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Research Article

Shea butter solid nanoparticles for curcumin encapsulation: influence of nanoparticles size on drug loading[†].

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Keywords: solid lipid nanoparticles, SLN, shea butter, curcumin, colloidal carrier, lipid polymorphism

Abbreviations: SLN, Solid Lipid Nanoparticles, **EE**, Encapsulation Efficiency, **XRD**, X-Ray Diffraction, **SAXS**, Small Angle X-ray Scattering, **WAXS**, Wide Angle X-Ray Scattering, **DSC**, Differential Scanning Calorimetry, **DLS**, Dynamic Light Scattering, **PdI**, Polydispersity Index, **MDOE**, Mixture Design Of Experiments, **RSM**, Response Surface Methodology.

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Abstract

In the present work, shea butter solid lipid nanoparticles (SLN) were prepared by sonication using nonionic surfactants as stabilizers without organic solvent. The mixture design methodology enabled to control particles size from 50 nm to more than 1 µm according to the mixture composition. Then, curcumin, a natural polyphenol, has been encapsulated in nanoparticles with a wide range of diameters (50-230 nm) and the encapsulation efficiency has been related to the particles sizes. The bigger the nanoparticles, the lower the encapsulation efficiency. The lipid structure at non dispersed state and under SLN form has been studied by Differential Scanning Calorimetry (DSC) and X-ray diffraction (XRD). From the obtained results, it seemed that encapsulated curcumin did not affect the lipid properties at the SLN state unlike at non dispersed state. This was consistent with a localization of the curcumin in the outer shell of the lipid nanoparticles which explained the evolution of the encapsulation efficiency according to the nanoparticles size.

Practical applications

Solid lipid nanoparticles (SLN) present a great interest in the delivery of both hydrophobic and hydrophilic drugs. Preparing such nanovectors with natural, non-expensive materials and easily available (i.e. shea butter) will promote their potential use in several applications like oral, parenteral, dermal or ocular delivery. Controlling the particles size from the formulation composition and also the drug loading from SLN properties is of main importance to optimize the use of SLN as colloidal carrier.

1. Introduction

Solid lipid nanoparticles (SLN), developed since the beginning of the 90's [1, 2], are an alternative carrier to liposomes, emulsions and polymeric particles promising for various applications as oral [3], dermal [4, 5], parental [4] delivery of either hydrophobic or hydrophilic actives. SLN are produced by cooling a nanoemulsion prepared at a temperature higher than the melting point of the composing lipid and thus are solid at room temperature. This property induces a better physical stability compared than liquid/liquid systems as emulsions or liposomes [6]. A wide range of lipid has been investigated for SLN preparation [3, 4]. Shea butter is an extensively used fat in West Africa and is employed in food, cosmetic and pharmaceutical industries. Shea butter is a good candidate for SLN production, taking into account its simple fatty acids composition (mainly stearic and oleic acids)[7] leading to a unique low melting temperature (ranging from 33 to 38 °C), its natural origin and its low price. In addition to the triacylglycerols, the presence of tocopherol and other phenolic compounds in Shea butter is moreover of special interest due to their antioxidant and anti-inflammatory properties highlighted in food and cosmetic uses [7]. Nevertheless, to the best of our knowledge, only one recent study reports the use of shea butter for the preparation of solid lipid nanoparticles [8].

Lipid nanoparticles have been already used to incorporate many actives compounds reported in several reviews [5, 6, 9, 10]. Some recent studies deal with the encapsulation of polyphenols such as quercetin or curcumin into SLN [11–13]. Curcumin is a natural hydrophobic polyphenol extracted from the roots of *Curcuma longa* and present in turmeric used as food additive. Several studies report the antioxidant, anti-inflammatory, antibacterial and also anti-cancer properties of curcumin [12, 14]. Its main drawback for an oral delivery is its low solubility in water and so, its poor bioavailability. To overcome this problem, several research teams focus their works on encapsulation of curcumin in nanovectors such as nanoliposomes, nanoemulsions, and solid lipid nanoparticles [12, 15, 16]. Curcumin is also often used as a lipophilic model compound for the development of drug carriers [16] thanks to its facility to be detected by its intensive yellow color (curcumin is also used as a food colorant) and a specific absorption at 425 nm.

Three models describing the distribution of the active molecule inside the nanoparticles are reported in the literature, i) homogeneous matrix model, ii) core-shell model, drug-enriched shell and iii) core-shell model, drug-enriched core [6, 9]. Obviously, the encapsulation efficiency (EE)

and the drug release depend on its distribution in the particle [17]. Controlling the nanoparticles properties should allow a better control of the drug encapsulation specificities. Mixture design methodology is a powerful technique to assess the nanoparticles properties according to the system composition. Indeed, it allows the determination of the influence of the system properties on several physico-chemical parameters using a minimum number of experiments. This methodology has been already used to study the effect of several parameters onto the properties of SLN prepared by emulsification-solvent evaporation or solvent diffusion methods [18, 19]. Solvent: lipid ratio, lipid concentration and temperature seem to be the key parameters to control the particles size.

In the present work, mixture design methodology has been used to control shea butter nanoparticles size prepared without organic solvent as a function of the ternary system composition (lipid, water, and nonionic surfactant fractions). Curcumin has been incorporated in different particles of different sizes to evidence the influence of the nanoparticles diameter on EE. The aim of this study was to obtain nanoparticles of different sizes from identical lipid contents to determine how the curcumin loading is influenced by the SLN size. Moreover, the effect of the encapsulated molecule on the structure of the lipid matrix is discussed from DSC and XRD experiments at low and wide angles and shed some light on its localization inside the nanoparticle.

2. Materials and methods

2.1.Materials

Shea butter was purchased from IeS Labo (Oraison, France). Main fatty acids were stearic (42.3 wt.%) and oleic (43.9 wt.%) acids confirmed by gas chromatography experiments (data not shown). Its melting point was determined from DSC measurements at 37 °C. Tween 80, Span 80 and curcumin were purchased from Sigma-Aldrich.

2.2.Methods

2.a. Shea butter nanoparticles preparation

The ternary system was composed of shea butter as lipophilic phase, a mixture of Tween 80 (hydrophilic surfactant) and Span 80 (lipophilic surfactant) (mass ratio 90/10) as emulsifiers and ultrapure water in several proportions (Table 1). First, the lipid was melted at 40 °C and mixed

with Span 80. Tween 80 was solubilized in ultrapure water at the same temperature under stirring (500 rpm) until the solution became transparent (micellar solution). The lipid phase containing Span 80 was then added dropwise to the micellar solution of Tween 80 at 40 °C under stirring at 500 rpm. The obtained pre-emulsion was homogenized using Ultra-Turrax system (IKA T25) during 5 min at 10,000 rpm and then sonicated in pulse mode (1s on, 1s off) using Vibracell 75115 from Bioblock Scientific at 40% of the maximal power during 6 min. The sample temperature was maintained at 40 °C all along the procedure. The obtained nanoemulsion was then cooled down slowly at 20 °C for the crystallization process and stored at 4 °C.

2.b. Mixture design methodology

Mixture Design Of Experiments (MDOE) is a methodology used to determine the optimum combination of constituents by using response surface methodology (RSM). Scheffé simplex centroid design was used with a three-component mixture, with lower and upper limit restrictions and test points. The studied variables were: shea butter (from 5 to 40 wt.%), water (from 48 to 92 t.%) and surfactant (from 1.5 to 19 wt.%). The nanoparticle hydrodynamic diameters and the polydispersity index of the dispersion were optimized using the NEMROD software (New Efficient Methodology for Research using Optimal Design).[20] The ternary mixture component ratios are given in Table 1. Data from the nanoparticles characterizations were used to calculate the special cubic equation for three components (Equation 1):

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad \text{(Eq 1)}$$

where y was the studied response (nanoparticle hydrodynamic diameters and polydispersity index), β_{ijk} were regression parameters and x_1, x_2 and x_3 represented the levels of three constituents. The validity of the model was tested by addition of further test points, which were incorporated into the design for analysis. The coefficients of the reduced cubic model were fitted to the data by least squares multi-linear regression and the goodness of fit was investigated by analysis of variance (ANOVA).

In the mixture design, the effect of the change of variables on the responses can be observed on the ternary contour map.

2.c. Curcumin solubility in shea butter

The EE was related to the maximal solubility of curcumin in the lipid phase. This solubility was determined as follow. Aliquot of 5 mg of curcumin was added to the 5 mL of melted lipid at 40 °C and kept under stirring for 3 days protected from light [21]. The blend then was centrifuged 15 min at 12100 *x g* at 40 °C. The non-dissolved (crystalline) curcumin was removed and the supernatant was diluted with methanol for absorbance measurements at 425 nm (UV-Vis 1605 spectrophotometer, Shimadzu, Japan). In parallel, a calibration curve was realized from different solutions of curcumin in methanol. The amount of curcumin solubilized in shea butter was calculated from the calibration curve equation. A sample without curcumin is used as blank to perform measurements. Experiments were done in triplicate.

2.d. Curcumin encapsulation into shea butter nanoparticles

Concerning the curcumin encapsulation, the procedure was similar to the nanoparticles preparation one despite the fact that the curcumin was added to the lipid phase (shea butter + Span 80). The curcumin quantity was calculated from its maximal solubility in the lipid phase and the mass of shea butter used for the formulation. The weight of curcumin could vary according to the system composition and was related to the shea butter content. The EE of the curcumin (see below) was calculated from its solubility in the lipid phase. Therefore, the results obtained with different compositions could be compared. After the preparation (homogenization by Ultra-Turrax and sonication), cooled samples (left enough time, more than one night, at room temperature to allow lipid crystallization confirmed by XRD experiments) were centrifuged at 3000 *x g* at room temperature during 5 min to precipitate free curcumin. The supernatant was then centrifuged in Centrisart ultrafilters (100 kDa molecular weight cut of, Sartorius) to remove remaining micelles which can incorporate a little amount of curcumin at 1400 *x g* during 20 min. The recovered SLN dispersion was diluted in methanol for absorbance analysis according to the calibration curve. The amount of encapsulated curcumin was calculated from the calibration curve (see section 2.c).

The curcumin amount used for encapsulation experiments was calculated from the weight of shea butter used and its solubility in the lipid phase. It corresponded to the maximal quantity of curcumin which could be dissolved in the lipid. Then, the EE was determined with the Equation 2.

$$EE(\%) = \frac{\text{encapsulated amount of curcumin } (mg.g^{-1})}{\text{solubility of curcumin in shea butter } (mg.g^{-1})} * 100 \qquad \text{(Eq.2)}$$

Therefore, an EE of 100% meant that the curcumin maximal solubility in the lipid was reached in the nanoparticles and that all the molecules remained in the particles after lipid solidification.

Even, if both compositions and curcumin weight were different, it was possible to compare the obtained EE as a function of the nanoparticles size.

2.e. Nanoparticles size analysis

The first part of the study consisted to control the size distribution of the lipid nanoparticles as a function of the system composition. The mean diameter (d.nm) and the polydispersity index (PdI) were measured at 20 °C by Dynamic Light Scattering using a Zetasizer Nano ZS (green laser at 532 nm) from Malvern Instruments analyzed by the CONTIN algorithm. Samples were diluted 100 times before the analysis to avoid detector saturation. The presented nanoparticles diameters came from the intensity weighing distribution which was the most accurate to describe the system. The presence of residual micelles in the SLN dispersion could indeed lower the z-average value obtained from DLS measurements which was then not representative of the SLN size. Measures were performed in triplicate.

2.f. Differential Scanning Calorimetry (DSC)

Calorimetric measurements were performed using a DSC1 differential scanning calorimeter equipped with a high sensitivity HSS8 probe from Mettler-Toledo. (Low temperature studies were achieved using a liquid nitrogen cooling system that allows reaching 123 K in the sample holder.) About 10 mg of powder was accurately weighted for each sample and sealed in aluminum pans with a mechanical crimp and at a constant scan rate of 5 $K.min^{-1}$. The temperature and energy were calibrating using indium, zinc, and mercury as references.

2.g. X-Ray diffraction analysis (XRD)

The XRD measurements were performed using a Panalytical X'Pert Pro diffractometer equipped with a Cu tube, a Ge (111) incident-beam monochromator (α = 1.5406 Å) and an X'Celerator detector. WAXS measurements were collected using 0.02 rad Soller slits, programmable divergence and anti-scatter slits, the irradiated area was fixed to 10mm $\times$ 10mm. The X'Celerator detector was used as "scanning line detector (1D)" with 2.122° active length. Data collection was carried out in the scattering angle range 2–30° with a 0.0167° step over 90 min. SAXS measurements were collected using 0.02 rad Soller slits, 1/16° fixed divergence and anti-scatter slits. The X'Celerator detector was used as "scanning line detector (1D)" with 0.518° active length. Data collection was carried out in the scattering angle range 0.8°–12° with a 0.0167° step over 90 min. Since the diffractometer is based on Bragg-Brentano θ-2θ geometry, the sample holder moves around θ causing any liquid sample to slip off the sample holder. As a consequence the

liquid SLN samples were filled into the X-ray holder, and covered with a thin aluminium foil. The aluminium foils presents diffraction peaks at higher angles, and causes a small absorption of the X-Ray intensity.

3. Results and discussion

3.1. Influence of the ternary system composition on shea butter nanoparticles size distribution from mixture design methodology

The mixture design methodology was applied to study the influence of the formulation composition on the shea butter nanoparticles diameter and polydispersity. The experimental area was limited to a domain defined by: 5-40 wt.% of shea butter ; 48-92 wt.% of water and 1.5-19 wt.% of surfactant.

SLN diameter (from the intensity weighing size distribution) and polydispersity index obtained from 23 experiments are presented in Table 1. Particles size distributions obtained from dynamic light scattering measurements of two different systems are presented on Fig. 1.A. Only the formulation composition varied between both samples. The SLN diameter varied from 70 nm to more than 1 µm over the 23 experiments. Fig. 1.B and C present the SLN sizes and related polydispersity index (PdI) responses according to the formulation composition. From a qualitative point of view, the size evolution upon the system composition was consistent with the expected results since smaller particles were obtained for low shea butter content and high surfactant concentrations and vice versa. Increasing the shea butter proportion or lowering the surfactant amount led to an increase of the SLN diameter. According to these results, the nanoparticle size was controlled by the amount of surfactant which could stabilize the interface between the water and the lipid phases. Higher the surfactant concentration, greater the interface, and so smaller the particles for a given lipid fraction. Likewise, for an identical surfactant content, the increase of the shea butter fraction led to bigger particles. Knowing the surface per polar head of the surfactant (60 $Å^2$ for Tween 80 from surface tension measurements) allowed to estimating the nanoparticle size as a function of the lipid and surfactant contents from a simple calculation. The polydispersity index (PdI) varied from 0.12 to 0.80 (Table 1). Nanoparticles dispersions were considered as monodispersed since PdI is lower than 0.2. The less polydispersed systems were recovered in the center of the studied domain (Fig. 1.C.).

The mixture design methodology allowed modeling the influence of the formulation composition on the studied responses (size and PdI). Then, it was possible to predict the SLN sizes as a function of the composition in the identical experimental range. The model depended on coefficients related to the fraction of each constituent and also from the interactions between them. The mixture design of experiments led to the following equations:

$$\text{Size (nm)} = -4090.x_{Sb} - 46^{*}.x_{w} + 7263.x_{s} + 9824.x_{Sb}.x_{w} + 22834.x_{Sb}.x_{s} - 8078.x_{w}.x_{s} - 65365.x_{Sb}.x_{w}.x_{s}$$

$R^2 = 0.86$ (Eq.3)

$$\text{PdI} = 2.65^{*}.x_{Sb} + 0.35^{***}.x_{w} + 10.85^{*}.x_{s} - 2.22.x_{Sb}.x_{w} - 22.69.x_{Sb}.x_{s} - 13.41.x_{w}.x_{s} + 1.49.x_{Sb}.x_{w}.x_{s}$$

$R^2 = 0.90$ (Eq.4)

This model enabled an easy preparation of shea butter nanoparticles of different diameters in order to study the influence of the SLN size on the curcumin encapsulation efficiency.

3.2.Curcumin solubility in shea butter

In order to calculate the curcumin EE in shea butter nanoparticles, it was necessary in a first step to determine its solubility in the lipid which corresponded to the maximum amount that could be incorporated in the nanoparticles. From absorbance measurements, the curcumin solubility in the lipid matrix at 40 °C (liquid lipid) was determined to 0.33 $mg.g^{-1}$ of shea butter (0.33 wt.%). The curcumin solubility was related to the averaged molecular weight of the lipid [16]. Short chain triglycerides favored curcumin solubility thanks to interactions between polar groups of both components, since short chain compounds had more polar groups per unit mass than long chain triglycerides. The value determined in this study was close to the one reported for corn oil (long chain triglycerides) of 0.30 $mg.g^{-1}$ of lipid by Ahmed *et al*.[16] Curcumin solubility was determined at 40 °C to evaluate the amount of curcumin remaining into the SLN after the lipid crystallization step.

3.3.Curcumin Encapsulation in SLN

From the mixture design experiments, several compositions leading to different nanoparticles sizes from 50 to 200 nm were chosen in order to evidence the effect of the nanoparticles diameter on the curcumin encapsulation efficiency. The EE was measured after the solidification of lipids. It then corresponded to the curcumin amount which remained in the lipid phase after emulsification and crystallization (see section 2.d). The biggest size was limited to 200 nm to focus the study on narrow particles population. Indeed, from the first set of experiments, the PdI increased upon the particles sizes (Table 1). The polyphenol mass added to the system was calculated from its

solubility in the lipid at 40 °C and from the mass of shea butter used. This way enabled to compare the influence of the nanoparticle size on the drug loading independently from the lipid concentration. The results obtained from these additional experiments are summarized in Table 2. Firstly, the measured SLN diameters were closed to the predicted ones (errors around 15%) except for one experiment. These additional preparations confirmed the numerical model coming from the mixture design part. Concerning the PdI, some differences between predicted and measured values were also important for several experiments but the PdI values remain lower or close to 0.2. Moreover, the SLN preparations were performed with and without curcumin. No significant differences were reported in terms of size and polydispersity for particles with and without the polyphenol. The presence of curcumin in the lipid did not modify the SLN size distribution whatever the nanoparticles diameter. The second and main result reported in Table 2 was the evolution of the curcumin encapsulation efficiency upon nanoparticles sizes. It decreased from 89 to 27 % when the nanoparticles size increased from 46 to 230 nm (Fig. 2.A). It seemed that greater the particles, lower the EE. Moreover, a significant result was that SLNs of different sizes prepared from identical shea butter contents but different surfactant and water fractions (comparison between experiments 25 and 26, or 27, 28 and 29 on Table 2) led to different values of EE. This meant that the present amount of curcumin in the SLN only depended on the nanoparticles sizes. The evolution of EE appeared to be linear upon SLN size (line on Fig. 2.A) in the studied nanoparticle diameters range according to the following equation:

EE (%) = -0.3743*nanoparticles diameter + 106 $R^2 = 0.93$ (Eq.5)

It was then possible to deduce the EE directly from the system composition according to Eq.3 (size upon formulation composition) and Eq.5.

Three models are reported in the literature concerning the localization of a guest molecule in solid lipid nanoparticles, i) homogeneous matrix, ii) shell-enriched and iii) core-enriched [9]. Depending on these models, the evolution of the encapsulation efficiency upon the nanoparticles diameter may vary differently. If the guest molecule is homogeneously distributed in the lipid matrix, the EE should not depend on the size of the nanoparticles. If the active compound is localized in a lipid shell around a pure lipid core, smaller the particles are, higher the EE should be since the surface of the particles increases when the diameter decreases for a constant mass. On the opposite, in the core-enriched model, bigger the particles, higher the EE. If curcumin is located in the corona, higher the specific surface area is, higher the amount of curcumin should be. For spherical

particles, the specific surface area is directly proportional to the inverse of the diameter. The evolution of the EE over the estimated SLN specific surface area is presented on Fig. 2.B.

Interactions between the curcumin polar groups and the hydrophilic part of the surfactants molecules could favor the presence of the polyphenol on the outer shell of the lipid nanoparticles and explain why the EE was more important when the surface area increased. To support the hypothesis that curcumin was mainly present in a corona at the particle edge and not in the SLN core, DSC and XRD experiments were performed to study the lipid structure and the effect of the polyphenol incorporation on it.

3.4.DSC analysis

The curcumin influence on the melting properties of shea butter at non-dispersed state and under SLN form was studied. Corresponding thermograms are presented on Fig. 3. At non dispersed state, both samples without and with curcumin presented an endothermic peak centered at 37 °C (Fig. 5.A). Under SLN form, this melting signal is shifted towards lower temperature with a maximum at 33 °C (Fig. 5.B). This lowering of the lipid melting point at nanodispersed state has been already reported in the literature [22]. This can be explained by the colloidal nature of the lipid. A different crystalline structure of the solid lipid can also explain this difference. Nevertheless, the main information coming from the DSC experiment was that the addition of the polyphenol did not modify the lipid melting properties whatever the lipid state, non-dispersed or nanodispersed.

3.5.X-ray diffraction analysis

The influence of the polyphenol on the lipid organization at long (longitudinal packing) and short distances (crystalline structure) (Fig. 4.A and B) was studied at both non-dispersed and nanodispersed state by X-ray diffraction (XRD). XRD diffractograms recorded at low angles for samples at non dispersed state are presented in Fig. 4.C. This diffraction angle range ($1° < 2\theta < 12°$) allowed the determination of the lipid bilayer structure (longitudinal packing) [23]. Two main configurations are reported in the literature depending on the repetition patterns of the lamellar structure and refers to the number of carbon chains which define the subcell ; *i.e* double-chain length (2L) and triple-chain length (3L) (Fig. 4.A). The chain packing is deduced from the repetition distance corresponding to the first reflections observed at small angle (double-chain length 2L: 40 – 45 Å and triple-chain length 3L: ~ 60 Å) [24]. These configurations depended on the fatty acid composition of the lipid mixture. Double chain length structures were indeed formed mainly by

saturated fatty acids while the presence of an unsaturation in the carbon chain could lead to the formation of triple chain bilayers (Fig. 4.A). Both 3L and 2L structures were observed for shea butter with specific diffraction lines at 64 ($3L_{001}$) and 45 Å ($2L_{001}$), respectively (Fig. 4.C). Nevertheless, the main conformation was the triple chain length one with at least 7 reflections. The peak at 64 Å corresponding to the first order of a 3L structure ($3L_{001}$) was indeed followed by 6 reflection lines around 32, 21, 16, 12.8, 10.7 and 8 Å were related to the second ($3L_{002}$), third ($3L_{003}$), fourth ($3L_{004}$), fifth ($3L_{005}$), sixth ($3L_{006}$) and eighth ($3L_{008}$) orders, respectively. Double-chain packing was also reported form the peaks located at 45, 21 and 15 Å which were related to the first ($2L_{001}$), second ($2L_{002}$ – superimposed with the $3L_{003}$) and third order ($2L_{003}$) of a double chain length conformation. The presence of these two coexisting phases has been already reported for milk fat [23]. The lowest number of reflections and intensities of these diffraction lines meant that the amount of 2L conformation was lower than 3L in the sample. The formation of 2L and 3L packing was consistent with the composition of shea butter because the main fatty acids were stearic and oleic acids. Diffraction lines detected at wide angles ($15° < 2\theta < 30°$) (Fig. 4.D) were related to the lateral packing (or cross-sectional packing) of the alkyl chains of acylglycerols in characteristic subcells (hexagonal α, orthorhombic perpendicular β' and triclinic parallel β forms) [25–27]. The α form led to one peak located at q values around 21° (4.2 Å). Characteristic β' patterns present three peaks at 20.5° (4.3 Å), 21.5° (4.1 Å) and 23.2° (3.8 Å) [28]. In this study, the presence of 8 diffraction peaks (shown in Fig. 4.E and reported Table 3) revealed that shea butter was crystallized into a β polymorph as reported for cocoa butter (Table 3) [28]. More precisely, the shea butter pattern was analogous (peak positions and intensities) to the one corresponding to the form V of cocoa butter (Table 3) [28–30].

The lipid crystalline structure after curcumin encapsulation inside the nanoparticles can bring informations on the localization of the polyphenol in the lipid matrix (shift of the repetition distances, modification of the peak intensities, different crystal structures, etc.) The curcumin influence on the lipid structure was firstly studied at non-dispersed state without water and surfactant at low angles (Fig. 4.C and D). Thus, the structures of pure shea butter and shea butter with curcumin at its maximum solubility (0.33 $mg.g^{-1}$, see previous section) were studied. The presence of the curcumin inside the lipid matrix did not strongly modify at first sight the diffraction signal. Similar diffractograms were indeed recorded for sample with and without curcumin (Fig. 4.C). Curcumin did not affect thus significantly the longitudinal chain packing of the lipid (2L and 3L longitudinal packings). In order to go further on the characterization of the lipid

longitudinal conformation and to obtain more accurate information, small angles XRD experiments ($1° < 2\theta < 4°$) were performed on the same systems (Fig. 4.D). Only the three first diffraction peaks were observed in this 2θ range corresponding to the $3L_{001}$ $2L_{001}$ and $3L_{002}$ reflections. A closer look on the peak positions revealed that curcumin addition to the lipid led to a shift towards lower angles corresponding to higher repetition distances. $3L_{001}$ reflection was for example shifted from 63.8 Å to 66.1 Å after curcumin addition. This could be related to the presence of the polyphenol between the carbon chains in the lamellar structure leading to an increase of the repetition distance. This increase (2.3 Å for the 3L structure) did not correspond to the curcumin size (close to 16 Å long and 4-5 Å wide) [31] but the repetition distances determined by XRD corresponded to an average of the lipid structure. A lower shift towards lower angles was also reported for the double chain length structure. Another difference between the two systems was recorded at low angle (Fig. 4.C) was the peak relative intensities and particularly between $3L_{001}$ and $2L_{001}$ reflections. This could be related to a difference of contrast (modification of the electronic densities) induced by the presence of the curcumin in the lipid structure.

The effect of the polyphenol addition on the crystalline structure of shea butter was also studied at wide angles as shown on Fig. 4.E. Curcumin did not modify the crystalline structure and the β phase was preserved. The only difference between samples with and without curcumin was that the presence of the polyphenol led to broader and less intense peaks. This could be related to a decrease of the crystalline domains size due to the presence of curcumin between them. The peak width depends indeed on the size of the crystallized domain according to the Scherrer's law [32];

$$D = K\lambda / B\ cos\theta \qquad \text{(Eq.6)}$$

With D: size of the crystalline domain (Å), K: Scherrer's constant depending on the geometry of the crystal (0.9 if the shape is not known), λ: X-rays wavelength (1.5418 Å), B: full width at half maximum in °, and θ: semi-angle of diffraction related to the peak position in °.

Thus, thinner the diffraction peak, larger the domain. From the XRD data recorded for non-dispersed samples, it seemed that curcumin was incorporated in the lipid structure. It is also important to notice here that curcumin was not under its crystal form and was really solubilized/dispersed inside the lipid matrix because no additional peaks were reported compares to pure Shea butter. Curcumin XRD pattern indeed showed a lot of diffraction peaks from 10 to 30° (data not shown).

X-ray diffraction experiments was also performed at the nanodispersed state (SLN) with and without curcumin encapsulated in the lipid matrix. The comparison of the XRD patterns at low and

wide angles of pure non dispersed shea butter (from Fig. 4.) and at nanodispersed state is presented in Fig. 5. without and with curcumin. At low angle (Fig. 5.A), the more intense reflections (3L packing) reported at non-disperse state were present under SLN form. The lower intensity and the peak broadening were due to the lower amount of lipid in the sample dispersed in water and/or to a lower crystal quality [33]. No diffraction lines corresponding to the 2L structure were observed but this could be related to the low intensities of the corresponding peaks. No shift of the peak position after curcumin encapsulation was reported at low angle unlike at non-dispersed state. It seemed that curcumin was not incorporated in the lipid matrix as at non-dispersed state. At wide angles (Fig. 5.B), the XRD signature of the lipid lateral packing was strongly different at nanodispersed state than in bulk. The more intense peak of the β phase recorded previously was not present in the patterns and only the reflections at 3.97, 3.87 and 3.69 Å could be related to a β polymorph but with unusual relative intensities. To the best of our knowledge, no analogous diffractograms are reported in the literature. The unusual intensities can be explained by the influence of the nanoparticles form factor which may present a minimum in this 2θ range leading to a disappearance of the peak at 19.2° and the increase of the intensity of the one at 24.0°. Nevertheless, the main information brought by these experiments was that both diffractograms without and with curcumin are identical. From the obtained results, the addition of curcumin did not affect the lipid structure at nanodispered state unlike at non-dispersed state. XRD data were thus consistent with the presence of curcumin in the outer shell of the nanoparticles since not difference was reported for sample with and without curcumin. The polyphenol was then not closely included in the lipid matrix and might interact with the surfactants molecules. This kind of interaction was reported in a study focused on the effect of curcumin on phospholipids bilayer properties. Authors reported that curcumin polar groups such as hydroxyl groups might interact with the polar headgroup region of the lipid [31]. The curcumin localization inside the nanoparticles was of main importance since it influenced the kind of release. In this case, shea butter nanoparticles favored a burst release of curcumin.

4. Conclusions

Shea butter solid lipid nanoparticles stabilized by non-ionic surfactants were prepared without the use of organic solvent. The evolution of the nanoparticles size upon system composition was determined using the mixture design methodology. The obtained mathematical models allowed

the preparation of SLN of a required size from the system composition. Curcumin, a natural polyphenol, was encapsulated in nanoparticles of several sizes. The encapsulation efficiency was related to the SLN diameter. Bigger the particles, lower the amount of encapsulated curcumin. From this result, it seemed that the polyphenol was located in the corona of the lipid particle. XRD experiments evidenced that the lipid structure was slightly modified by the curcumin addition at non dispersed state while no differences were detected at SLN state. This was consistent with the fact that the polyphenol was not closely incorporated in the lipid matrix but interacted with the surfactants molecules at the nanoparticles surface which explain the linear decrease of the encapsulation efficiency upon SLN diameter. It was then possible to predict the EE upon the nanoparticles sizes and directly, using the models coming from the mixture design methodology, from the ternary system composition.

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Tables

Table 1: Mixture design plan of 23 shea butter nanoparticles formulations

N°.exp.	Shea butter fraction	Water fraction	Surfactant fraction	Diameter* (nm)	Polydispersity Index (PdI)
1	0.05	0.76	0.190	84	0,21
2	0.40	0.58	0.015	408	0,48
3	0.05	0.92	0.030	150	0,23
4	0.06	0.92	0.015	305	0,31
5	0.40	0.48	0.120	304	0,26
6	0.33	0.48	0.190	208	0,15
7	0.05	0.84	0.110	72	0,22
8	0.19	0.62	0.190	265	0,26
9	0.23	0.75	0.015	301	0,26
10	0.40	0.53	0.067	1094	0,80
11	0.05	0.92	0.022	1624	0,60
12	0.36	0.48	0.150	201	0,16
13	0.21	0.69	0.093	871	0,50
14	0.13	0.72	0.140	470	0,42
15	0.30	0.63	0.054	316	0,40
16	0.13	0.8	0.061	214	0,20
17	0.14	0.8	0.050	193	0,18
18	0.30	0.58	0.100	170	0,18
19	0.27	0.58	0.140	419	0,19
20	0.10	0.87	0.020	189	0,17
21	0.20	0.76	0.017	194	0,27
22	0.30	0.68	0.038	203	0,26
23	0.40	0.57	0.028	204	0,35

* SLN diameter is expressed in intensity

Table 2: Compositions, predicted and experimental SLN sizes and PdI with and without curcumin and related curcumin encapsulation efficiency.

N°.exp	SLN composition			Mass of curcumin (mg)	Predicted SLN size (nm)	Experimental SLN size (nm)	Error on size (%)	Predicted PdI	Experimental PdI	Error on PdI (%)	Encapsulation Efficiency (%)
	Shea butter content (wt.%)	Surfactant content (wt.%)	Water content (wt.%)								
24	4.2	7.6	88.2	- **0.5**	50 **-**	44 ± 2 **46 ± 1**	13.6 -	0.18 -	0.21 0.20	16.7 -	- **89.0 ± 5.0**
25	4.7	1.0	94.3	- **0.6**	180 **-**	180 ± 6 **170 ± 4**	0.0 -	0.22 -	0.15 0.14	31.8 -	**-** **30.6 ± 4.3**
26	4.7	4.8	90.5	- **0.6**	100 **-**	91 ± 2 **104 ± 3**	9.9 -	0.19 -	0.20 0.20	5.3 -	**-** **72.8 ± 6.8**
27	7.6	6.4	86.0	- **1.0**	100 **-**	113 ± 3 **116 ± 3**	11.5 -	0.18 -	0.22 0.21	22.2 -	**-** **61.0 ± 5.1**
28	7.6	9.3	83.1	- **1.0**	100 **-**	86 ± 1 **91 ± 2**	16.3 -	0.18 -	0.22 0.23	22.2 -	**-** **68.0 ± 1.4**
29	7.6	4.3	88.1	- **1.0**	200 **-**	136 ± 4 **141 ± 2**	47.1 -	0.22 -	0.18 0.19	18.2 -	**-** **56.2 ± 0.3**
30	13.7	24.1	62.2	- **1.8**	200 **-**	230 ± 5 **223 ± 5**	13 -	0.18 -	0.19 0.18	5.6 -	**-** **27 ± 4**

Table 3: Wide angle reflections measured for shea butter compared to cocoa butter β polymorph (form V) from ref [28]

2θ (°)	Shea butter Short spacing (Å)	Cocoa butter Short spacing (Å)	Intensity
16.33	5.41	5.40	m
17.29	5.11	5.15	w
19.25	4.59	4.58	vs
-	-	4.23	vvw
22.10	4.00	3.98	s
22.71	3.89	3.87	w
23.43	3.77	3.75	s
24.00	3.68	3.67	s
26.28	3.39	3.39	vw

vvw: very very weak ; vw: very weak ; w: weak ; m: medium ; s: strong ; vs: very strong

Figure legends

Figure 1: (A) Size distribution of two nanoparticles (experiments 7 (dotted line) and 9 (full line)), (B) experimental range and variation of the particles hydrodynamic diameter (C) and Polydispersity Index (PdI) (D) responses as a function of the formulation composition.

Figure 2: Evolution of the curcumin encapsulation efficiency upon (A) nanoparticles diameter and (B) calculated specific surface area. Experiments have been done in triplicate.

Figure 3 : DSC thermograms of shea butter without (continuous line) and with curcumin (dashed line) at (A) non dispersed state and (B) at SLN state. Curcumin content corresponded to its maximal solubility in the lipid.

Figure 4: Representations of the solid lipid organization: (A) at long distances (longitudinal packing), double chain and triple chain length configurations detected at small angles and (B) at short distances, crystalline structure detected at high angles (top and longitudinal view of a β triclinic phase). XRD patterns of Shea butter without (bottom line) and with curcumin (top line) at non dispersed state recorded at small (C and D) and (E) high angles.

Figure 5 : (A) Low and (B) wide angles diffraction patterns of Shea butter at non dispersed state (bottom line) at SLN state (middle line) and SLN state with curcumin (top line). Experimental q-range is different for SLN samples due to a specific configuration of the apparatus (see materials and methods section 2.g)

Figure 1

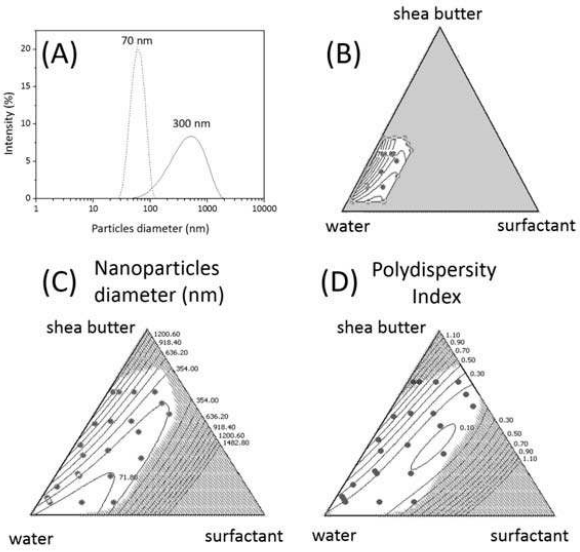

(A)
70 nm
300 nm
Intensity (%)
Particles diameter (nm)
(B)
shea butter
water
surfactant
(C) Nanoparticles diameter (nm)
shea butter
water
surfactant
(D) Polydispersity Index
shea butter
water
surfactant

Figure 2

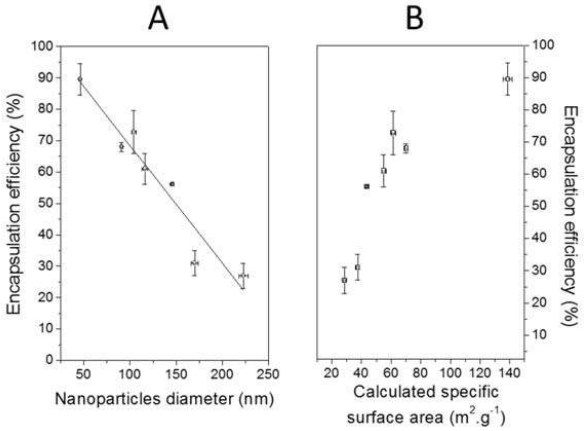

A
B
Encapsulation efficiency (%)
Nanoparticles diameter (nm)
Calculated specific surface area ($m^2.g^{-1}$)
Encapsulation efficiency (%)

Figure 3

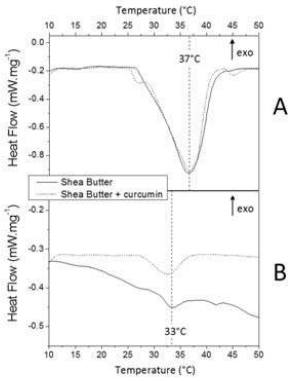

Temperature (°C)
Heat Flow (mW.mg-1)
37°C
exo
A
Shea Butter
Shea Butter + curcumin
exo
B
33°C
Temperature (°C)

Figure 4

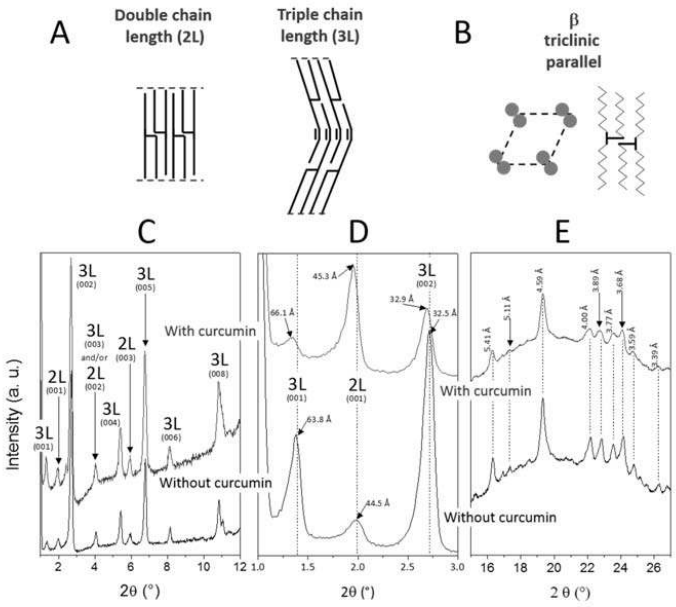
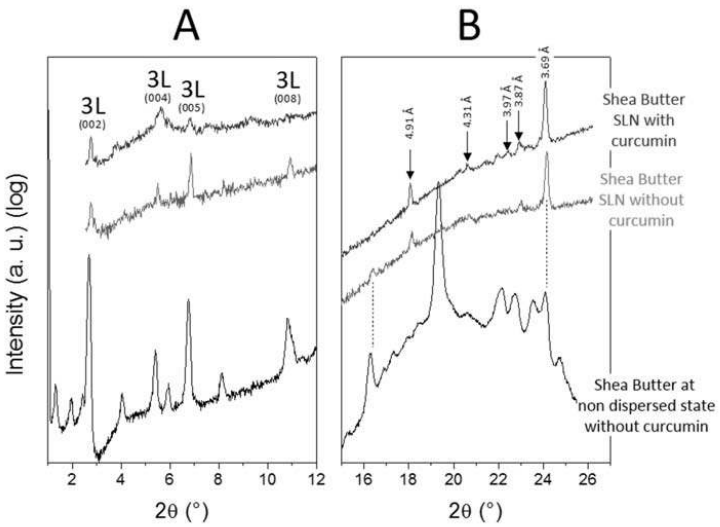

A
Double chain length (2L)
Triple chain length (3L)
B
β triclinic parallel
C
D
E
Intensity (a. u.)
With curcumin
Without curcumin
2θ (°)
2 θ (°)

Figure 5


A
B
3L (002)
3L (004)
3L (005)
3L (008)
4.91 Å
4.31 Å
3.97 Å
3.87 Å
3.69 Å
Shea Butter SLN with curcumin
Shea Butter SLN without curcumin
Shea Butter at non dispersed state without curcumin
Intensity (a. u.) (log)
2θ (°)
2θ (°)