

PROCESS PARAMETERS AND WORKING CONDITIONS FOR OBTAINING LIPID NANOSTRUCTURES BASED ON FISH OIL

Elena MITREA¹, Aurelia MEGHEA²

The paper aims to optimize the process parameters and working conditions for the synthesis of aqueous nanostructured lipid dispersions (NLC) based on fish oil, by using a mixture of lipids such as glyceryl monostearate, carnauba wax, fish oil and a mixture of surfactants such as Tween 80, soybean lecithin and poloxamer 407. Preliminary characterization was performed based on photon correlation spectroscopy (dynamic light scattering technique) to assess particle size distribution by determination average diameters and the polydispersity index. The experiments were finalized with optimizing the composition and the concentration of surfactants mixture and of the homogenization time of the precursor pre-emulsion of NLCs.

Keywords: nanostructured lipid dispersions, fish oil, parameter optimization

1. Introduction

Lipid based nanoparticles have been considered as appropriate systems for encapsulation and delivery of poorly soluble drugs in the pharmaceutical field [1]. Nanostructured lipid carriers (NLCs) are the latest generation of nanoscale encapsulation systems, developed by Muller since 2000 [2]. Many of the lipid nanoparticle compounds are physiologically accepted concerning their excipients status and have well-established safety profiles and toxicological data [3, 4]. Up to present, the application of lipid nanoparticles for active food ingredient delivery has been limited, although recent studies have reported the main features and advantages of the lipid nanoparticles for oral distribution of active food nutrients and their utility for the food sector [5, 6, 7].

As drug delivery nanosystems have been widely researched in the pharmaceutical field, more and more attentions were paid to use appropriate delivery systems in food industry. The nanoencapsulation of bio-active food compounds is an essential requisite for the production of functional food [8, 9,10].

Within the category of natural oils with significant biological benefits, the fish oils have a high content of essential fatty acids omega-3 and omega-6. These

¹ Phd, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Ch.eng., Biofarm S.A., Bucharest, Romania, e-mail: mitrea.elena@yahoo.com

² Prof., Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Romania, e-mail: a.meghea@gmail.com

organic acids are particularly important in the metabolism of some body systems such as cardiovascular, reproductive, immune and nervous system. Moreover, these unsaturated fatty acids have functional properties such as positive effects on blood lipids, cardio protective properties [11], rheumatoid arthritis and cancer prevention [12, 13].

The quality and effectiveness of lipid nanoparticles are influenced by the surfactant properties and mainly by their concentration, particle size, parameters of preparation process, crystallinity, dispersion stability [14, 15]. It is already known that the mixtures of surfactants reduce the interfacial tension in a higher extent than one surfactant formulations, resulting in the formation of smaller and steadier particles.

Therefore, for the purpose of this research a mixture of surfactants has been used: Tween 80 (the main surfactant, nonionic, polyoxyethylenesorbitan monooleate), soybean lecithin (as an additional ionic surfactant) and poloxamer 407 (co-surfactant, Polyethylene and polypropylene glycol, block copolymer).

For the lipid core formation of NLCs, a mixture of solid lipids has been used: glyceryl monostearate (GS) and carnauba wax (CW), given the fact that a highly ordered crystal lattice can not accommodate large amounts of bio-active components; for the lipid liquid a natural lipid was chosen, that is fish oil (FO), essential fatty acids concentrated in omega-3 component. In all formulations, the lipid phase composition (10%) was made of: 70% blend solid fat (glyceryl monostearate, carnauba wax, the mass ratio GS : CW = 1:1) and 30% liquid lipid (fish oil - FO).

The aim of this study was to optimize the process parameters and working conditions in the synthesis of nanostructured lipid carriers that will be used for encapsulation of bio-active components.

In this context, in order to determine the optimal composition used in the preparation of aqueous dispersions, the following parameters are crucial: average diameter and polydispersity index of self-organized structures consisting in a mixture of a nonionic surfactant, an ionic surfactant and a co-surfactant in aqueous phase. Moreover, in order to establish the optimal concentration of the surfactant mixture, the time of the pre-emulsion mixing and the preparation techniques (high shear homogenization and sonication) for aqueous dispersions, the values of average particle diameter and polydispersity index of aqueous dispersions are highly important.

2. Materials

Polyoxyethylenesorbitan monooleate (Tween 80) was supplied by Merck (Germany); Poloxamer 407 (block copolymer of polyethylene and polypropylene glycol) was supplied by BASF Chem Trade GmbH (Germany), Soybean lecithin

from Cargill Texturizing Solutions Deutschland GmbH & Co. (Germany). The fish oil (FO) with a composition of minim 30% eicosapentanoic acid (EPA), minim 20% docosahexanoic acid (DHA) and a total amount of ω -3 fatty acids of 63% was supplied by Henry Lamotte Oils GmbH (Germany). Carnauba wax (CW) was obtained from Kahl Wachsraffinerie (Germany) and Glyceryl Stearate (GS) from Cognis GmbH.

3. Methods

3.1. Synthesis of Lipid Nanocarriers

The lipid nanocarriers were synthesized by using the melting emulsification coupled with the high shear homogenization technique [7, 16]. The experimental protocol follow the scheme presented in figure 1. Lipid phase composed of GS, CW and FO was heated by stirring at 85°C, to ensure a complete melting. The aqueous phase consisting of various compositions and various concentrations of surfactant mixture was also heated under stirring at 85°C. The surfactant mixture is composed of Tween 80 (as main surfactant), Soybean lecithin and Poloxamer 407.

The lipid phase was added into the aqueous phase and stirred at 85°C and has been homogenized. There have been two scenarios based on the homogenization time: 1h and 2h.

The hot emulsion resulted by mixing the lipid and aqueous phases at 85°C was exposed to an external mechanical energy by high shear homogenization with a Lab rotor-stator Homogenizer (High-Shear Homogenizer SC 250 type; 0~30.000 rpm; power of 250 W, PRO Scientific, USA), by applying 25 000 rpm for 10 minutes. The obtained emulsion was allowed to cooling down at room temperature, with formation of NLCs dispersions. NLCs aqueous dispersion was then subjected to ultrasonic technique for 9 minutes.

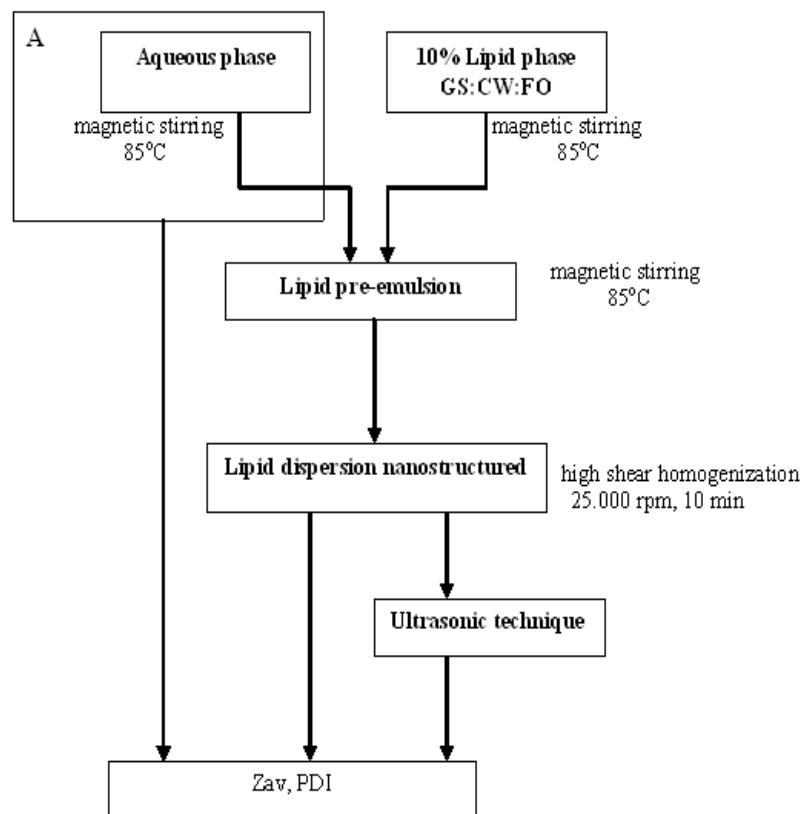


Fig. 1. Synthesis of Lipid Nanocarriers
A – Preparation aqueous phase

3.2. Determination of average diameters and polydispersity index

The particle size parameters of nanoparticles given by the hydrodynamic diameters, $Z_{average}$ and polydispersity index, PDI of each self-organized structures of surfactants or NLC dispersion were determined by using dynamic light scattering (DLS) technique (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.), at a scattering angle of 90° and a temperature of 25°C . Prior to the measurements, each dispersion was analyzed after appropriate dilution with deionised water to an adequate scattering intensity prior to the measurement. The particle size analysis data were evaluated using intensity distribution. The average diameters (based on Stokes-Einstein equation) and polydispersity index were calculated from the three individual measurements.

4. Results and discussion

4.1 Optimization of surfactants mixture composition

In order to establish the optimal composition of surfactant / co-surfactant mixture various synthetic experiments of the aqueous phase were conducted on different systems by modifying the contents of each surfactant / co-surfactant component (Table 1).

Table 1

Composition and concentration of surfactant mixture						
Composition	Surfactant			Concentration of surfactant mixture (% w/w) / Aqueous phases		
	Tween 80	Soybean lecithin	Poloxamer 407	3	4	5
C ₁	70	15	15	F ₁	-	-
C ₂	70	10	20	F ₂	-	-
C ₃	80	10	10	F ₃	-	-
C ₄	80	5	15	F ₄	F ₈	F ₁₁
C ₅	85	5	10	F ₅	F ₉	-
C ₆	90	2,5	7,5	F ₆	F ₁₀	-
C ₇	95	-	5	F ₇	-	-

Using these compositions of the surfactant mixture (C₁ ÷ C₇), different aqueous phases (F₁ ÷ F₇) were prepared with the same initial concentration of 3% surfactant mixture (figure 1 A).

The results obtained from DLS measurements for aqueous phases F₁ ÷ F₇ are shown in figure 2.

One can observe that by increasing the content of Tween 80 from 70 to 80% and decreasing the content of soybean lecithin and that of poloxamer 407 from 15% to 10%, the average diameter decreased from 524.8 nm (F₁/ C₁) to 296.6 nm (F₃/ C₃), but have the same polydispersity index PDI = 0.5;

For the same content of Tween 80 (80%) and soy lecithin content decreasing from 10% to 5%, while increasing poloxamer 407 content from 10% to 15%, the average diameter decreased significantly, from 296.6 nm (F₃/ C₃) to 91.73 nm (F₄/ C₄), but the polydispersity index remained the same (PDI = 0.5)

Moreover, by increasing the content of Tween 80 from 80 to 90% while soy lecithin content decreased from 5% to 2.5%, and that of poloxamer 407 from 15% to 7.5%, the average diameter decreased significantly from 91.73 nm (F₄/ C₄) to 8.19 nm (F₆/ C₆). Polydispersity index decreased to 0.177, which indicates a relatively narrow distribution of the population size of nanoparticles.

Based on the previous results, three optimal composition of the mixture of surfactants have been selected: C₄, C₅ and C₆, for which the average diameter is less than 100 nm. Therefore, in subsequent experiments these optimal composition of the surfactant mixture have been used:

- C₄ = 80% Tween 80, 5% soybean lecithin and 15% poloxamer 407
- C₅ = 85% Tween 80, 5% soybean lecithin and 10% poloxamer 407
- C₆ = 90% Tween 80, 2.5% soybean lecithin and 7.5% poloxamer 407.

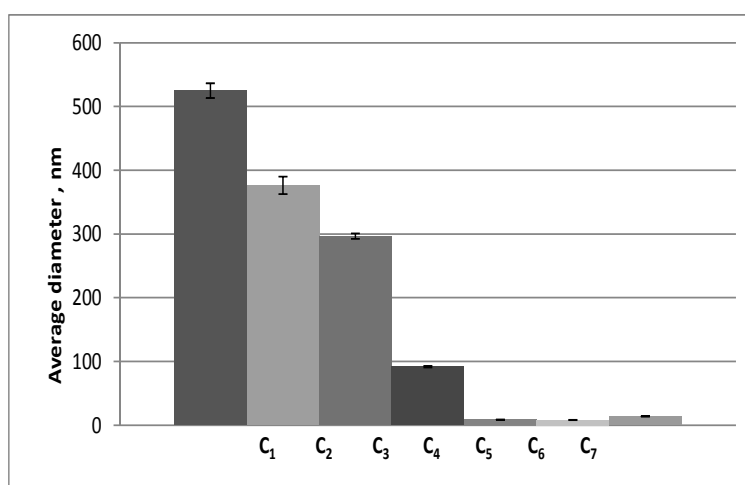


Fig. 2 Average diameter depending on the composition of the surfactant mixture

3.2. Establishing the optimal concentration of surfactant mixture in the aqueous phase

Three other aqueous phases (F₈ ÷ F₁₀) were prepared (table 1) by increasing the concentration of surfactant mixture up to 4% for the best three compositions for mixed surfactants: C₄, C₅, C₆.

Analyzing the measurement results for 4% concentration of surfactant mixture (aqueous phases F₈ ÷ F₁₀) (figure 3), it has been noticed that for the aqueous phase F₈ (4% C₄) particle size decreased to 8.7 nm, as compared to the aqueous phase F₄ (3% C₄, $Z_{av} = 91.7$ nm). For aqueous phase F₉ (4% C₅) the particle size increased to 56.2 nm, as compared to aqueous phase F₅ (3% C₅, $Z_{av} = 8.7$ nm) and for aqueous phase F₁₀ (4% C₆) the particle size increased to 15.36

nm, as compared to the aqueous phase F_6 (3% C_6 , $Z_{av} = 8.19$ nm). Polydispersity index showed the following values: 0.179 (F_8), 0.512 (F_9) and 0.473 (F_{10}).

Out of 4% mixed surfactant concentration in the aqueous phase, only C_4 variant was selected as the optimum composition of the surfactant mixture (aqueous phase F_8). Indeed, for C_4 spectacular values were obtained for both the average diameter of the particles and the polydispersity index.

As a result, the optimal composition mixture of surfactants was established as C_4 and therefore for this particular composition the concentration was increased from 4% to 5%, resulting the F_{11} aqueous phase.

The measurements of F_{11} aqueous phase (5% C_4) (figure 3) have shown an increase in particle size up to 11.8 nm, and compared with the aqueous phase F_8 (4% C_4), the particles had an average diameter of 8.7 nm; polydispersity index is 0.238.

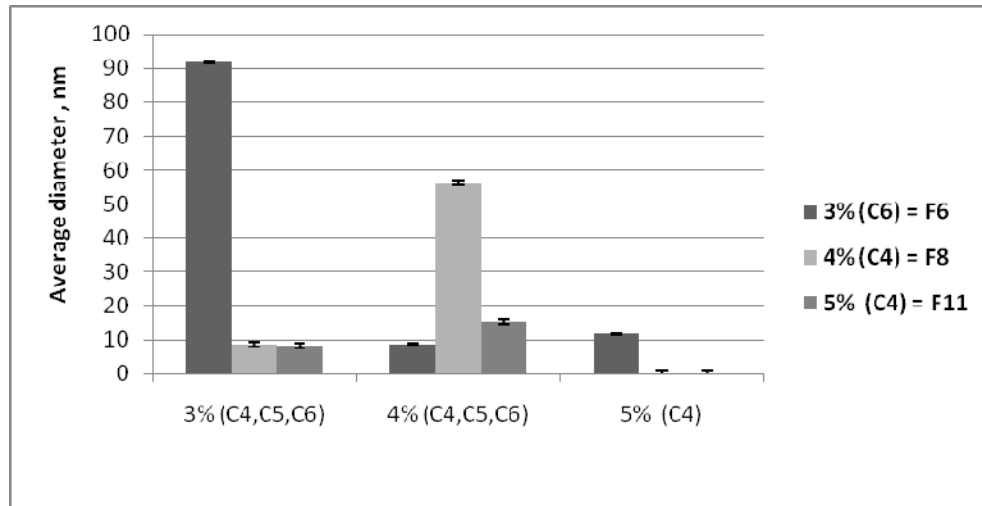
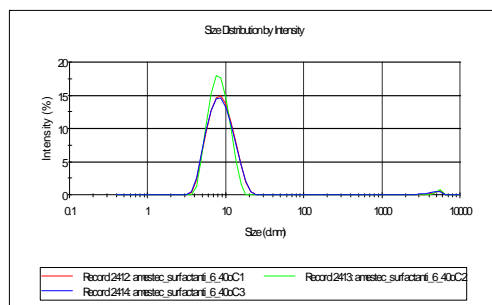
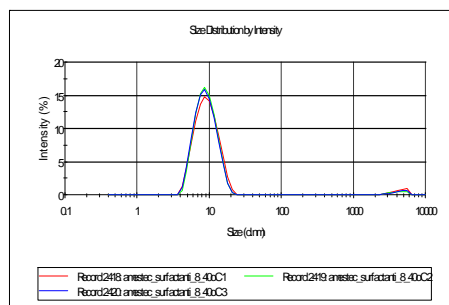


Fig. 3. Average diameter depending on the concentration of the surfactant mixture

The results obtained by dynamic light scattering technique showed that aqueous phase present a monomodal profile of size distribution, with mean particle size of 8.2 nm for F_6 (3% C_6) (figure 4) and 8.7 nm for F_8 (4% C_4) (figure 5).

Fig. 4 Particle size distribution of F₆Fig. 5 Particle size distribution of F₈

In the specialty literature [17] it is stated that for each lipid mixture used in the synthesis of nanoparticles the optimal concentration of the mixture of surfactants has to be determined.

Therefore, in order to determine the optimal concentration for the surfactant mixture used in the synthesis NLCs mixture of GS, CW, FO lipids, a number of three NLCs aqueous dispersions were prepared using three consecutive concentrations of the surfactant mixture (3, 4 and 5%), and for each concentration the optimal composition was used which having the lowest average particle diameter (C₆, C₄, C₄) (Table 2).

Table 2

Aqueous dispersion of NLCs			
NLCs*	Concentration of surfactant mixture (% w/w)	Composition of surfactant mixture	Aqueous phases
NLCs-1	3	C ₆	F ₆
NLCs-2	4	C ₄	F ₈
NLCs-3	5	C ₄	F ₁₁

*All NLCs formulations have been prepared with 10 % (w/w) lipids mixture, in a ratio 70% of GS:CW = 1:1 and 30% FO

The measurement results are shown in figure 6. By comparing the three particle size of NLCs aqueous dispersions it can be noticed that for NLCs-2 (4% surfactant mixture) and NLCs-3 (5% surfactant mixture) smaller particle size was obtained (below 250 nm), while for NLCs-1 (3% surfactant mixture) particle size is over 300 nm. Therefore, increasing the concentration of surfactant mixture over 4% does not affect the particle size ($Z_{av} = 243.9$ nm for NCLs-2 and $Z_{av} = 241.8$ nm for NCLs-3). Polydispersity index ranged from 0.227 (for NCLs-2), 0.238 (for NCLs-3) and 0.262 (for NCLs-1) indicating good homogeneity of dispersions.

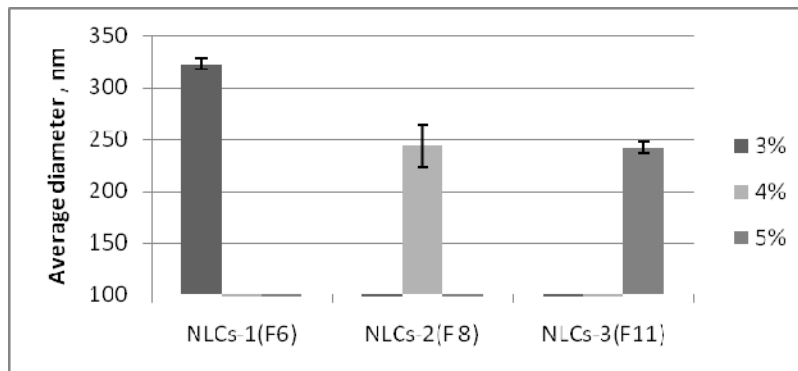


Fig. 6 Effect of surfactant mixture concentration on particle size aqueous dispersion

Based on these results, the 4% concentration for the surfactant mixture C₄ has been selected as the optimum concentration.

3.3. Establishing the optimum parameters for homogenization and ultra sonication time

This stage of the research was focused on identifying an optimal time to maintain pre-emulsion formed after contacting the two phases (aqueous and lipid) in thermal regime. For this purpose, two identical pre-emulsion were prepared (precursor of NLCs) that were kept for 1h and 2h respectively, at a temperature of 85°C, before being subjected to operation with high shear mixing. Results showed that by increasing the time to maintain the temperature from 1h to 2h, there was a slight increase in particle size of NLCs (from 243.9 nm to 274.1 nm) and in index polydispersity from 0.227 to 0.249 (figure 7).

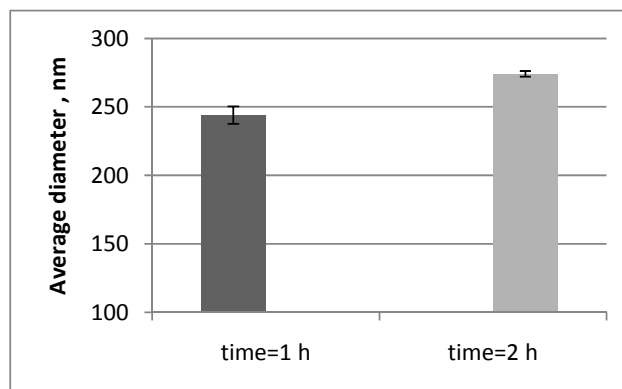


Fig. 7. Average particle diameter depending on the time of homogenization

Based on these results the best time for the homogenizing the pre-emulsion in thermal regime, precursor of NLCs, was set to one hour. Following later stages of optimization of the concentration and composition of the mixture of surfactants / co-surfactant and of time required to maintain the pre-emulsion precursor NLCs at thermal regime, there was a step that took into account the influence of ultra sonication time on the average diameter of NLCs. Thus, two working modes have been compared:

- 1) the melting emulsification coupled with the high shear homogenization technique (HSH) and
- 2) the melting emulsification coupled with the high shear homogenization technique (HSH) followed by ultrasonic technique (US).

Measurements (figure 8) revealed that NLCs aqueous dispersion prepared according to operating mode 1 resulted in average diameter value (Z_{av}) = 271.1 nm and polydispersity PDI = 0.239. NLCs aqueous dispersion was then subject to 9 minutes ultra sonication (according to the work mode 2). The particle size was measured after each 3 minutes of ultra sonication time as is illustrated in figure 8.

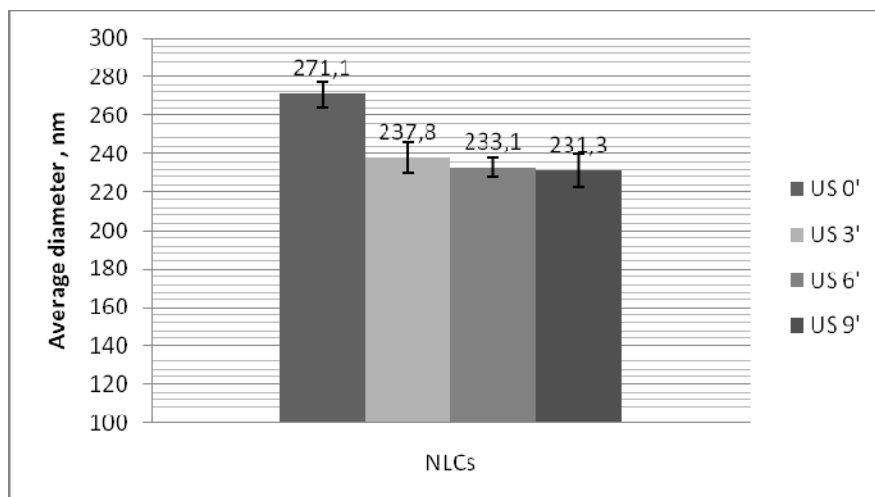


Fig. 8. Average particle diameter depending on the ultra sonication time

Analyzing the obtained results, it has been noticed that for the technique of high shear mixing with HSH followed by nine minutes of ultra sonication, the average diameter of NLCs aqueous dispersion decreased from 271 nm to 231 nm. It can be concluded that the influence of ultra sonication time on the average diameter is insignificant. Consequently, working mode no 1 has been chosen as optimal solution.

4. Conclusion

A systematic study has been performed on composition of surfactants and lipid components in order to establish the optimal conditions for obtaining lipid nanostructures based on fish oil as efficient nanocarriers for hydrophobic bioactive compounds.

As referring to surfactant mixture, by gradually increasing the relative content of Tween 80 from 70 to 90%, while tapering soybean lecithin content from 15 to 2.5% and poloxamer 407 from 15 to 7.5%, specific self-assembled structures of surfactants having average diameter less than 100 nm and a relatively narrow distribution of the nanoparticles population size (< 0.18) have been obtained.

Moreover, by increasing the concentration of surfactant mixture, from 3 to 4 and 5%, it appears that 4% concentration of surfactant mixture results in self-assembled structures of surfactants with average diameter less than 10 nm. For higher lipid content the particle size is not longer changed.

Another approached referred to optimization of process parameters is related to the techniques used. It was found that the melting emulsification coupled with the high shear homogenization technique yielded average particle diameter less than 280 nm NLCs, while ultra sonication does not affect the particle size in a significant extent.

Therefore, the process parameters and operating conditions optimized for the synthesis of lipid nanocarriers to be loaded with bio-active components using the mixture of lipids consisting in glyceryl monostearate, carnauba wax, fish oil stabilized with Tween 80, soybean lecithin and poloxamer 407, are:

- the mixture of surfactants consists in 80% Tween 80, 5% soybean lecithin, 15% poloxamer 407;
- concentration of surfactant mixture - 4%;
- maintaining pre-emulsion to thermal treatment for one hour;
- melting emulsification coupled with the high shear homogenization technique, while sonication brings no significant contribution to process optimization.

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