

Development of a New Process for the Manufacture of Nanostructured Particles for UV Filter Encapsulation

Adriano M. Oliveira^{*1}, Natália N.P. Cerize¹, Fernanda C.P. Ferreira¹, Juliana N. Schianti¹, Wagner Aldeia¹

¹Bionanomanufacturing Center, Institute for Technological Research, 532, 05508-901, Sao Paulo, Brazil

^{*1}amarim@ipt.br

Abstract- This study describes a successful process using high pressure homogenization (HPH) and interfacial polymerization to produce nanoencapsulation of a UV-filter. Actually, this kind of agent has some limitations mainly related to photostability, degradation and toxicity, and then the process of encapsulation promises to overcome these drawbacks by protecting the active substance. Octyl-methoxycinnamate (OMC) was used as UV-filter model to develop the nanostructured particles. The nanocapsules were prepared by emulsification (using HPH) of an organic phase containing 4,4'-methylene-diphenyl-di-isocyanate (MDI) (a hydrophobic monomer), medium-chain triglycerides, Span 80 and OMC, in an aqueous solution composed of sodium lignosulfonate (stabilizing agent), Pluronic F127 and ethylene glycol (a hydrophilic monomer). In sequence, the polymerization of MDI and ethylene glycol at the water-oil droplet interface was performed at 40 °C during 4 hours. The nanocapsules dispersions were characterized with respect to their particle size (DLS), zeta potential (electrophoretic mobility), morphology (SEM-FEG), efficiency for UV absorption (UV spectrometry) and FTIR. The nanostructured particles developed here have diameter in a nanometric scale (between 251 and 661 nm), very low polydispersity index (around 0.1 to 0.3), negative zeta potential (about -46,0 mV), fluid aspect and colloidal stability during more than 2 months. The nanocapsules dispersion in a 0.005% (w/w) concentration absorbed around 70% of UV light incident, demonstrating the increase of UV-filter efficacy. In conclusion, the HPH and interfacial polymerization processes allowed producing nanostructured particles to encapsulate UV-filters, presenting a promising system to protect against UV radiation.

Keywords- Sunscreen; Nanoencapsulation; Interfacial Polymerization; High Pressure Homogenization

I. INTRODUCTION

Topical sunscreens are recommended to protect human skin against acute and chronic adverse effects of solar radiation. The main reported effects of solar radiation are acute sunburn, many forms of photoallergy, premature photoageing, melanoma, non-melanoma cutaneous neoplasia and pre-neoplastic disorders. A modern sunscreen with a broad anti-UVB and anti-UVA spectrum and a long-lasting effect should prevent erythema and skin inflammation (mediated by ROS species) better than sunscreens usually used 10-20 years ago.

Sunscreens preparations are usually applied to large skin areas and they should stay on skin surface and penetrate as little as possible [1]. As we know, sunscreens usually need a long residence time on the skin to absorb/reflect the UV radiation. However, the risk of systemic distribution should be considered as already demonstrated for broad-spectrum sunscreens [1], and they can lead to toxicological problems such as estrogenic and antiandrogenic activity [2, 3] as well as to a carcinogenic effect [4]. Furthermore, they are also well-known photosensitizers, photoirritants and photoallergens [5]. One of the sunscreens most used are cinnamates, as octyl-methoxycinnamate (OMC), that absorbs radiation in the 290-320 nm region and benzophenones as benzophenone-3 (BZ-3) that absorbs in 320-340 nm.

Colloidal carriers, such as microemulsions, nanoemulsions [5], and polymeric nanocapsules [6] have been proposed to improve topical administration, based on their capacity to encapsulate highly lipophilic pharmaceutical and cosmetic active compounds, in relation to the usual topical compositions, such as emulsions and gels. Besides this, the nanoencapsulation of sunscreens improves both its photostability and effectiveness compared to the non-encapsulated sunscreen [7] and consequently reduces the toxicological effects.

In this context, some attempts have been made to increase the stability and efficacy of sunscreen products and decrease their local intolerance by using new formulation strategies such as nanoparticles, microparticles, cyclodextrins or liposomes [8-12]. For example, encapsulation has been shown as an effective way to avoid OMC photodegradation in formulations [13-15], to slow down its skin penetration leading to an SPF enhancement and to decrease systemic absorption [16-18]. Moreover, nanoparticles of lipid type (Solid Lipid Nanoparticles) could increase the efficacy of sunscreens by providing a good epidermal targeting effect [19, 20].

Vettor et al. evaluated the release and the distribution of OMC nanoencapsulated in PLA on the skin layers and they related that more than 80% of OMC encapsulated was retained on stratum corneum. An example of a sunscreen formulation with benzophenone-3 nanoencapsulated in chitosane was evaluated [21] and penetration profiles showed that higher amount of

benzophenone-3 remained at the skin surface and a lower amount was found in the receptor compartment after the application of the formulation containing chitosan-coated nanocapsules compared to a formulation containing its free form.

Some studies have demonstrated the viability of development of new products based on nanoencapsulated actives ingredients as can be confirmed by inventions patented cited here (WO 2004069216 A1 [22] and WO 2011113129 A2 [23]). The first describes a novel stabilized cinnamic ester sunscreen composition, relating a method of enhancing the photostability of an encapsulated cinnamate derivative in a topical sunscreen. The second presents a nanostructured sun protection agent and the process of particles synthesis and this document comprises of core-shell-type nanoparticles, consisting of a wall formed by oxide nanoparticles and a core consisting of polymers and solar radiation protection chemicals, said system providing broad-spectrum solar protection, ranging from UVA to UVB.

In this study, the authors describe a new process to prepare nanostructured particles for UV filter encapsulation by using a new approach based on high pressure homogenization. Basically, the best conditions to produce nanocapsules dispersion in water with colloid stability and particle sizes in nanometric scale were identified, appropriated to sunscreen application.

II. EXPERIMENTAL SECTION

A. Materials

For interfacial nanoencapsulation, the 4,4-diphenylmethane diisocyanate (MDI) (Sigma-Aldrich) was utilized as a hydrophobic monomer, and as a hydrophilic monomer the ethylene glycol (Carlo Erba). Sorbitan monooleate Span 80 (Oxiten), Pluronic F127 (BASF S.A.) and sodium lignosulfonate (LSNa) were used as surfactants (purchased from Melbar Products of Lignin Ltda.). The sunscreens used were octyl-methoxycinnamate (OMC) (supplied by Henrifarma) and benzophenone-3 (BZ-3) (supplied by Delaware). Calendula oil and caprylic/capric triglycerides oil were used as a vehicle to active agents (Mygliol) (purchased from Henrifarma).

B. Nanoencapsulation Methods

The nanoencapsulation method adopted was based on a simple emulsification process by Ultra Turrax mechanical agitation (during 2 minutes) followed by six high pressure homogenization cycles, operating with 700 bar on the first valve and 100 bar on the second valve (using APV 2000 equipment). The nanoencapsulation process was finalized with the monomers reaction through mechanical agitation per 4 hours and it was performed at two different temperatures (25 and 50 °C). The organic phase was composed of the sunscreen chemical agent (OMC or BZ-3), SPAN 80 and one vehicle (calendula oil and caprylic/capric triglycerides oil) and the aqueous phase was composed of water, LSNa, Pluronic F127 and ethylene glycol. The mass ratio of organic and aqueous phase varied between 10 and 20 % for all experiments. Table 1 presents the formulations used in the nanoencapsulation process. All the reactions were performed using high pressure homogenization (HPH), except for the NPFS02 which was prepared without using this mixture technique.

TABLE 1 DESCRIPTION OF THE FORMULATIONS PREPARED THROUGH THE NANOENCAPSULATION PROCESS

Sample	Mixture technique	Composition (% w/w)								
		LSNa	Aqueous Phase		Composition (% w/w)			Organic Phase		
			Pluronic	Water	Span 80	MDI	OMC	BZ-3	Mygliol	Calendula
NPFS 01	HPH	3	1	96	0.80	0.50	-	4	16	-
NPFS 02	-	3	1	96	0.80	0.50	-	4	16	-
NPFS 03	HPH	3	1	96	0.80	0.50	4	-	16	-
NPFS 04	HPH	3	1	96	0.80	0.50	4	-	16	-
NPFS 05	HPH	3	1	96	0.80	0.50	-	-	16	-
NPFS 06	HPH	3	1	96	0.80	0.50	2	2	16	-
NPFS 07	HPH	3	1	96	0.40	0.5	2	-	8	-
NPFS 08	HPH	7	1	77.5	0.50	1	13	-	-	-
NPFS 09	HPH	3	1	96	0.60	0.5	-	8	12	-
NPFS 10	HPH	1	1	98	0.80	0.5	4	-	16	-
NPFS 11	HPH	3	1	96	0.20	0.5	4	-	-	-
NPFS 12	HPH	7	1	77.5	0.50	1	13	-	-	-
NPFS 13	HPH	7	1	77.5	0.50	1	8	-	-	5
NPFS 14	HPH	7	1	77.5	0.50	1	-	-	-	5
NPFS 15	HPH	3	1	96	0.80	0.5	4	-	16	-

C. Nanocapsules Characterization

1) Particle Size and Polydispersity Index

The particle size distributions (PSD) were determined by the means of the laser diffraction technique using a Beckman Coulter Analyzer model DelsaTM Nano C. A dilution required to adjust the obscuration range compatible with the technique was performed by adding distilled water. The mean particle diameter (D50) and the span $[(D90-D10)/D50]$, in which may be considered as a representative value of the polydispersity index, were evaluated in a comparative mode to analyze the obtained results.

2) Zeta Potential

The Laser Doppler Electrophoresis technique was used to determine the velocity of charged particles under the influence of an applied electric field (electrophoretic mobility). All measurements were performed in a single point mode and repeated fifteen times to ensure a representative value expressed by its mean and a standard deviation. Zeta potential was calculated from the measured electrophoretic mobility considering the Smoluchowsky theory. The equipment used was a Malvern Zeta Master S. Distilled water was used as the dispersing medium and each sample was appropriately diluted to ensure a significant signal/noise ratio detected by the photon correlator.

3) pH

After the polymerization process, the pH was immediately determined for each sample, without any dilution. The analyses were performed using a Mettler Toledo pH meter, model MP225.

4) UV-Visible Spectroscopy

The UV light absorption property of nanocapsules was determined by applying a UV-Visible spectrophotometer Cintra 10, model GBC. The wavelength ranged from 190 up to 900 nm.

5) FTIR

The technique of Infrared was used to evaluate the residual MDI (monomer that forms the polymeric wall) as an indication of nanocapsules formation. The spectrums in transmission mode were obtained with a Fourier transmission infrared spectrophotometer (FTIR), Nicolet model 6700 FT-IR.

6) High Resolution Scanning Electron Microscopy

The morphological aspects of the nanoparticles were further characterized using the high resolution scanning electron microscopy technique (FEG-SEM). A field emission microscope model Quanta 3D provided by FEI Instruments was used and the system operated at high vacuum mode. The samples were sputter coated with gold and an accelerating voltage of 20 kV was set to prevent any charge-up effect.

III. RESULTS AND DISCUSSION

A. Influence of Process

Aiming to evaluate the influence of the process on the colloidal stability of the nanostructures, two formulations in the same proportions were prepared as can be seen in Table 1, where NPFS01 was carried out using high pressure homogenization (HPH) and NPFS02 was performed without HPH. The results obtained (Table 2) showed the importance of the use of mixing homogenizer equipment on the emulsification stage of organic and aqueous phase on the obtaining of nanometric system. It was observed that when the high pressure homogenization process was not used, the particles mean diameter (DP) was 3.4 μm while the particles mean diameter using high pressure homogenizer decreased to 242 nm (data shown in Table 2). In addition, the reaction obtained with higher mean diameter (DP) showed phase separation after 24 hours (Fig. 1), while the reaction with mean diameter of 242 nm remained stable per 4 months.

Fig. 1 depicts an image of the obtained products in this study and it is possible to observe that the phase separation is clear for the reaction NPFS02. This phase separation occurred in the flotation form, because the capsules were filled with oil (Mygliol) that is less dense than water, therefore the particles also stayed less dense than water, causing the flotation in the NPFS02, which was prepared without using HPH.

Differences in the zeta potential of the particles were also observed, evidencing a higher value (in module) for the reaction that suffered phase separation (coagulation). The zeta potential of nanocapsules always presented negative values. These results are associated with the negative character of LSNa used as stabilizing agent on the preparation of nanocapsules, staying anchored on the nanostructures surface and render those values of zeta potential.

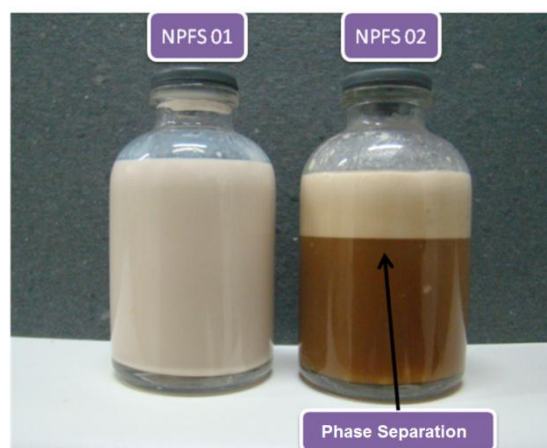


Fig. 1 Visual aspect of samples NPFS01 (using HPH) e NPFS02 (without HPH) stored for 24 h for comparing the influence of the mixture process

TABLE 2 RESULTS OBTAINED FROM THE REACTIONS USED IN THE STUDY OF TYPES OF MIXTURE PROCESS

Sample	Mixture Process	Zeta Potential (mV)	Sizes		pH	Visual Aspect
			Diameter (nm)	Polydispersity		
NPFS 01	HPH	- 38.1 $\pm$ 0.35	242.6	0.131	6.33	Stable
NPFS 02	-	- 54.1 $\pm$ 1.9	3470.2	1.183	6.25	Coagulation

Table 3 presents the characterizations of the stability of samples NPFS01 and NPFS02, 4 months after the preparation. A decrease of zeta potential on NPFS02 was observed, while sample NPFS01 did not present a significant variation on the zeta potential value, which can influence the changes on the interface with dispersing medium, because of the dissociation of functional groups on particle surface or species adsorption presents in aqueous medium.

Another observation that deserved attention was the formation of some crystals of BZ-3 in the NPFS01 reaction. This fact is associated with the presence of sunscreen agent not encapsulated in aqueous medium. The BZ-3 has low solubility in water, so the agent was not encapsulated and re-crystallized. This problem can be solved by varying the sunscreen active concentration in the encapsulation process.

TABLE 3 RESULTS OBTAINED FROM THE STABILITY STUDY OF PROCESS INFLUENCE AFTER 4 MONTHS

Sample	Zeta Potential (mV)	Size		pH	Visual Aspect
		Diameter (nm)	Polydispersity		
NPFS 01	- 34.5 $\pm$ 1.0	220.2	0.129	6.34	Instable a
NPFS 02	- 47.9 $\pm$ 1.7	2686.9	0.761	6.26	Instable b

a – Precipitation and crystals formation

b – Coagulation

B. Influence of Temperature

The processing temperature is an important parameter for interfacial polymerization process, therefore higher temperature leads to better performance of the polymerization rate of monomers. In this study, an effect of temperature on the capsule wall formation that depends on the MDI polymerization was observed. Two temperatures of reaction were tested with the sample NPFS03, at 25 °C and NPFS04 at 50 °C.

Table 4 presents the results of this temperature evaluation, and as can be observed there was not any significant difference in particle mean diameter (DP), zeta potential, pH or stability of the two reactions (NPFS03 e NPFS04). However, a difference can be observed in the infrared characterization (FTIR analysis) presented in Figs. 2, 3 and 4. Fig. 2 shows a MDI FTIR spectrum, monomer responsible for wall nanocapsules formation. It was observed in the MDI spectrum a highlighted band in 2250 cm⁻¹, a characteristic isocyanate band.

Comparing the spectrums of NPFS03 and NPFS04, it was observed that for the reaction performed at 25 °C (NPFS03), there was a signal in the region of 2250 cm⁻¹, indicating a MDI residual not polymerized higher than for the reaction NPFS04 (reaction performed at 50 °C), where this signal disappeared completely. This result indicates the necessity of heating the reaction of nanoencapsulation by interfacial polymerization to accelerate the MDI polymerization, because this monomer, even in residual concentration, can cause some type of skin irritation. This study was performed qualitatively and the MDI quantification will be measured in the next study.

TABLE 4 RESULTS OBTAINED FROM REACTIONS USED IN THE STUDY OF PROCESS TEMPERATURE OF NANOENCAPSULATION

Sample	Zeta Potential (mV)	Size		pH	Visual Aspect
		Diameter (nm)	Polydispersity		
NPFS 03	- 43.4 $\pm$ 0.7	291.1	0.127	6.33	Stable
NPFS 04	- 40.2 $\pm$ 0.4	226.0	0.124	6.38	Stable

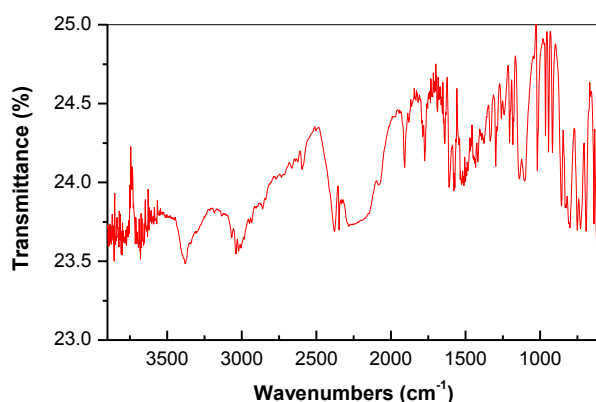


Fig. 2 MDI FTIR spectrum

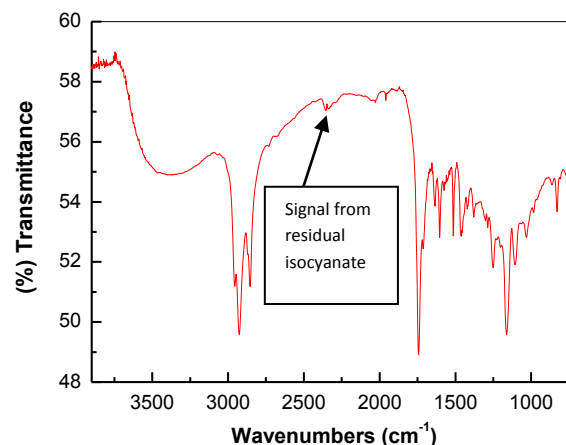


Fig. 3 FTIR spectrum of the product obtained in reaction 3

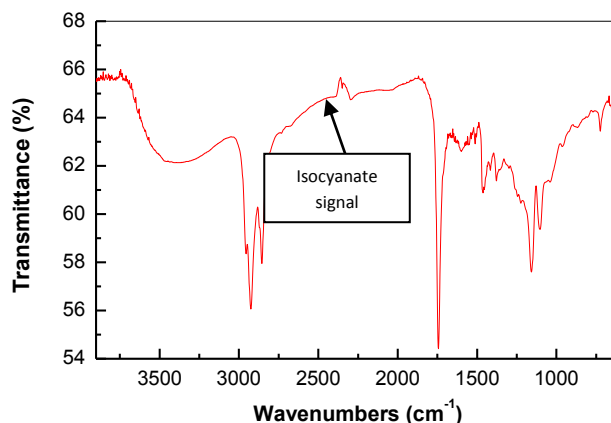


Fig. 4 FTIR spectrum of the product obtained in reaction 4

As we can see in Table 5, the samples 03 and 04 presented a colloidal stability after 4 months confirmed by measurements of zeta potential, particle size, pH and visual aspect.

TABLE 5 RESULTS OBTAINED IN THE STUDY OF INFLUENCE STABILITY OF TEMPERATURE CHANGES AFTER 4 MONTHS

Sample	Zeta Potential (mV)	Size		pH	Visual Aspect
		Diameter (nm)	Polydispersity		
NPFS 03	-38.0 $\pm$ 0.5	218.7	0.135	5.94	Stable
NPFS 04	- 38.8 $\pm$ 0.5	230.2	0.132	6.34	Stable

C. Influence of Protection Agent

With the purpose to evaluate the formulation efficiency of different actives, two types of chemical agents of light absorption, the OMC and the BZ-3, were tested. These actives were chosen because they are widely utilized by cosmetics industries in the preparation of sunscreen. In addition, these actives have distinct physical properties; the OMC is liquid at room temperature and the BZ-3 is solid at the same condition, which can influence in important way the encapsulation process. The formulations applied in this study varying the type (BZ-3 and OMC), the sample NPFS01 presented 4% of BZ-3, the sample NPFS03 presented 4% of OMC, the sample NPFS05 was the placebo (without UV filter), and the sample NPFS06 presented 2% of BZ-3 and 2% of OMC.

Table 6 presents the results obtained in this study (influence of the type of protection agent). For these conditions the results of DP were similar, without significant variations in function of active changes. It was observed that the BZ-3 encapsulation efficiency depends on its solubility in the oil used as vehicle (in this case Mygliol), because when 4% of BZ-3 was encapsulated, crystals formation occurred in the aqueous phase, indicating that part of BZ-3 added to the system were free,

i.e. decrease of encapsulation efficiency. This crystal formation did not occur when part of BZ-3 was substituted by OMC, which in this case is liquid, probably by the fact of increasing liquid fraction and decreasing solid fraction.

TABLE 6 RESULTS OBTAINED IN THE STUDY OF INFLUENCE OF TYPE OF ACTIVE ON THE NANOENCAPSULATION PROCESS

Sample	Zeta Potential (mV)	Size		pH	Visual Aspect
		Diameter (nm)	Polydispersity		
NPFS 01	- 38.2 $\pm$ 0.3	216.0	0.135	6.66	Stable*
NPFS 03	- 43.4 $\pm$ 0.7	219.1	0.127	6.33	Stable
NPFS 05	- 46.8 $\pm$ 0.7	211.3	0.127	6.64	Stable
NPFS 06	- 46.4 $\pm$ 0.6	236.6	0.124	6.55	Stable

*Precipitation and crystals formation

These results demonstrated that in the case of solid agents, it is important to have an oil for solubilize completely the solid active in the nanoencapsulation process and makes possible the encapsulation process. In the case of OMC, this problem was minimized, because OMC is liquid at the encapsulation temperature, which facilitates the solubilization in the Mygliol and also the emulsification process that occurs before the encapsulation step, increasing the encapsulation efficiency.

The sample stability showed in Table 7 presents a destabilization of the colloidal system for the samples 01, 05 and 06, although the particle diameter did not present an expressive variation. A precipitation of the sample 01 was also observed, with BZ-3 crystals formation that they were not encapsulated. BZ-3 at room temperature is solid, and for encapsulation process the BZ-3 solubilization in caprylic/capryc triglycerides oil is necessary. However, this method was not efficient. Although in the sample 06, there was also the presence of BZ-3, the amount was reduced by half and the quantity of OMC was added in the same proportion, so the amount of oil increased and the solid active decreased, increasing the solubility in this system. After 4 months, a slight coagulation occurred, as the oil is less dense than water, the particles stayed on superior part.

TABLE 7 RESULTS OBTAINED IN THE STUDY OF STABILITY OF INFLUENCE OF THE TYPE OF PROTECTION AGENT BEFORE 4 MONTHS

Sample	Zeta Potential (mV)	Size		pH	Visual Aspect
		Diameter (nm)	Polydispersity		
NPFS 01	-34.5 $\pm$ 1.0	220.2	0.129	6.34	Instable b
NPFS 03	- 38.0 $\pm$ 0.5	218.7	0.135	5.94	Stable
NPFS 05	- 40.8 $\pm$ 0.5	212.6	0.128	6.78	Instable c
NPFS 06	- 39.1 $\pm$ 0.4	234.6	0.144	6.56	Instable d

b- Precipitation with the crystals formation

c- Light precipitation

d- Coagulation with a light upper film

D. Influence of Active Content

Evaluating the active content that an encapsulation process is capable to store is a fundamental step for the development of product economically feasible. Therefore, higher encapsulation capacity of the system leads to lower operation costs and higher product efficiency. In this way, the formulation applied in this study aimed to evaluate the influence of active content to be encapsulated. The sample NPFS01 was added 4% of BZ-3, the NPFS03 was added 4% of OMC, and the NPFS05 was not added any type of active. The NPFS 07 was added 2 % of OMC, NPFS08 was added 13% of OMC, and finally 8% of BZ-3 was added to the sample NPFS09.

The results of this study are demonstrated in Table 8. As can be observed, although there was an increase of active concentration, the nanocapsules diameter did not change significantly and the stability was maintained. This result can be considered an excellent result, because as mentioned before, greater amount of embedded asset on the nanocapsules leads to better product efficiency and lower production costs.

For the case of BZ-3 nanoencapsulation, the increase of asset concentration potentiates the crystals formation in aqueous phase. As discussed previously, the crystals formation is associated with the active solubility in the Mygliol (oil used as a vehicle), which can be overcome by oil substitution by some materials in which the BZ-3 presents higher solubility. It is important to consider also that the crystals formed in the aqueous phase are micrometric and can be observed by naked eyes, i.e. the mean diameter presented in Table 8 did not take into account the diameter of those crystals, because they decant rapidly at the end of encapsulation reaction and can be separated by filtration.

TABLE 8 RESULTS OBTAINED IN THE STUDY OF INFLUENCE OF TYPE OF ACTIVE ON THE NANOENCAPSULATION PROCESS

Sample	Zeta Potential (mV)	Size		pH	Physical Aspect
		Diameter (nm)	Polydispersity		
NPFS 01	- 38.2 $\pm$ 0.3	216.0	0.135	6.66	Stable*
NPFS 03	- 43.4 $\pm$ 0.7	219.1	0.127	6.33	Stable
NPFS 05	- 46.8 $\pm$ 0.7	211.3	0.127	6.64	Stable
NPFS 07	- 45.1 $\pm$ 0.6	247.6	0.122	6.55	Stable
NPFS 08	- 45.8 $\pm$ 1,1	357.6	0.198	6.66	Stable

NPFS 09	- 43.9 ± 0.7	212.9	0.121	6.19	Stable*
---------	--------------	-------	-------	------	---------

* Precipitation and crystals formation

E. Study of Absorption Properties of UV Light by Nanocapsules

This study consisted in the dilution of samples of active nanoencapsulated for a concentration by 0.005% in weight, followed by reading of light transmittance of the sample in a wavelength range of 190 up to 900 nm. The principle of the test consisted in evaluating how much a sample of nanoencapsulated absorbs the light within a specified range of wavelength (UV wavelengths) and the values were expressed in percentage of absorbed light, for the measuring in transmittance.

For all reactions presented in this work, the UV light absorption was studied. However, with the purpose of synthesizing the best condition obtained, this paper presents only the results that illustrate better the concept that when working with chemical actives of UV light absorption nanoencapsulated, an increase of efficiency of solar protection occurred, and the LSNa and calendula oil contribute to this efficiency.

Fig. 5 presents a percentage curve of transmitted light by samples of nanocapsules, of pure OMC and of the LSNa. As can be seen, when comparing to blue and green curves, it is possible to evaluate the influence of nanoencapsulation process on the efficiency of OMC in absorbing light. For the OMC dispersed in water the light transmission (in the UVA and UVB region) is approximately 50%, while this value became less than 20 % when the same concentration of OMC was nanoencapsulated. Thus it is possible to have an estimation of how the nanotechnology tools can contribute to the optimization of physical and chemical phenomenon that can be potentiated when the material are reduced to nanometric scale. In Fig. 5, it is possible to verify the LSNa capacity of light absorption in UV region, as is shown by the yellow curve. Comparing to the blue and green curves, it is possible to see that at wavelengths below 300 nm, the absence of LSNa caused an increase in the light transmittance.

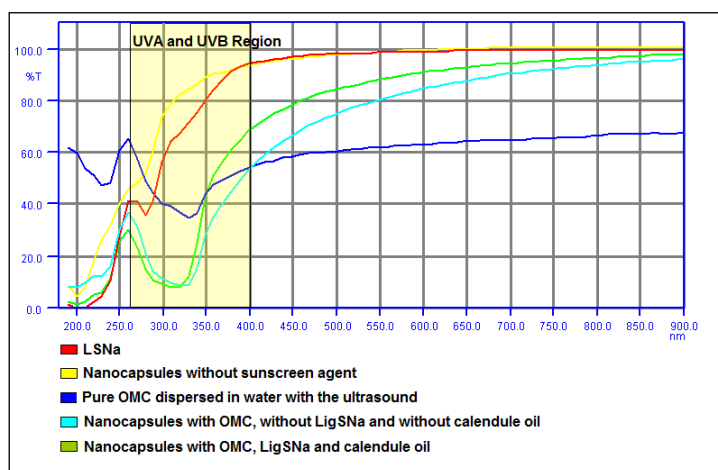


Fig. 5 Transmittance Spectra in function of light wavelength comparing the raw materials and nanocapsules

Fig. 6 presents a comparison of transmittance curves of the products containing calendula oil. The yellow curve presents a nanostructured system containing only calendula oil and it is possible to verify that 5 % of the mass of the system oil was capable to absorb part of the light. However, the best result was obtained associating the oil with OMC, represented by the blue curve, where 8% in mass of OMC and 5% in mass of calendula oil were utilized. This association of oil with OMC produced a result of UV light absorption more interesting than that by 13% of pure OMC, indicating that part of OMC can be substituted by calendula oil without affecting the sunscreen efficiency. This substitution of part of solar protection chemical agents by natural oils can be an alternative to reduce the toxicity of these products and also decrease the final cost of product.

The SEM image of the NPFS13 is shown in Fig. 7. The surface shows that the nanocapsules prepared from high pressure homogenization and interfacial polymerization (as described before) tend to be regular and with spherical morphology, with average size in a nanometric scale.

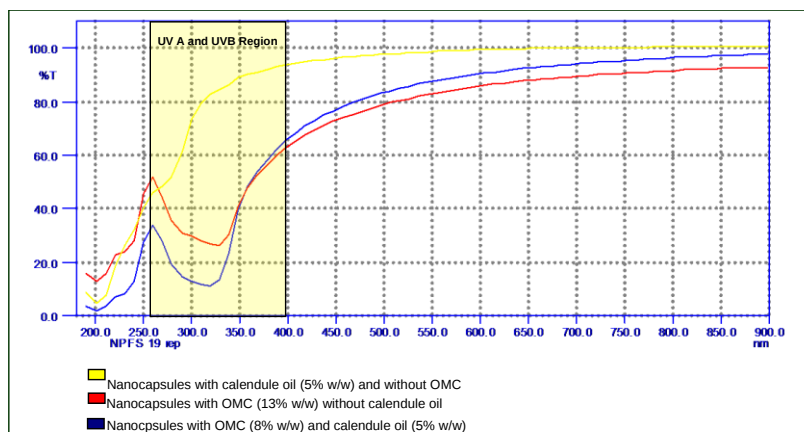


Fig. 6 Transmittance Spectra in function of light wavelength for the samples containing calendule oil

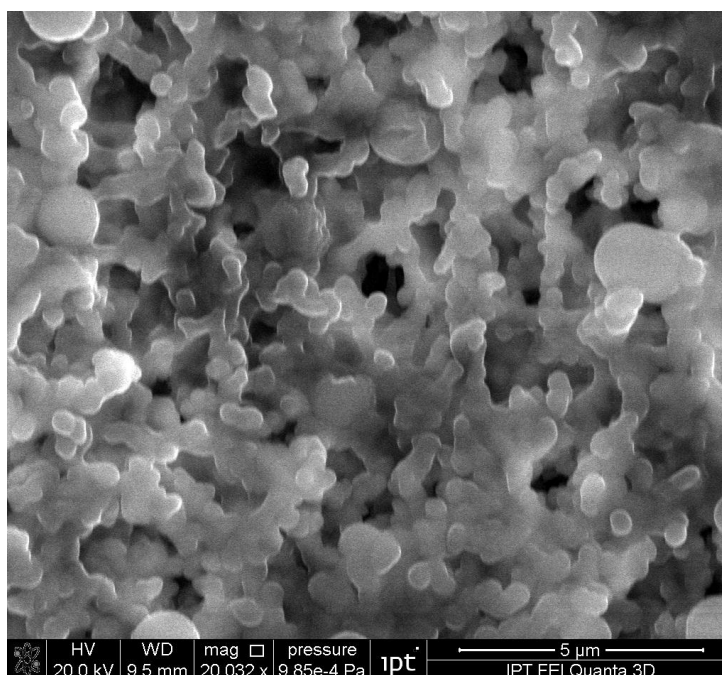


Fig. 7 FEG-SEM image of OMC-loaded nanocapsules

IV. CONCLUSIONS

This study showed a successful process using high pressure homogenization (HPH) and interfacial polymerization to produce nanoencapsulation of a UV-filter. The study identified the importance of an efficient mixture process such as high pressure homogenization, and the necessity of temperature control on the interfacial polymerization step for preparing the nanostructured system with a low residual monomer.

The nanostructured particles developed in this study have diameter in a nanometric scale (between 250 and 600 nm), very low polydispersity index (around 0.2), negative zeta potential (about -40.0 mV), fluid aspect and colloidal stability during more than 2 months. The nanocapsules dispersion in a 0.005% (w/w) concentration absorbed around 70% of UV light incident, demonstrating the increase of UV-filter efficacy. This encapsulation process allows the use of liquid or solid agents, providing an alternative oil vehicle for solubilization of solid actives as benzophenone-3. Liquid UV filter can be selected as oil contributing to the light absorption with the advantage of using a nature compound (calendula oil). Knowing the solid active solubility in oil can be important information for the optimization of encapsulation efficiency. On the other hand, with liquid active, the oil can be dispensed, increasing the percentage of active in encapsulation process, enhancing the efficiency and decreasing the costs of nanoencapsulated production.

The use of products of nature origin that present properties of light absorption can bring advantages for sunscreen systems and the most valued information in this way was the increase of light absorption properties of the product when it was reduced to nanoscale range, demonstrating the advantages of working with nanostructured systems, mainly the nanoparticles. This new process allows producing nanostructured particles to encapsulate UV-filters, presenting a promising system to protect against UV radiation.

ACKNOWLEDGMENT

The authors thank IPT for the financial support.

REFERENCES

- [1] M. M. Rieger, "Efeito protetor dos filtros solares contra patologias da pele," *Cosmet. Toilet.*, vol. 1, pp. 33-40, 1989.
- [2] I. Karlsson, L. Hillerström, A. Stenfeldt, J. Mårtensson, and A. Börje, "Photodegradation of dibenzoylmethanes: potencial cause of photocontact allergy to sunscreens," *Chem. Res. Toxicol.*, vol. 22, pp. 1881-1892, 2009.
- [3] R. M. Lavker, G. Gerberick, D. Veres, C. J. Irwin, and K. H. Kaidbey, "Cumulative effects from repeated exposures to suberythral doses of UVB and UVA in human skin," *J. Am. Acad. Dermatol.*, vol. 32, pp. 53-62, 1995.
- [4] B. Gilchrest, "A review of skin ageing and its medical therapy," *Br. J. Dermatol.*, vol. 135, pp. 867-875, 1996.
- [5] L. Perioli, V. Ambrogio, B. Bertin, M. Ricci, M. Nocchetti, L. Latterini, and C. Rossi, "Anionic clays for sunscreen agent safe use: Photoprotection, photostability and prevention of their skin penetration," *Eur. J. Pharm. Biopharm.*, vol. 62, pp. 185-193, 2006.
- [6] G. K. Borghetti, "Development and evaluation of physical stability from O/ W lotions containing sunscreens," *Braz. J. Pharm. Sci.*, vol. 42, pp. 531-537, 2006.
- [7] M. Burnett and S. Wang, "Current sunscreen controversies: a critical review," *Photomed., Photodermatol. Photoimmunol.*, vol. 27, pp. 58-67, 2011.
- [8] M. Jiménez, J. B. Pelletier, and M. Martini, "Influence of encapsulation on the in vitro percutaneous absorption of octyl methoxycinnamate," *Int. J. Pharm.*, vol. 272, pp. 45-55, 2004.
- [9] B. Olvera-Martínez, J. Cázarez-Delgadillo, S. Calderilla-Fajardo, R. Villalobos-García, A. Ganem-Quintanar, and D. Quintanar-Guerrero, "Preparation of polymeric nanocapsules containing octyl methoxycinnamate by the emulsification-diffusion technique: penetration across the stratum corneum," *J. Pharm. Sci.*, vol. 94, pp. 1552-1559, 2005.
- [10] E. Ramon, C. Alonso, L. Coderch, A. De La Maza, O. Lopez, J. Parra, and J. Notario, "Liposomes as alternative vehicles for sun filter formulations," *Drug Delivery.*, vol. 12, pp. 83-88, 2005.
- [11] A. Molinari, R. Tursilli, A. Casolari, A. Maldotti, and S. Scalia, "Influence of complexation with cyclodextrins on photo-induced free radical production by the common sunscreen agents octyl-dimethylaminobenzoate and octyl-methoxycinnamate," *Pharmazie*, vol. 61, pp. 41-45, 2006.
- [12] R. Tursilli, G. Piel, L. Delattre, and S. Scalia, "Solid lipid microparticles containing the sunscreen agent, octyl-dimethylaminobenzoate: effect of the vehicle," *Eur. J. Pharm. Biopharm.*, vol. 66, pp. 483-487, 2007.
- [13] P. Perugini, S. Simeoni, S. Scalia, I. Genta, T. Modena, B. Conti, and F. Pavanetto, "Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate," *Int. J. Pharm.*, vol. 246, pp. 37-45, 2002.
- [14] N. Anumansirikul, M. Wittayasuporn, P. Klinubol, A. Tachaprutinun, and S. Wanichwecharungruang, "UV-screening chitosan nanocontainers: increasing the photostability of encapsulated materials and controlled release," *Nanotechnology*, vol. 19, pp. 19, 2008.
- [15] VOL. Weiss-Angeli, F. Poletto, L. Zancan, F. Baldasso, A. Pohlmann, and S. Guterres, "Nanocapsules of octyl methoxycinnamate containing quercetin delayed the photodegradation of both components under ultraviolet A radiation," *J. Biomed. Nanotechnol.*, vol. 4, pp. 80-89, 2008.
- [16] R. Alvarez-Román, G. Barré R. Guy, and H. Fessi, "Biodegradable polymer nanocapsules containing a sunscreen agent: preparation and photoprotection," *Eur. J. Pharm. Biopharm.*, vol. 52, pp. 191-195, 2001.
- [17] P. Klinubol, P. Asawanonda, and S. Wanichwecharungruang, "Transdermal penetration of UV filters," *Skin Pharmacol. Physiol.*, vol. 21, pp. 23-29, 2008.
- [18] M. Vettor, P. Perugini, S. Scalia, B. Conti, I. Genta, T. Modena, and F. Pavanetto, "Poly(D,L-lactide) nanoencapsulation to reduce photoinactivation of a sunscreen agent," *Int. J. Cosmet. Sci.*, vol. 30, pp. 219-227, 2008.
- [19] S. Lombardi-Borgia, M. Regehly, R. Sivaramakrishnan, W. Mehnert, H. Korting, K. Danker, and M. Schafer-Korting, "Lipid nanoparticles for skin penetration enhancement-correlation to drug localization within the particle matrix as determined by fluorescence and piezoelectric spectroscopy," *J. Contr. Rel.*, vol. 110, pp. 151-163, 2005.
- [20] H. Chen, X. Chang, D. Du, W. Liu, J. Liu, T. Weng, and X. Yang, "Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting," *J. Contr. Rel.*, vol. 110, pp. 296-306, 2006.
- [21] N. M. Siqueira, "Technological development and evaluation of penetration skin of BZ-3 from the chitosan coated nanocapsules," Thesis, 2008.
- [22] K. Berg-Schultz, V. Schehlmann, and H. Westenfelder, WO2004069216A1, "Novel stabilized cinnamic ester sunscreen compositions," Sep. 25, 2003.
- [23] A.M.Oliveira, K.L.Guimarães, and N.N.P. CERIZE, WO2011113129A2, "Nanostructured sun protection agent and process," Mar. 21, 2011.