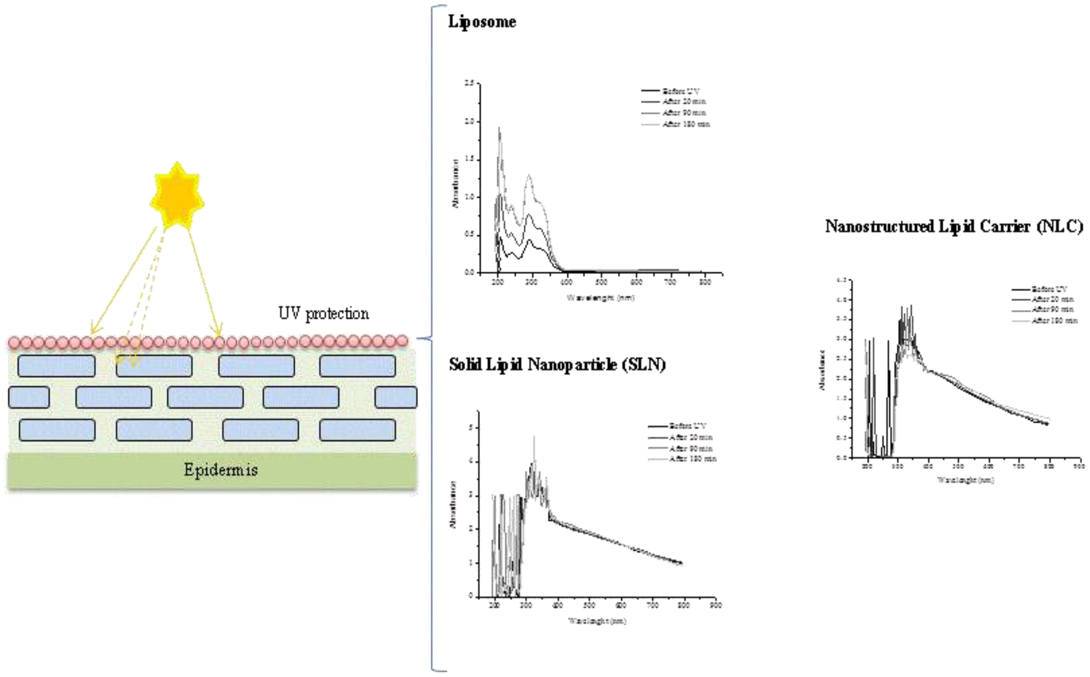
ABSTRACT

The aim of this work was to compare in vitro photoprotection effect of benzophenone-3 (BZN) loaded in three distinct lipid nanocarriers, namely, elastic liposomes, solid lipid nanoparticles (SLN) and nanostructured Lipid Carriers (NLC). Lipossomes were produced by Bangham method and SLN/NLC by solvent diffusion method. All formulations were characterized by mean particle size, polydispersity index (PI), zeta potential (ZP), encapsulation efficiency (EE), and in vitro photoprotection capacity. The size of lipid nanoparticle range 100 to 800 nm and the polydispersity index range 0.2 to 0.8. The zeta potential range to -9 a -36 mV and encapsulation efficiency was 18 to 100 %. UV protection in vitro essays showed increase UV absorption over the time. Conclusion, it was observed type of matrix lipid influence in UV photoprotection. Solid matriz showed more promising results compared liposome.

KEYWORDS: sunscreen, lipid nanoparticle, solar radiation, solid lipid nanoparticle, nanostructured lipid carrier and liposome.

Graphical Abstract



1. INTRODUCTION

Solar radiation produces benefits, but also encounters several risks to human health. The benefits are related to the synthesis of vitamin D, which regulates many functions in the body as lowering blood pressure, the risk of heart attack and stroke. The greatest risks are linked to skin cancer and premature aging.[1] Therefore, skin protection with sunscreens needs to be part of the daily routine is important prevent cancer and aging.[2]

The efficiency of sunscreen in the topical application depends on their binding capacity to the skin, the penetration through the pores and prolonged retention at the application site. In general, bioactive compounds become more efficient in relation to its free form when encapsulated in nanoparticles.[3] These systems promote greater surface area contact and interaction with the skin, controlled release of the encapsulated bioactive, and they are susceptible to penetration of the skin layers, without the risk of reaching the dermis.[4]

Among the different controlled release systems composed of lipid materials, liposomes[5], solid lipid nanoparticles (SLNs)[4] and nanostructured lipid carriers (NLCs)[6] have demonstrated to be suitable as physical sunscreen agents. Lipid nanoparticles (both SLNs and NLCs), are produced with a mean particle size between 50 nm and 1000 nm, and have been widely used in dermatology and cosmetology.[7] Lipid nanoparticles are biodegradable, non-toxic and biocompatible. They allow the modification of the skin barrier function to a greater controlled drug delivery and specific targeting. A clear advantage of the use of SLNs and NLCs, as drug delivery systems is the fact that the matrix is composed of physiological components, that is, excipients with Generally Recognized as Safe (GRAS) status for topical administration.[7] There are various studies about chemical sunscreens loaded in SLNs and NLCs [8-12]. Benzophenone-3 (BZ3) an aromatic ring with the absorption range between 288 and 325 nm, is approved by the U.S. Food and Drug Administration to be used as sunscreen.

In this work, we propose to study the effects of lipid nanoparticles (liposomes, SLN and NLC) empty and loading BZ3. The physicochemical properties (mean particle size, polydispersity index, zeta potential, and encapsulation efficiency) have been checked and the fractions of absorbed ultraviolet radiation have been assessed by in vitro studies.

MATERIAL

Benzophenone-3 (BZ3) was obtained from Delaware (Porto Alegre, Brazil); Egg phosphatidylcholine (Lipoid E-80) was purchased from Lipoid (Campinas, Germany). 1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 (PEG_{2000}) and 1,2-Distearoyl-sn-glycero-3- Phosphatidylethanolamine-[Poly(ethyleneglycol)-500 (PEG_{5000}) were obtained from Genzyme (Campinas, Brazil);. Ethanol was provided from Synth (São Paulo, Brazil); 0.9 % NaCl solution was purchased from Fresenius Kabi Merck (Campinas, Brazil); PEGylated stearic acid (AE peg), stearic acid (AE) and Polyoxyethylene-Polyoxypropylene Block Copolymer (Pluronic® F68) were purchased by Sigma, EUA. Medium chain triacylglycerols (C8–C10) (GTCC Crodamol® was donated by Croda. Ultrapure water (Milli-Q Plus, Millipore) was used throughout.

2. METHODS

2.1. Preparation of the nanocarriers

Liposomes were prepared according to the Bangham's method.[13] This method requires solubilizing the lipids in an organic solvent, following the evaporation of the solvent and rehydration of the dry lipid film with saline solution 0.9 % for the formation of liposomes. Egg phosphatidylcholine, PEG and BZ3 were dissolved in 10 mL ethanol. The ethanol was removed from the lipid film under rotary evaporator at vacuum (650 mmHg, 200 rpm and 1 h). For the complete removal of the solvent, the thin lipid film remained under vacuum at 250 rpm rotation for more than 1 h. The film was then rehydrated with 10 mL of saline solution 0.9 %. Table 1 describe the composition of liposome produced.

Table 1. Formulation composition of liposome

Formulation	Lipoid® E-80	PEG_{2000}	PEG_{5000}	BZ3
L1	0.02			
L2	0.02			0.01
L3	0.019	0.001		
L4	0.019	0.001		0.01
L5	0.019		0.001	
L6	0.019		0.001	0.01

*Values in percentage

SLN and NLC were prepared by the solvent diffusion method in an aqueous system reported in our previous study [14, 15]. Briefly, lipid (stearic acid or stearic acid pegylated) and/or BZ3 were dissolved in 3 mL of ethanol in a water bath at 70 °C. The resultant organic solution was quickly dispersed into aqueous solution Pluronic® (0.1 %, w/v) under mechanical stirring at 600 rpm for 5 min in 70 °C in water bath. Then, the emulsion was cooled to room temperature. Table 2 describe the composition of SLN/NLC produced.

Table 2. Formulation composition of SLN and NLC.

Formulation		Lipid phase				Aqueous phase	
	AE	AEpeg	BZ3	Crodamol®	Ethanol (mL)	Pluronic®	Water (mL)
S1	0.06				5	0.1	100
S2	0.06		0.01		5	0.1	100
S3		0.06			5	0.1	100
S4		0.06	0.01		5	0.1	100
N1	0.051			0.009	5	0.2	100
N2	0.051		0.01	0.009	5	0.2	100
N3		0.051		0.009	5	0.2	100
N4		0.051	0.01	0.009	5	0.2	100

*Values in percentage; ** S:SLN; N:NLC

2.2. Particle size, polydispersity index and zeta potential analysis

The mean size of liposomes, SLN and NLC was determined through dynamic light scattering (DLS, Zetasizer Nano NS, Malvern, UK). This instrument also allows determining the electrophoretic mobility to assess the surface electrical charge of particles. The samples were diluted with ultra-purified water to weaken the opalescence before measuring the particle mean diameter and polydispersity index). Zeta potential values of liposomes were also measured in purified water adjusting conductivity (50 µS/cm). The zeta potential was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation. The analysis was performed using the software included in the system.

2.3. Encapsulation efficiency

For the determination of encapsulation efficiency was done by ultrafiltration method. Samples was collected and analyzed in the UV spectrophotometer (Malvern, S-MAM 5005; UK) at 285 nm and 325 nm for its free active content, against a calibration curve. Encapsulation efficiency was determined using the Equation 1.[5]

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total amount benzophenone} - \text{free benzophenone}}{\text{Total amout benzophenone}} \text{x } 100$$

Equation 1

2.4. Study of photoprotection

Photoprotection was checked exposing benzophenone-3 loaded all formulations produced under UV light at different time intervals (20 min, 1.5, and 3 h). A solution of free benzophenone-3 in ethanol was used as reference. The absorbance spectrum was measured. For this testing, BZ3 loaded liposome, SLN and NLC and free BZ3 were used. The samples were exposed under UV light for the predetermined time intervals and then measured via UV spectrophotometer scanning (Malvern, S-MAM 5005; UK).

3. RESULTS AND DISCUSSION

SLN, NLC and liposomes were prepared with lipids and phospholipids. The body's physiological biocompatibility were the criteria of choose. Others materials were chosen to obtain a stable formulation. The objective was development a stable formulation that delay active penetration, without penetrating the skin, reducing the toxic potential of a conventional product.

For this, the size and encapsulation efficiency is essential evicting penetrating bloodstream, avoiding possible risks to health. Lipossomes are more useful to loading hydrophilic drug[16], while lipid matrices (SLN/NLC) is more appropriate to incorporate lipophilic drugs.[17]

From the results obtained, shown Figure 1, the size and polydispersity index was determined through Dynamic Light Scattering for all formulations. The size of lipid nanoparticle range 100 to 800 nm and the polydispersity index range 0.2 to 0.8.

It was observed loading BZF in lipid nanoparticle alter the size and the polispersity index. This difference can be attributed hydro and lipophilic characteristics of lipid nanoparticle and BZF. Formulations coating by PEG (L5, L6, S3, S4, N3 and N4) showed similar results, it was observed that adding BZF reduced size and polydispersity index. In formulation that observe increase of size suggesting the BZF coating the nanoparticle forming a core shell.[18]

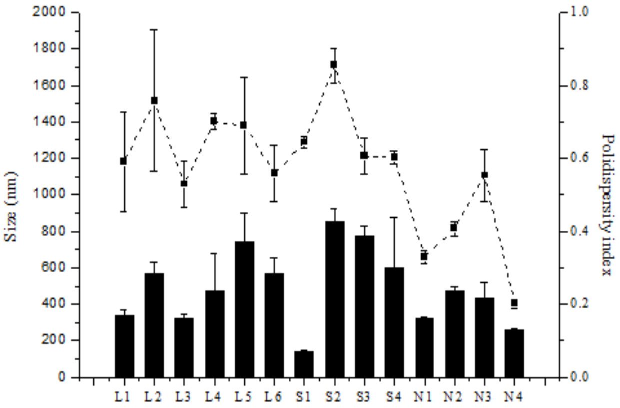


Figure 1. Particle size and polidispersity index of lipid nanocarriers (liposomes, SLN and NLC).

Charge of surface of nanoparticle is denominated Zeta Potential. This parameter predict the stability of system.

High values of zeta potential, positive or negative, produced stable formulation. Studies suggesting aggregation occur with zeta potential ZP (>|20|).

Table 3 shows zeta potential showed interesting results, predicting a good long-term stability. The formulation SLN and NLC coating by PEG that showed low zeta potential however it is expect due to PEG. Therefore, the Encapsulation efficacy was higher in SLN/NLC compared liposome. These characteristics also can attributed hydro and lipophilic characteristics of lipid nanoparticle and BZF.

BZF shows to peaks of absorption in ultraviolet spectrophotometry (λ=285 nm and λ=325 nm) [19, 20]. To evaluate Encapsulation efficiency was done a calibrations curves in both peaks ($y=70.876x + 0.0124$, $r^2 = 0.9952$ (λ=285 nm) and $y = 47.85x + 0.0124$, $r^2 = 0.995$ (325 nm). Where y stands for the absorbance of standard solutions and x the respective concentrations given in µg/mL. The concentration of BZF evaluated was 0.002 – 0.014 µg/mL.

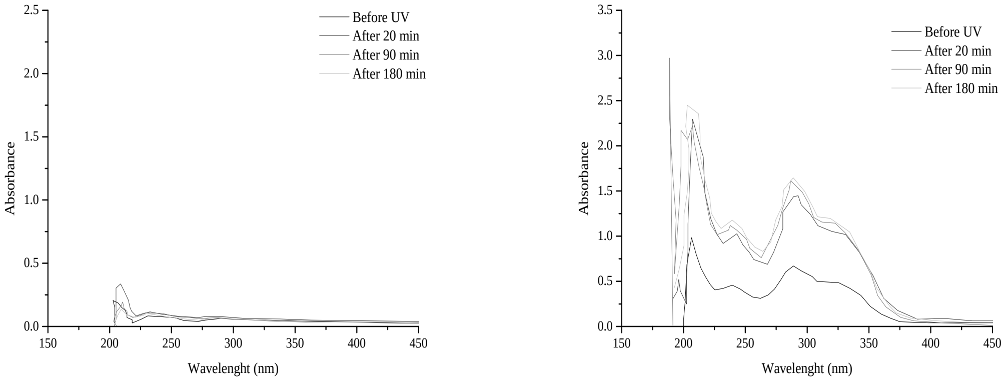
Table 3. Zeta potential and encapsulation efficiency characteristics of lipid nanocarriers (liposomes, SLN and NLC).

Formulation	Zeta Potential (mV)	Encapsulation efficiency (%)	
		285 nm	325 nm
L1	-36.30 ± 0.850	-	-
L2	-32.00 ± 2.340	60.83	56.61
L3	-30.90 ± 1.160	-	-
L4	-22.00 ± 2.070	18.50	18.99
L5	-33.10 ± 0.321		
L6	-32.00 ± 0.252	17.09	21.08
S1	-32.10 ± 0.611		
S2	-26.10 ± 0.781	80.30	81.99
S3	-9.24 ± 3.170		
S4	-9.52 ± 2.670	82.42	87.21
N1	-42.3 ± 4.690		
N2	-26.20 ± 0.252	91.30	88.76
N3	-18.40 ± 0.551		
N4	-13.0 ± 0.577	100	100

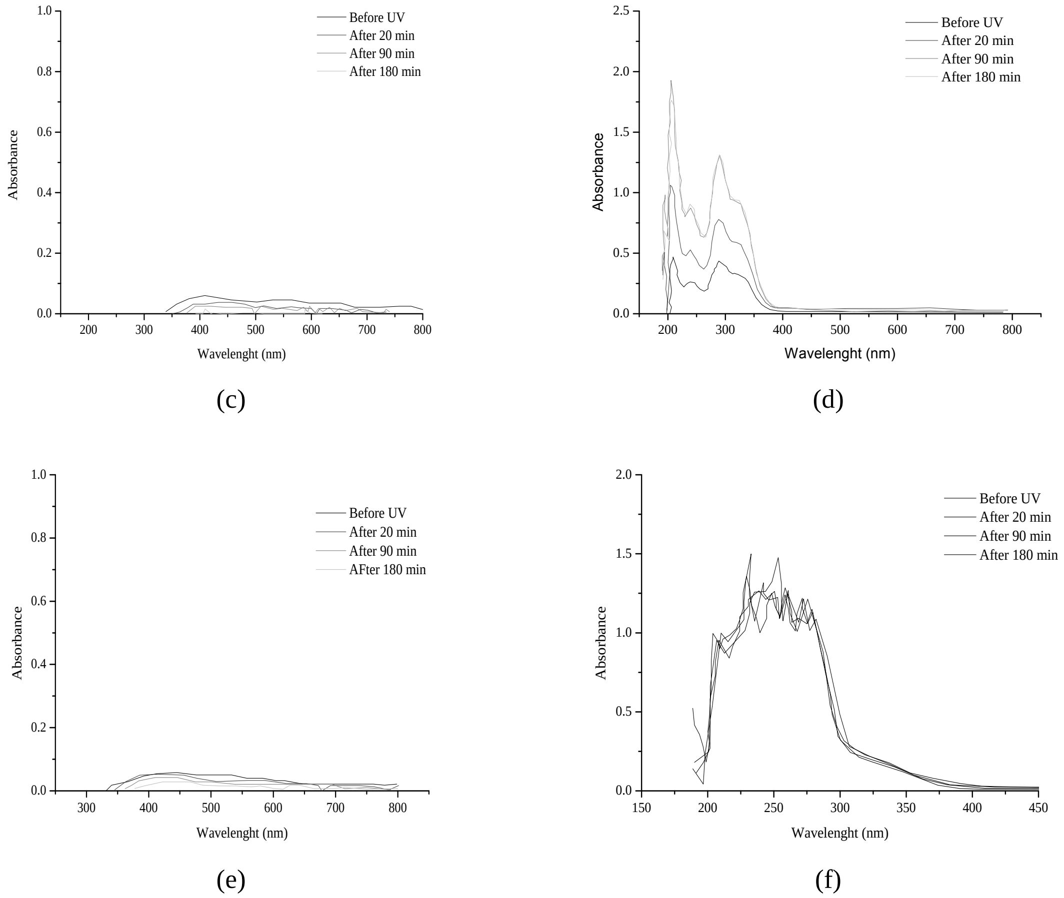
*L: liposomes; S: solid lipid nanoparticles and N:nanostructured lipid carriers

Figure 2, 3 and 4 showed increase of UV light absorption of loading in all formulations. Observing that lipid nanoparticles contributing increase photoprotection. The lipid components and the type de matrix influence to increase UV spectro. Therefore, in the skin nanoparticle are responsible to forming layer occlusion on the skin. Others works have been observing this.[21,22]

Figure 2 it was observed liposomes formulations with and without BZF. It was observed conventional liposome showed higher absorbance in the time studied. Then, 5000PEGylated liposomes not showed considerable change in absorption over time.



(a) (b)



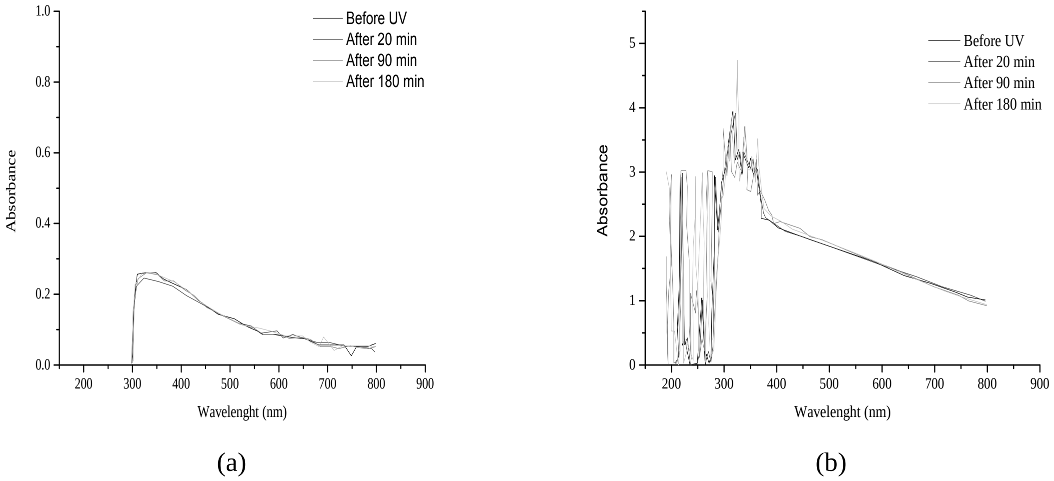
(c) (d)

(e) (f)

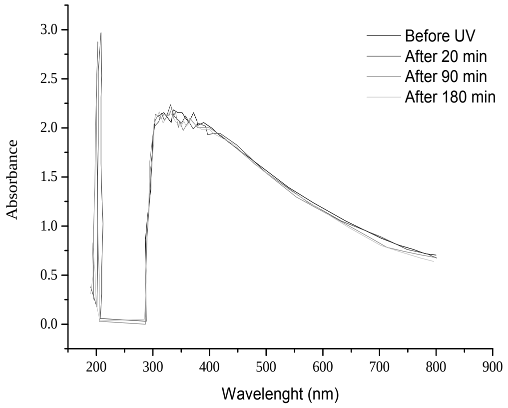
Figure 2. UV absorption of liposomes: (a) empty liposomes, (b) conventional liposome loading BZ3, (c) empty 2000PEGylated liposomes, (d) 2000PEGylated liposomes loading BZ3, (e) empty 5000PEGylated liposomes, (f) 5000PEGylated liposomes loading BZ3.

Figure 3 and 4 shows SLN and NLC samples. Empty SLN and NLC showed UV absorption demonstrating that lipid or mixture of lipid in solid matrix is responsible photoprotection. Excepting PEGylated-SLN that not was possible analyzing without BZF. The BZF promoting increasing in all formulation.

These dates suggest that SLN and NLS formulation obtain a more efficient UV photoprotection properties compared lipossomes due solid state of matrix. Similar results was observed by.[23,24]

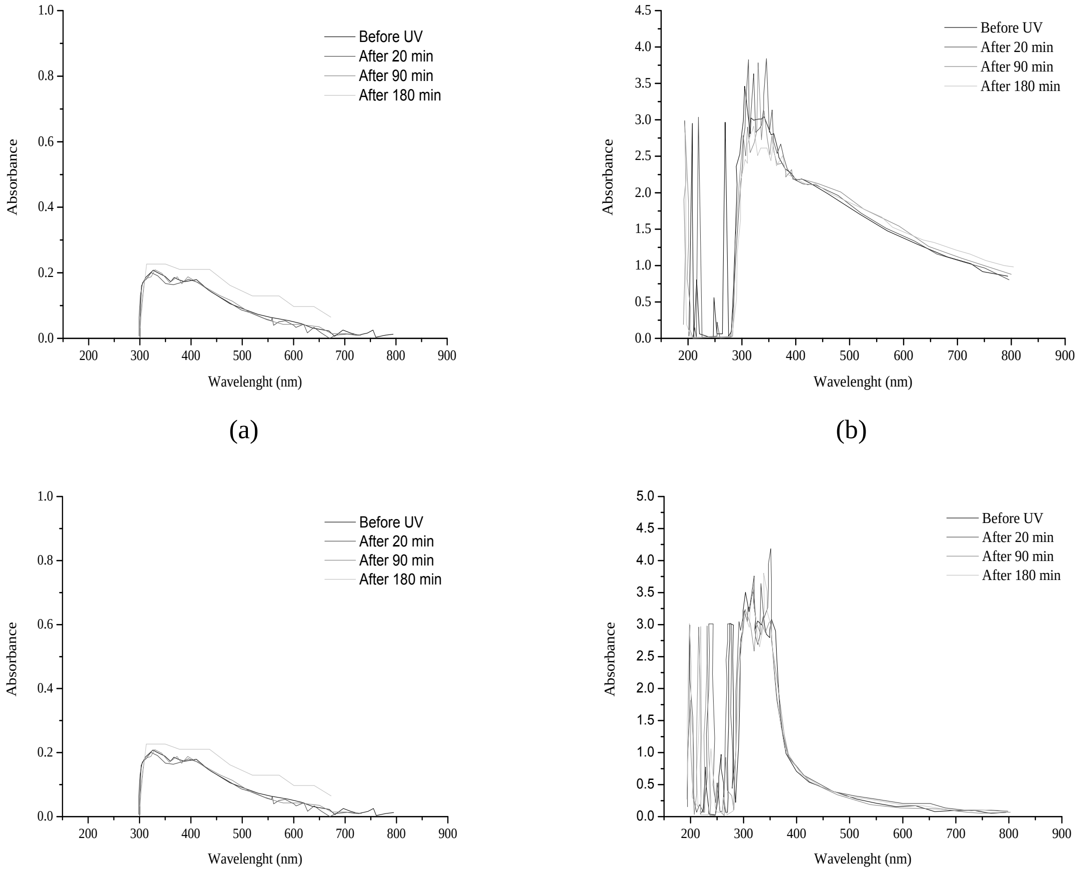


(a) (b)



(c)

Figure 3. **UV absorption of stearic acid-SLN: (a) empty SLN, (b) SLN loading BZ3, (c) PEGylated-SLN loading BZ3.**



(a) (b)

(c) (d)

Figure 4. UV absorption of NLC (a) NLC empty, (b) NLC loading BZ3, (c) NLC PEGylated empty, (D) NLC PEGylated loading BZ3.

4. CONCLUSIONS

The results show the potential of liposomes and lipid nanoparticles modulate the effect of ultraviolet radiation on the human body. It was a promising nanotechnology to incorporating in semi solid formulation. Finally, more studies of liposomes, SLN and NLC formulations is necessary

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