Characterization of Encapsulation Process of Avobenzone in Solid Lipid Microparticle Using a Factorial Design and its Effect on Photostability

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ARTICLE INFO

ABSTRACT

Avobenzone (AVO)liposphereswere obtained using as encapsulating material carnauba wax and applying the method of hot emulsification. A factorial design 2^3 was performed to optimize and recognize the influence of the initial amount of AVO, the stearyl alcohol (SA) and polysorbate (PS-20)concentrationsin the AVO loading, mean and D90 particle size. The results showed a variation of AVO loading between 1.8 and 20%, which was mainly affected by initial amount of AVO. The mean particle size D [4.3] ranged from 11.11 to 37.15 μm, this was affected by the levels of SA and PS-20, while D90 was influenced by three variables under study. Lipospheres highly loaded and under a narrow particle distribution was obtained when initial amount of AVO, SA and PS-20 were set in the maximum level. The study showed that the encapsulation process enhanced the photostability going from 31.5% for free AVO to 16.2% for the encapsulated AVO.

INTRODUCTION

The population occupying the equatorial regions is subjected to the most intense UV radiation all the year round, because the UV radiation reaches the surface of the earth at the most direct angle in this region. Therefore low latitude regions are considered areas of greatest risk, which is demonstrated by high UV Indexes.

Nowadays, more people have increased their awareness about the harmful effects of UV-sunlight on human skin, hence sunscreens have been included in the daily care routine. Some sunscreens contain inorganic compounds with a high refractive index acting as a physical barrier to light, and others contain UV filters that display ability of absorbing UV radiation. It is usually used a combination of UV filters to provide broad-spectrum protection from 280-315 (UVB) to 315-400 (UVA) (Barel *et al.,*

2009). The combination more extensively used in commercial sunscreens is 4-tert-butyl-4´-methoxydibenzoylmethane, AVO (UVA filter)and octylmethoxycinnamate, OMC (UVB filter) (Trotta *et al.,* 2013). Since to users fully trusts in sunscreens to prevent damages from solar radiation, the sunscreens must be effective and safe. However, when a molecule absorbs UV energy, an excited state is formed, then it returns to its basal state by radiative and non-radiative decay mechanisms,some UV filters, such as AVO, suffer degradation reactions as non-radiative decay mechanisms, giving rise to new compounds (Afonso *et al.,* 2014). These new compounds don`t have protective activity (not absorb the UVA radiation). For this reason, the photostability at the present time is a new quality criteria for development of sunscreens (Jansen *et al.,* 2013). The sunscreens are evolving toward formulations containing filter-carriers, to protect them against photodegradation and to reduce their penetration into the skin and photosensitization of bio-components causing photo-toxic and photo-allergic reactions (Puglia *et al.,* 2014).

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Several carrier systems such as cyclodextrins, clays, liposomes, polymer and lipid microparticles or nanoparticles have been proposed as filter-carrier (Blasi *et al.,* 2011). Concomitantly an intense discussion is carried out on the safety of nanoparticles in cosmetics, mainly in particles with sizes below 100nm (Robertson *et al.,* 2010, Jatana and DeLouise, 2014). Solid lipid microparticles also known as lipospheres(LPs), are systems characterized by its lipid core and its high loading capacity for hydrophobic molecules. They are good carriers that provide chemical stability to the encapsulated compound, high physical stability, easy incorporation in cosmetic matrices, skin compatibility, low cost of the ingredients; easy preparation and scaling without use solvents.Scalia et al have reported the encapsulation of AVO into LPs, they have used materials as triestein, glycerylbehenate, carnauba wax and hydrogenated phosphatidylcholine(Iannuccelli *et al.,* 2006, Scalia *et al.,* 2011a, Scalia *et al.,* 2011b).AVO also has been encapsulated into solid lipid nanoparticle. (Nesseem, 2011, Niculae *et al.,* 2012). In these developments, the authors have found that UVA efficacy rise and percutaneous penetration and photodegradation are reduced compared to AVO conventional emulsions. Up to now, carnauba wax has been used in the preparation of solid lipid microspheres and nanospheres of several drugs e.g.ketoprofen (Kheradmandnia, 2010). In the case of sunscreen, it has been reported that there is a synergistic effect with TiO_2 to achieve a higher sun protection factor (SPF) (Villalobos and Müller, 2006). The aim of this study was develop an AVO-carrier to augment the photostability of the filter, the AVO encapsulation into LPs obtained by hot emulsion technique was chosen due to lipophilic character of AVO and because the technique involves production equipment relatively simple, cheap and easy to scale-up. A 2^3factorial design was used to study the influence ofAVO initial amount, stearyl alcohol concentration (SA) as lipid modifier and polysorbate 20 concentration (PS-20) as emulsieragent, on the AVO loading(LOAD %) and size of formed LPs. Size target range was 1-30 um because size below 1 um may pass through the skin, and size above 30 μm can generate gritty feel to any cosmetic formulation. This study is looking for contributing to the advanced formulation of sunscreen products more effective and safe.

MATERIAL AND METHODS

Carnauba wax (Protoquímica), stearylalcoholand Polysorbate 20 (Tween 20) (Protoquímica), AVO (Parsol 1789 DSM Nutritional Products AG), OMC (Parsol MCX, DSM Nutritional Products AG) were purchased from a local supplier (L&F Ltda, Medellín-Colombia). The chloroform and methanol were analytical grade (Merck), and the potassium phosphate monobasic (J. T. Baker). Water used was deionized.

Experimental design

A 2^3 randomized factorial design was used to evaluate the influence of the initial amount of AVO (X1), concentration of SA (X2) and concentration of PS-20 (X3) in the mean particle size of the LPs, μm, (Y_1) and encapsulation efficacy, load % (Y_2).The factorial design consisting of 8 runs carried out in duplicate as described in Table 1 (Armstrong, 2006, Castillo, 2007). The fixed variables were: the carnauba wax amount (5000 mg), stirring speed (3500 rpm) and the volume of aqueous phase (200 mL).

The statistical model was represented by **Equation 1:**

$$Y=\beta_0+\beta_1\ X_1+\beta_2\ X_2+\beta_3\ X_3+\beta_{12}\ X_1\ X_2+\beta_{13}\ X_1\ X_3+\beta_{23}\ X_2\ X_3+\beta_{123}\ X_1X_2X_3+\varepsilon$$

Where Y is the measured response, βi is the regression coefficient for Xi, Xi is the level of the independent variables and ε is a random error component that is normally distributed with zero mean and variance σ^2. The statistical software used was Design-Expert ® version 7.0.0 (Stat-Ease, Inc) and analysis of variance (ANOVA) was performed.

Liposphere preparation

LPs were prepared following the method of hot emulsification proposed by Mehnert and Mäder, 2001, which was chosen to avoid the use of solvents. Lipid phase was composed of carnauba wax (5000 mg), stearyl alcohol and filter (AVO).The lipid materials were melted at 85°C, and then the filter was added and dissolved. Aqueous phase was water (200mL) with polysorbate 20, which was also heated at 90°C. The amount of stearyl alcohol, polysorbate 20 and AVO were set according to the design (Table 1). The lipid phase was poured to aqueous phase under constant stirring with a homogenizator (IKA Ultra-Turrax T18 Basic) at 3500 rpm for 5 minutes, the temperature was set at 85°C. An O/W emulsion was obtained. The formation of LPs was achieved by slowly addition of cold water at 4°C (300 mL) to the emulsion, without agitation. LPS were isolated by filtration, washed with water and dried by lyophilization (Labconco).

Quantification of the entrapped AVO

An aliquot of 25 mL was taken from the liposphere preparation mixture and 50 mL methanol were added (as washing agent to dissolve the free AVO). Then, it was filtered on a Millipore ultrafiltration equipment 8400 through a cellulose membrane (cut off 10000 Daltons), until only 50 ml was left unfiltered and those were washed with 25.0 mL of methanol and filtered until left 50 mL of the unfiltered mixture again. This process was repeated twice. The last unfiltered 50 mL were dried using a rotary evaporator (IKA RV10). The resultant residue was dissolved in chloroform in a 50.0 mL volumetric flask. A 1:25 dilution was made with methanol and it was analyzed by HPLC.

A chromatographer UFLC Shimadzu LC20 with autosampler, diode array detector, and a chromatographic cartridge LiChro CART ® 250-4 RP-18 (5μm) was used. The mobile phase used was methanol-water, 92-8, with a flow of 1 mLmin-1 and the measurement wavelength was at 358 nm to measured AVO and 310 nm to OMC. A calibration curve was developed using 7 solutions ranging from 0.010 to 0.150 mgmL-1.

The percentage of AVO loading (% w/w) were determined according to the following equation:

$$AVO\,loading\;\% = \frac{actual\,AVO\,mg}{liposphere\,smg}\,x100 \qquad \textbf{Eq. 2}$$

Differential Scanning Calorimetry (DSC)

DSC analysis was used to evaluate the process of incorporating of AVO into the LPs using a calorimeter TA Instruments DSC 2920. For these assays were used AVO, plainLPs and LPs containing AVO. The thermal behavior was assessed in a temperature range of 0 -200°C at a heating speed of 5 ° C min-1.

Morphology and particle size distribution

Morphological characterization was performed by scanning electron microscopy, using a microscope SEM - JEOL JSM-6490LV (Oxford Link). The particle size distribution of LPs was determined using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd) with attachment Hydro 2000S (A). The reading of the sample was done in triplicate.

Photostability of AVO into liphosferes

Two emulsions (o/w) were prepared containing each one free AVO orencapsulated AVO, the concentrations was 1.4% of AVO or its equivalent amount of LPs. To explore the incompatibility of AVO and OMC (Octyl-methoxycinnamate, filter UVB) were also prepared two emulsions, the former contained free AVO and free OMC, and the latter contained encapsulated AVO and free OMC. Both emulsions were prepared at 1.4% AVO and 7%OMC.

The formulation excipients were: EDTA tetrasodium 0.1%, phenoxyethanol0.2%, Emulgade 4%, steryl alcohol 2%, stearyl alcohol 2%, mineral oil 4%, polysorbate 20 2%, silicon 3%, water 74,6%. These emulsions were prepared heating the oil phase at 72°C and the aqueous phase at 76°C and mixed with Ultraturrrex. LPs and UV filters were incorporated into the oil phase.

Tramitance % and UVA/UVB ratio was determined in vitro for each emulsion, according to the method Stars boots. A transmittance analyzer (Labsphere UV-2000S, USA) and PMMA plates (Helioplates HD, 5um) were used. Two plates were prepared for each formulation, which were analyzed immediately after preparation, and were subjected to irradiation in a solar simulator Solarbox 1500e (Erichsen-Italy) equipped with a xenon lamp and an optical filter to remove wavelengths less than 290 nm, for 1 hour at 500 Wm^2. The extent of photodegradation was measured by comparing the tramitance % and UVA/UVB ratiobefore and after irradiating the plates.

RESULT AND DISCUSSION

In the first assays was observed that percentage of fatty alcohol and PS-20, condition in cooling and homogenization processes, and the relation oil/aqueous, v/v, are important in the size particle distribution. It was observed than stirring during cooling process leads to multimodal distributions not desirable,

with 90% of the particles below 2 µm, probably the shear forces favor the formation of smaller emulsion drops. The adding slowly of 300 mL of cold water (at 4° C) to solidify the emulsion drops, resulted in a monomodal distribution of LPs. The speed of the homogenizer at 7500 rpm yielded microspheres with sizes ≤ 2 µm, while at 3500 rpm was favored the formation of LPs ≥ 10 µm without exceeding the limit of 30µm and under a monomodal distribution. Concerning the relation of the O/W phases, the amount of carnauba wax was set at 5 g, and the amount of water was ranged from 100 to 200 mL. It was found than with 200 mL were obtained LPs with size close to 10 µm andmonomodal.

Based on these preliminary assays, the following variables were set: speed stirring at 3500 rpm, stirring time for 5 min and volume aqueous phase 200 mL.

Particle sizes obtained for the experimental design were higher 1 µm, D[4,3] values (volume mean diameter) were in the range 11.11-37.15 µm (Table 1)without taking into account trial 9 because this experiment exhibited a bimodal trend which is different from other three repliques in the central point of design, and D[3,2] (average value in surface) ranged from 6.97 and 29.65 µm. This broad variation evidences that the particle size was strongly dependent on the selected variables. ANOVA was applied after thatthe assumption of constant variance was satisfied through normal probability plot of the residuals, and the Box-cox plot did not suggest any transformation. The fitted model indicated that the particle size was affected by each one of studied variables and its interactions (equation 3)

Particle size=$22.57+4.44X_1-0.36X_2-3.44X_3-2.47X_1X_2-2.89X_1X_3-4.00X_2X_3-2.72X_1X_2X_3$ **Eq. 3**

The probability value ($p_{model} < 0.0001$) and the correlation coefficient (R^2 0.9881) indicate good fit of the regression model. The statistical parameters of R^2, and adjusted R^2 and -predicted R^2were 0.9881, 0.9816 and 0.9831 respectively indicating that this model can explain the 98% variability in the particle size,and also since the difference (predicted R^2-adjusted R^2) is no major to 0.2 units this model (Equation 3) can be used for prediction.

The response surface plot (Figure 1 a. and b.) showed thatLPs mean size was significantly ($p < 0.0001$) affected by initial AVO amount and PS-20. The size diminished when the PS-20 concentration (X_3) increased, as was suggested by the negative coefficients in equation3and statistical analysis, which showed that factor X_3caused a significant change in the particle size ($p < 0.0001$), it may be explained because a higher PS-20 concentration mean a better packaging in the drops wall of the O/W emulsion formed before the solidification, the packaging favors the drop curvature and avoid the coalescence, furthermore the high concentration of tensoactive depresses the interfacial tension. It was expected that PS-20 (HLB 16.7) had a good performance as emulsifier because it can satisfy the requirements of HLB of O/W emulsion with carnauba wax (HLB 14.5). (Gomma et al, 2010)

The ANOVA test revealed no significant effect for SA concentration as individual factor (X_2), however Figure 1a shows that an increase in SA concentration resulted in an increase in

theLPssize when AVO concentration is keeping in low level, while Figure 1b exhibits a negative effect for SA concentration when AVO concentration is keeping in high level, indicating the significance of the interactions X_1X_2, (p< 0.0001); in addition the influence of varying the lipid modifier concentration (X_2)on LPs size was most evident with lower levels of PS-20 and AVOconcentration (Figure 1a), whereas the opposite effect was observed in Figure 1b, indicating significant interaction between variables $X_1X_2X_3$(p< 0.0001) and the significant influence of initial AVO amount (p< 0.0001).

Figure 2 showed that particles size distribution were different in each trial, suggesting that studied variables affect strongly the polydispersability, and since our target range was narrow (10-30 µm), was relevant to assess what factors affect the polydispersibility, for this reason particle size D90 was analyzed. The data were fit into the following equation:

Particle size D90= $41.28+10.37X_1-3.64X_2-3.72X_3-7.75X_1X_2-3.16X_1X_3-8.64X_2X_3-6.36X_1X_2X_3$. **Eq4.**

The ANOVA indicated that the P_{model} was significant, less than 0.001, and the correlation coefficient was 0.9988. The ANOVA also showed that all variables and their interaction were significant (p< 0.0001).Response surface plot generated using the equation4 is presented in Figure 3 Lipospheres with sizes less than 30 µm can be achieved by decreasing the concentration of SA, when working at low levels of AVO ascan be seen in Figure 3a, while, at high AVO concentrations, it is necessary to increase levels of SA and PS-20 (Figure 3b). Probably in the first case, the viscosity of lipid phase might have a notable influence on the dispersability; and in the second case the packing in the wall of the emulsion drop,where SA act as co-surfactant,(Gasco, 1993) might explained the uniformity of particle size. This result is interesting because showed that the polydispersability not onlydepends on technology to apply shear force in the emulsification process but also in the compositional formula.

The AVO loading for all formulations ranged from 1.8 to 20%, (Table 1).These data clearly indicated that load % was strongly affected by the process variables. A linear statistical model, eq5, was generated by regression analysis in order to evaluate the AVO loading %.

Load %=$10.62062+8.81437X_1+0.085625X_2-0.075625X_3$ **eq. 5**

The p_{model}< 0.0001 and the correlation coefficient (R^2 0.9994) indicated good fit of the regression model. The ANOVA results showed that load % is strongly affected by initial AVO amount (p< 0.0001) and SA concentration (p< 0.0001) in a minor extent.Figure 4 exhibited any interactions were not significant, and the highest load % were found when AVO was keeping at a high level. Similar results were found in the optimization of lipospheres of oxibenzone, were the drug content was only depend on initial drug loading. (Gomma et al, 2010). The positive effect of SA was expected because SA can promote the loading due to an increase in the affinity between lipid matrix and AVO. According to the statistical results in the experimental region the narrow distribution

size and high load % was found when the initial amount of AVO was found in the maximum level (2000 mg), the SA in the maximum level (1%) and the PS-20 in the maximum level (3%) (run1). This finding is consistent with those reported previously using lipid material different from carnauba wax, where AVO loading % ranged from 10 - 21% and the particle size was into the range 10-40 µm.(Iannuccelli *et al.,* 2006, Scalia and Mezzena, 2009a, Scalia and Mezzena, 2009b)However, LPspreviously obtained using carnauba wax and phosphatidylcholine, yield a AVO loading rather high (40.1-48.5%, w/w), however the particle size washigher than reported herein, 86% of population was between 20 and 60 µm (Albertini *et al.,* 2009). The morphology of LPs obtained under condition of trial 1, wasanalyzed by scanning electron photomicrographs, it was observed that they were spherical with a rough surface (Figure 5) and with an approximate average diameter of 8-15 µm. The DSC analysis (Figure 6) showed that the peak at 83.71°C from pure AVO was close to that typical peak (at 84.4°C) reported to carnauba wax, (Gomma et al, 2010), as was seen in the thermogram of the plain liposphere, herein other peak is observed at 40°C probably due to presence of SA (Gandolfo *et al.,* 2003). The AVO-loaded LPs thermogram exhibited a broad peak at 83-92°C and a new peak is observed at 58°C. These findings suggested that AVO could be dispersed into LPs and a possible transformation in the amorphous form of AVO could be happened during preparation process of LPs. The photostability study showed the formulation with free AVO had a higher percentage of decay of tramittancethat formulation with AVO encapsulated in LPs (Figure 5), the rate of decay of the free AVO was 31.1%, where the maximum transmittance pre-irradiated at 358 nm was 0.389 and post-irradiated was 0.579, while the rate of decay of encapsulated AVO was 16.2%, with maximum transmittance unirradiated at 358 nm was 0.357 and irradiated was 0.461. The UVA/UVB ratio also was modify from 0.82 to 0.68 before and after irradiation, in the formulation containing free AVO; while in the test with encapsulated AVO, this was maintaining in 0.53-0.52.This indicates that the process of encapsulation in LPs decreases almost half the degradation of the AVO in a cosmetic formulation. These results are comparable to the extent of degradation previously reported by Albertini *et al.,* 2009, which was $38.6\pm3.6\%$ for free AVO and $32.1\pm4.3\%$ for AVO-loaded microparticles obtained by melt dispersion, and $15.4\pm4.1\%$ to microparticles obtained by spray congealing. The photostability study of OMC in the formulation contained AVO+OMC showed that the encapsulation process did not affect the rate of decay and there is no statistically significant difference between the formulation with free AVO+OMC and encapsulated AVO+OMC, where the maximum decay was 10.0% for both formulations (Figure 6).

The photostability study of the OMC in the formulation showed that the encapsulation process does not affect the decay rate of OMC and there is no statistically significant difference between the formulation with AVO+OMC and encapsulated AVO+OMC, where the maximum decay was 10.0% for both formulations.

Table 1: Experimental arrangement and results.

Factors		Levels		
		Minimum	Central	Maximum
		-	0	+
X1	Initial amount of Avobenzona, mg	100	450	1000
X2	Concentration of stearyl alcohol, % w/w	1.0	2.0	3.0
X3	Concentration of polysorbate 20, % w/w	1.0	2.0	3.0

run	Coded Variables			Mean Particle size (μm), D[4,3] values [+]	Particle size D90 values [+]	AVO loading (%)*
	X1	X2	X3			
1	+	+	+	11.11 ± 0.08	18.27 ± 0.21	19.7230 ± 0.0006
2	0	0	0	16.94 ± 0.12	34.56 ± 0.31	8.7460 ± 0.0002
3	+	-	+	29.63 ± 1.02	69.44 ± 3.05	18.8340 ± 0.0014
4	-	+	-	22.30 ± 0.41	38.65 ± 1.38	1.7763 ± 0.0233
5	-	-	-	15.27 ± 0.04	25.37 ± 0.51	1.8204 ± 0.0002
6	-	-	+	16.74 ± 0.01	28.49 ± 0.02	1.8654 ± 0.0002
7	0	0	0	20.17 ± 0.06	36.42 ± 0.79	8.7820 ± 0.0004
8	+	-	-	29.35 ± 0.33	54.35 ± 1.12	19.6181 ± 0.0027
9	0	0	0	203.80 ± 34.16	1140.13 ± 124.85	8.6959 ± 0.0009
10	-	+	+	18.30 ± 0.23	32.00 ± 0.39	1.7596 ± 0.0005
11	0	0	0	19.97 ± 0.24	35.51 ± 0.99	8.7905 ± 0.0001
12	+	+	-	37.15 ± 0.15	61.99 ± 0.26	19.5675 ± 0.0002

[+] Mean value ± S.D. (n=3)
* Mean value ± S.D. (n=2)
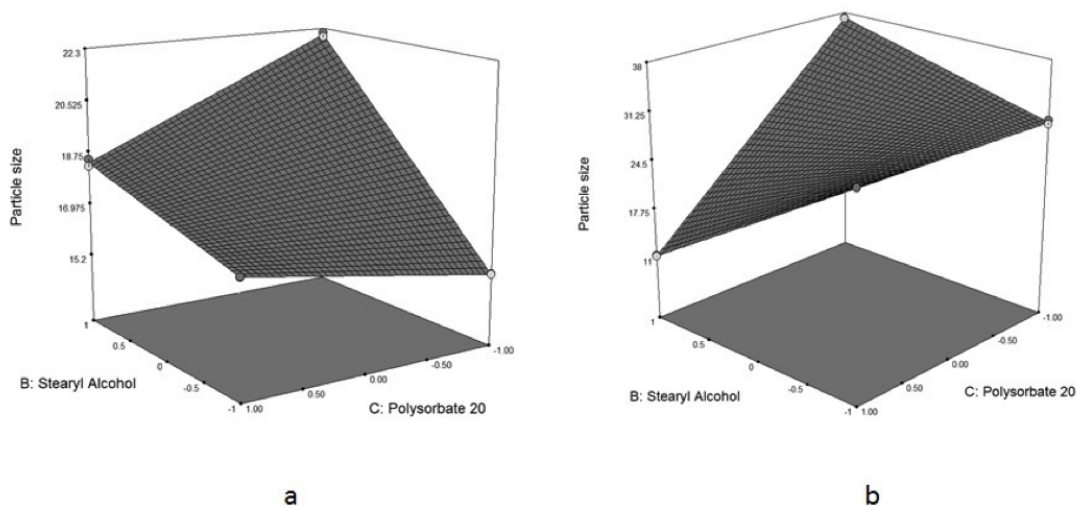
Fig. 1: Response surface plot for particle size mean, when the AVO quantity is keeping in the minimum level (a) and in the maximum level (b).
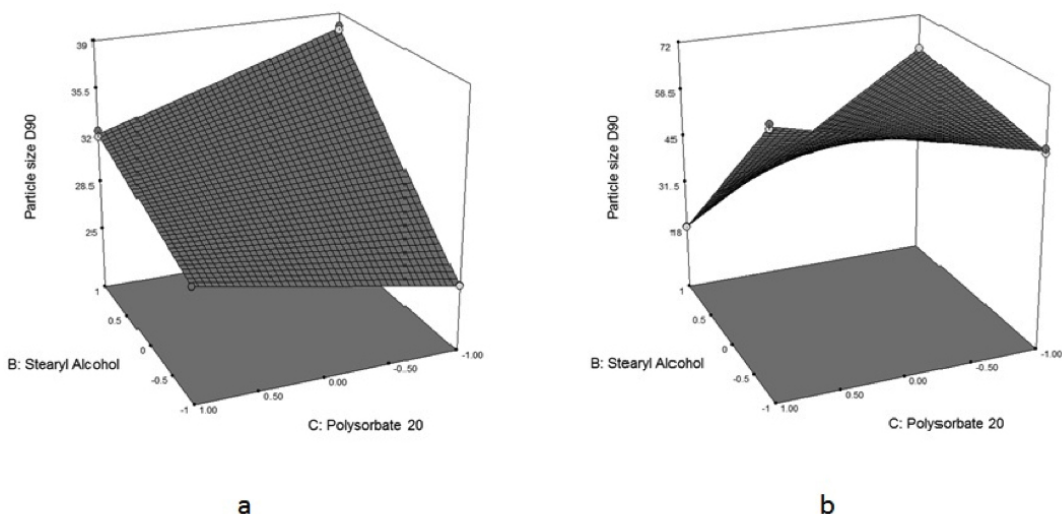

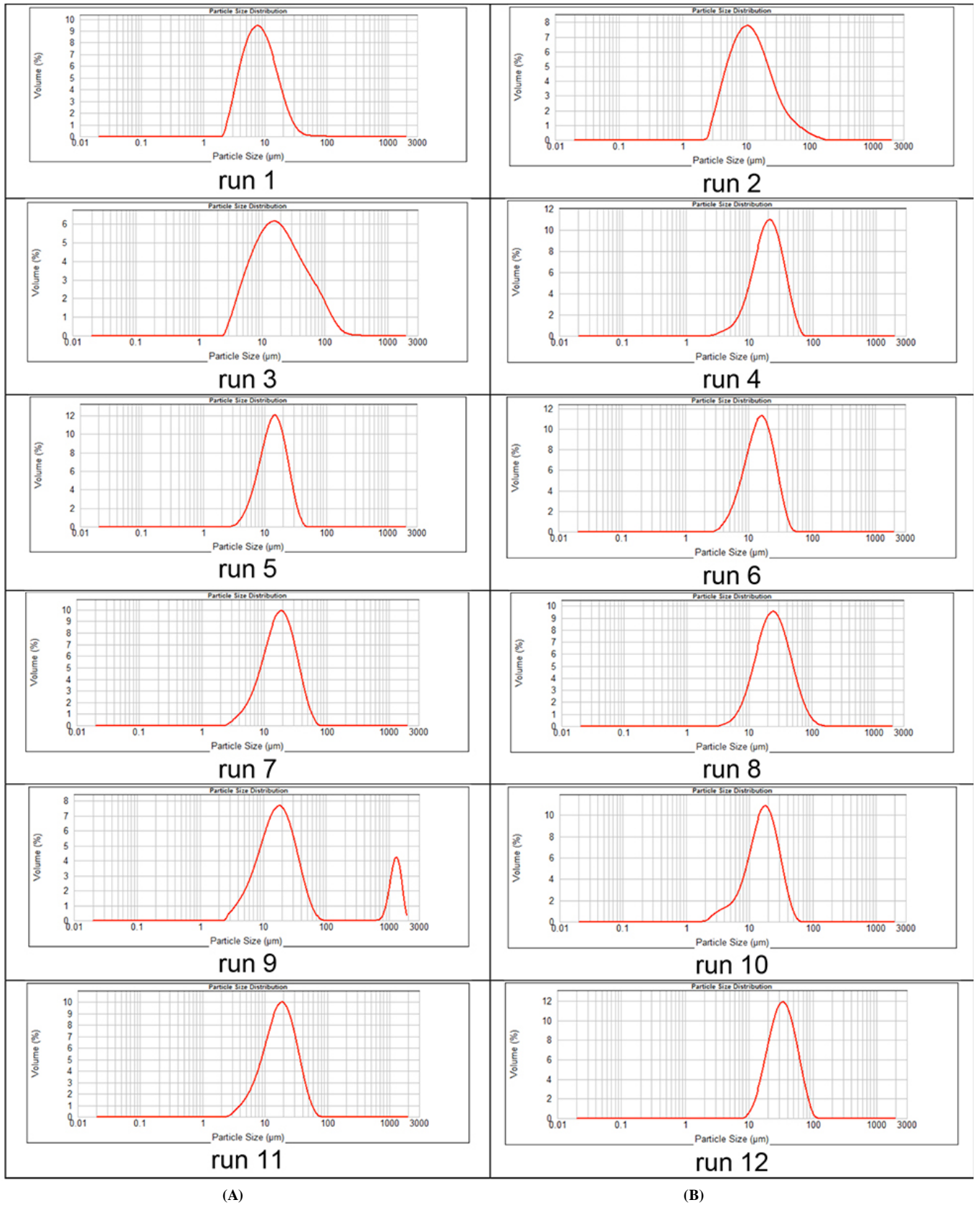**Fig. 2:** Particles size distributions according trials describe in table 1.

Fig. 3: Response surface plot for particle size at 90%, when the AVO quantity is keeping in the minimum level (a), maximum level (b).
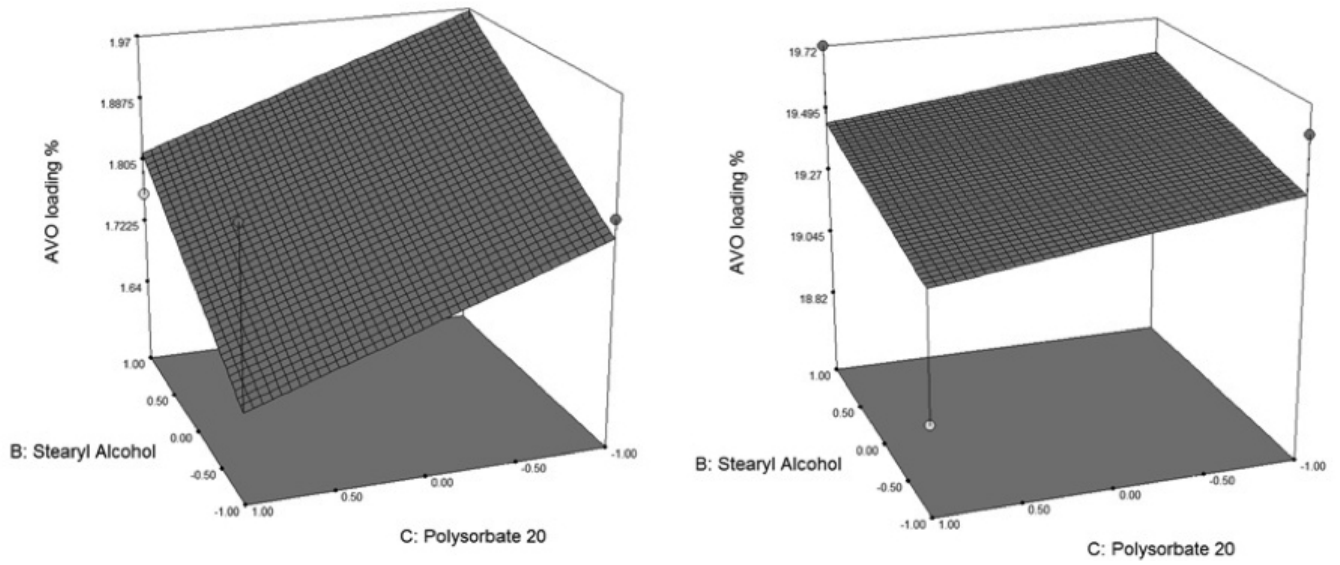
Fig. 4: Response surface plot for encapsulation efficiency, when the AVO quantity is keeping in the minimum level (a), maximum level (b).
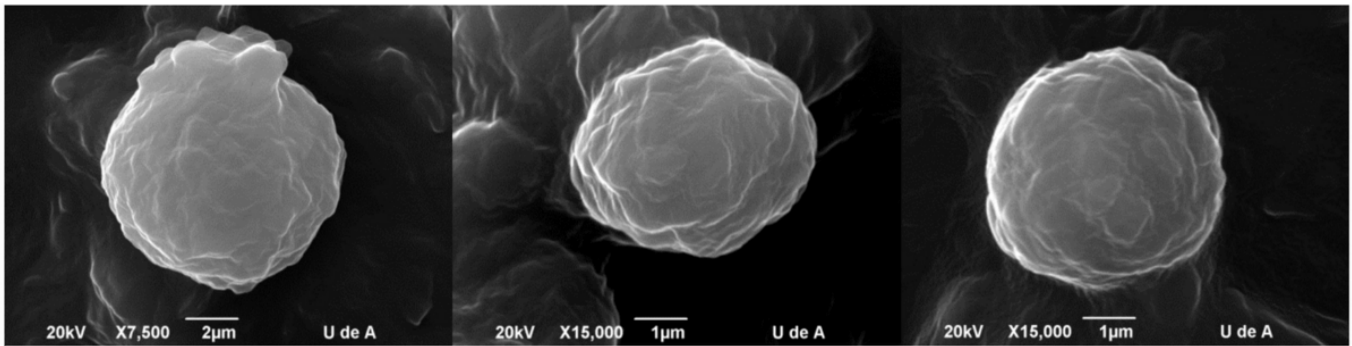

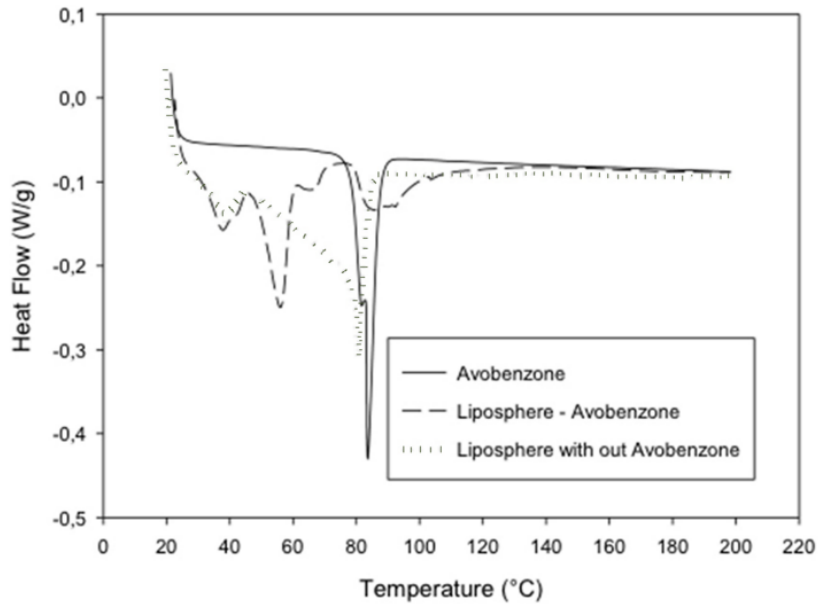**Fig. 5:** Scanning electron photomicrographs (SEM) of lipospheres dry by lyophilization.

Fig. 6: DSC thermogram. AVO raw material (-), AVO encpasulated in lipospheres (- - -), and liposphere without AVO (…).
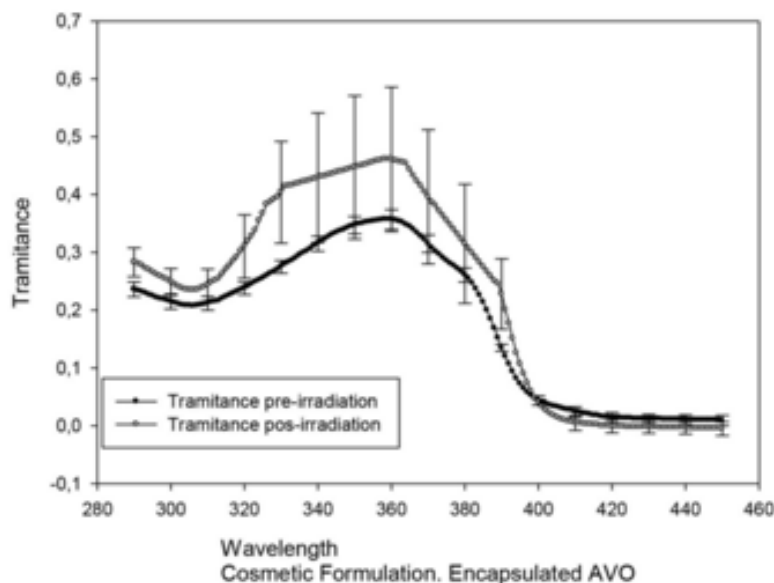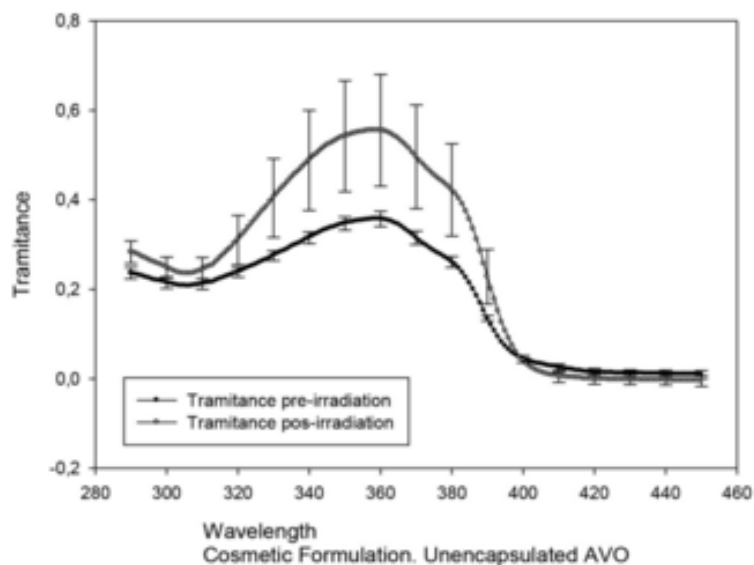
Fig. 7: Tramitance analysis.

CONCLUSION

The LPs were prepared using carnauba wax as lipid material, following anexperimental design, which showed that AVO loading was significantly affectedby AVO concentration, while mean and D90 particle size was influenced by AVO, SA and PS-20 concentration, and their interactions with each other. The application of statistical designs allowsunderstanding how variables can affect the properties of LPs. It was possible to demonstrate that the encapsulation processes favor stability of UVA sunscreen AVO to prevent photodegradation, achieving diminish the decomposition induced by light to the half when AVO is not being encapsulated. It was obtained LPs with particle size into the range10-30 µm, which is suitable for topical preparations.

ACKNOWLEDGEMENT

REFERENCES

Afonso S, Horita K, Sousa e Silva J, Almeida I, Amaral M, Lobão P, Costa P, Miranda M, Esteves da Silva K, Sousa Lobo J. Photodegradation of Avobenzone: Stabilization effect of antioxidants. Journal of Photochemistry and Photobiology B: Biology, 2014; 140: 36-40

Albertini B, Mezzena M, Passerini N, Rodriguez L, Scalia S. Evaluation of spray congealing as technique for the preparation of highly loaded solid lipid microparticles containing the sunscreen agent, avobenzone. Journal of pharmaceutical sciences, 2009; 98(8): 2759-2769.

Armstrong N. 2006. Pharmaceutical Experimental Design and Interpretation. 2da ed. Boca Raton: Editorial Taylor and Francis Group.

Barel A, Paye M, Maibach H. 2009. Handbook of cosmetic science and technology. 3rd ed. New York: Informa Healthcare.

Blasi P, Schoubben A, Giovagnoli S, Rossi C, Ricci M. The real value of novel particulate carriers for sunscreen formulation. Expert Review of Dermatology, 2011; 6(5): 509-517

Castillo E. 2007. Process Optimization. A Statistical Approach. Pennsylvania, USA: Springer.

Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of LPs as carriers for bioactive compounds. Biomaterials, 2002; 23(11): 2283-2294.

Gandolfo F, Bot A, Flöter E. Phase diagram of mixtures of stearic acid and stearyl alcohol. Thermochimicaacta, 2003; 404(1): 9-17.

Gasco M. Method for producing solid lipid microspheres having a narrow size distribution. U.S. Patent No 5,250,236, 5 Oct. 1993.

Gomaa Y, Darwish I, Boraei N, El-Khordagui L. Formulation of wax oxybenzonemicroparticles using a factorial approach. Journal of microencapsulation, 2010; 27(7): 628-639

Iannuccelli V, Sala N, Tursilli R, Coppi G, Scalia S. Influence of liposphere preparation on butyl-methoxydibenzoylmethanephotostability. European Journal of Pharmaceutics and Biopharmaceutics, 2006; 63: 140–145.

Jansen R, Osterwalder U, Wang S, Burnett M, Lim H. Photoprotection: Part II. Sunscreen: Development, efficacy, and controversies. Journal of the American Academy of Dermatology, 2013; 69(6): 867.

Jatana S, DeLouise L. Understanding engineered nanomaterial skin interactions and the modulatory effects of ultraviolet radiation skin exposure. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2014; 6(1):61-79.

Kheradmandnia S, Vasheghani-Farahani E, Nosrati M, Atyabi F. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. Nanomedicine: Nanotechnology, Biology and Medicine, 2010: 6(6): 753-759.

Mehnert W, Mäder K. Solid lipid nanoparticles. Production, characterization and applications. Drug Delivery Rev, 2001; 47: 165-196.

Nastruzzi C. 2005. LPs in drug targets and delivery: approaches, methods, and applications. Boca Raton, USA: CRC Press.

Nesseem D. Formulation of sunscreens with enhancement sun protection factor response based on solid lipid nanoparticles. International journal of cosmetic science, 2011; 33(1): 70-79.

Niculae G, Lacatusu I, Badea N, Meghea A. Lipid nanoparticles based on butyl-methoxydibenzoylmethane: in vitro UVA blocking effect. Nanotechnology, 2012; 23(31): 315704.

Puglia C, Damiani E, Offerta A, Rizza L, Tirendi G, Tarico M, Perrotta R. Evaluation of nanostructured lipid carriers (NLC) and nanoemulsions as carriers for UV-filters: Characterization, in vitro penetration and photostability studies. European Journal of Pharmaceutical Sciences, 2014; 51: 211-217.

Robertson T, Sanchez W, Roberts M. Are commercially available nanoparticles safe when applied to the skin? Journal of biomedical nanotechnology, 2010; 6(5): 452-468

Scalia S, Mezzena M. Incorporation in lipid microparticles of the UVA filter, butyl methoxydibenzoylmethane combined with the UVB filter, octocrylene: effect on photostability. AAPS PharmSciTech, 2009; 10(2): 384-390.

Scalia S, Mezzena M. Co-loading of a photostabilizer with the sunscreen agent, butyl methoxydibenzoylmethane in solid lipid microparticles. Drug development and industrial pharmacy, 2009; 35(2): 192-198.

Scalia S, Coppi G, Iannuccelli V. Microencapsulation of a cyclodextrin complex of the UV filter, butyl methoxydibenzoylmethane: In vivo skin penetration studies. Journal of pharmaceutical and biomedical analysis, 2011; 54(2): 345-350.

Scalia S, Mezzena M, Ramaccini D. Encapsulation of the UV filters ethylhexylmethoxycinnamate and butyl methoxydibenzoylmethane in lipid microparticles: effect on in vivo human skin permeation. Skin pharmacology and physiology, 2011; 24(4): 182-189.

Trotta V, Goios F, Monteiro H, Almeida I, Scalia S. Influence of lipid microparticle encapsulation on in vitro efficacy, photostability and water resistance of the sunscreen agents, octylmethoxycinnamate and butyl methoxydibenzoylmethane. Drug development and industrial pharmacy, 2013; 40(9): 1233-9

Villalobos J, Müller C. Sun protection enhancement of titanium dioxide crystals by the use of carnauba wax nanoparticles: the synergistic interaction between organic and inorganic sunscreens at nanoscale. International journal of pharmaceutics, 2006; 322(1): 161-170