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ORIGINAL ARTICLE

Optimization of the emulsification and solvent displacement method for the preparation of solid lipid nanoparticles

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Abstract

Objective: The essential aim of this article is to prepare solid lipid nanoparticles (SLNs) by emulsification and solvent displacement method and to determine the best process conditions to obtain submicron particles. *Methods*: The emulsification and solvent displacement method is a modification of the well-known emulsification–diffusion method, but without dilution of the system. The extraction of the partially water-miscible solvent from the emulsion globules is carried out under reduced pressure, which causes the diffusion of the solvent toward the external phase, with subsequent lipid aggregation in particles whose size will depend on the process conditions. The critical variables affecting the process, such as stirring rate, the proportion of phases in the emulsion, and the amount of stabilizer and lipid, were evaluated and optimized. *Results*: By this method, it was possible to obtain a high yield of solids in the dispersion for the lipids evaluated (Compritol® ATO 888, Geleol®, Gelucire® 44/14, and stearic acid). SLNs of up to ~20 mg/mL were obtained for all lipids evaluated. A marked reduction in size, between 500 and 2500 rpm, was seen, and a transition from micro- to nanometric size was observed. The smaller particle sizes obtained were 113 nm for Compritol® ATO 888, 70 nm for Gelucire® 44/14, 210 nm for Geleol®, and 527 nm for stearic acid, using a rotor–stator homogenizer (Ultra-Turrax®) at 16,000 rpm. The best phase ratio (organic/aqueous) was 1 : 2. *Conclusions*: The process proposed in this study is a new alternative to prepare SLNs with technological potential.

Key words: Emulsification and solvent displacement, emulsification–diffusion method, lipids, nanoparticles, solid lipid nanoparticles

Introduction

Nanoparticles (NPs) are solid colloidal particle dispersions of submicron size, which may contain active substances and are produced by mechanical or chemical means. Depending on the preparation method and the materials involved, nanospheres or nanocapsules may be obtained[1,2]. Nanospheres are characterized by a dense polymeric matrix, in contrast to nanocapsules, which consist of an oily core covered with a polymeric membrane.

Currently, there is a trend to use lipids instead of polymers for the elaboration of NPs. The term solid lipid nanoparticles (SLNs) has been coined to designate NPs obtained from lipids. The reasons for using lipids are their low toxicity, high biodegradability, and the possibility of modifying the bioavailability of some drugs. However, among the disadvantages of SLNs are expulsion and a low drug entrapment efficiency[3–8].

The method of choice to prepare SLNs is high-pressure homogenization (HPH), in which high efficiency devices are used to disperse the system at high shear forces, breaking the particles in the submicron range. In addition, this technique has two modalities: hot and cold homogenization (H-HPH and C-HPH); in both cases, it is necessary to melt the lipid to incorporate the drug[6,9]. In the solvent emulsification–evaporation (SEE) technique, the production of SLNs by means of lipid precipitation in an oil-in-water (o/w) emulsion is proposed[10,11]. The lipid material is dissolved in a nonwater-miscible solvent (e.g., cyclohexane); this is emulsified in an aqueous

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phase containing a stabilizer with the aid of a conventional stirrer, followed by HPH. The solvent is subsequently evaporated and the lipid is precipitated in the aqueous phase, and particles whose size will depend on homogenization intensity are obtained. Preparation of SLNs by microemulsion (μE) consists in precipitating the lipid from a hot microemulsion (o/w) in a cold aqueous medium under mechanical stirring[12]. The μE is constituted of lipid (e.g., stearic acid), surfactant (e.g., polysorbate 20, polysorbate 60, and soya phosphatidylcholine), cosurfactant(s) (e.g., butanol), and water, with which a transparent and thermodynamically stable system is formed at high temperatures (65–70°C), provided that the components are found in adequate proportions to form the μE. This μE is added to a cold aqueous medium (2–3°C) under stirring, causing lipid precipitation with particle sizes very similar to the globules of its predecessor μE[13,14]. Besides the specific problems with formulation, the μE method has the disadvantage of requiring high surfactant (>15%) and cosurfactant (>10%) concentrations.

Recently, Quintanar-Guerrero[15] demonstrated the feasibility of preparing lipidic nanospheres by the emulsification–diffusion method using four model lipids. Traditionally, the method had been used to prepare polymeric NPs. The process consists in dissolving the lipid in a partially water-soluble solvent (previously saturated with water) at room temperature or at controlled temperature, depending on the lipid's solubility in the solvent. This organic phase is emulsified with an aqueous solution (saturated with solvent), which contains the stabilizing agent, with conventional mechanical stirring at the same temperature used to dissolve the lipid. This o/w emulsion is then diluted with excess water at controlled temperature for prompting the diffusion of the internal phase toward the external phase, causing lipid aggregation in the form of SLNs.

The emulsification–diffusion method has the following advantages: (1) conventional laboratory equipment can be used; (2) pharmaceutically acceptable solvents are used; (3) solvent recycling is possible; (4) it is adaptable to several types of polymers and lipids; and (5) it is easily upscaled; furthermore, it has a high degree of reproducibility and efficiency. However, one of the main imputations of this process is that dispersions with a low solid concentration are obtained because of the large dilution performed to produce the diffusion of the solvent.

Considering the low efficiency achieved in obtaining SLNs by the emulsification–diffusion method, in this article it is proposed to evaluate the feasibility of causing the diffusion of the solvent, and consequently, the formation of NPs by direct displacement of the emulsion solvent under reduced pressure, a proposal originally made for the preparation of pseudolatexes with high solid content[16]. Then it is possible to increase the lipid concentration to obtain dispersions with lipid amounts comparable to that obtained with other methods such as HPH.

Four model lipids were used, such as Compritol® ATO 888, Geleol®, Gelucire® 44/14, and stearic acid, optimizing the preparation process through the evaluation of four critical preparatory variables: (a) stirring rate, (b) proportion of phases, (c) amount of lipid, and (d) amount of stabilizer.

Materials and methods

Materials

Glyceryl behenate (Compritol® ATO 888), glyceryl monostearate (Geleol®), and lauroyl polyoxylglycerides (Gelucire® 44/14) (Gattefossé) were generously donated by Noveon (Estado de Mexico, Mexico), and stearic acid by Sigma (Steinheim, Germany). The stabilizing agents tested, Poloxamer 188 (P-188, Pluronic® F-68) and Poloxamer 407 (P-407, Pluronic® F-127), were gift samples from BASF (Estado de Mexico, Mexico), and poly(vinyl alcohol) (PVAL) with a molecular mass of 26,000 (Mowiol® 4–88) was obtained from Hoechst (Frankfurt-am-Main, Germany). The partially water-miscible solvent studied was methyl ethyl ketone (analytical grade) supplied by Fermont (Monterrey, Mexico). Distilled water was of Milli-Q quality (Millipore, Bedford, MD, USA). All other reagents were of analytical grade and were used without further purification.

Methods

Solubility of lipids in the water-saturated solvent

The partially water-miscible solvent was saturated with water for 15 minutes. Approximately 400 mg of lipid was added to 20 mL of saturated solvent. The samples were sealed and stirred for 12 hours. The ability of the solvent to dissolve the lipid was considered when the content appeared transparent after visual observation. In case of lipid insolubility, the temperature required to achieve complete lipid dissolution was determined by heating the vials from 25°C to 70°C at 5°C intervals.

Preparation of SLNs

The solvent and water were mutually saturated for 15 minutes at room temperature before use to ensure the initial equilibrium of both liquids. When heating was required to solubilize the lipid, the saturation step was performed at this temperature using a water bath. Typically, 400 mg of lipid was dissolved in 20 mL of water-saturated solvent and this organic phase (internal phase) was emulsified with 40 mL of the solvent-saturated aqueous solution containing 5% (w/v) of stabilizer (dispersion medium), using a mechanical stirrer (Caframo RZR-l; Caframo, Ontario, Canada; propeller: IKA l381, IKA Werke GmbH & Co. KG, Staufen, Germany) at 2200 rpm for 10 minutes.

Following the formation of an o/w emulsion, the solvent is extracted without dilution by means of a

rotavapor. The bath temperature was as that used for lipid solubilization, with a constant vacuum of 70 mmHg and rotavapor speed of 30 rpm.

Particle size analysis

The average size and polydispersity index were determined by the laser light scattering technique (Coulter N4, Coulter, Florida, USA). Measurements were obtained at a 90° fixed angle for 180 seconds, at a temperature of 25°C. The scattering intensity data were analyzed by a digital correlator under a unimodal analysis mode. Dispersions were diluted with water to ensure that the light scattering signal, as indicated by particle counts per second, was within the instrument's sensitivity range. Measurements were made in triplicate for all batches prepared.

Evaluation of process variables

To optimize SLN preparation by the emulsification–solvent displacement method, different process variables were evaluated using the model lipids.

Influence of stirring rate. Compritol® ATO 888, Geleol®, Gelucire® 44/14, and stearic acid SLNs were prepared using different stirring rates (664, 1000, 1400, 1800, 2200, 11,000, and 16,000 rpm), with a variable speed stirrer and an Ultra-Turrax apparatus (T25 IKA; Labortechnik, Wilmington, DE, USA). The amount of lipid (400 mg) and the stabilizer concentration [5% (w/v) of P-127 for Compritol® ATO 888, Geleol®, Gelucire® 44/14, and 5% (w/v) of PVAL for stearic acid] were kept constant.

Influence of proportion of phases. SLN batches of the four model lipids were prepared, varying the proportion of the organic phase; the solvent/aqueous phase ratios were 1:8, 1:4, 1:2, 3:4, and 5:8. The stirring rate (2200 rpm), the amount of lipid (400 mg), and the amount of surfactant (5%, w/v) were kept fixed.

Influence of stabilizer and lipid amount. Based on the previous results, the decision was made to carry out a factorial design to determine the optimal conditions and to optimize the SLNs elaboration process by the emulsification–solvent displacement method; therefore, batches were prepared increasing the amount of lipid (200, 400, 600, 800, 1000 mg) with the aim of determining the maximum amount of lipid that can be transformed into NPs. Also, the amount of surfactant was varied (0.5, 1.0, 2.5, 5, 10%) and the stirring rate (2220) and organic/aqueous phase ratio (1:2) variables were kept fixed. PVAL was used as surfactant to prepare stearic acid SLNs, and P-127 was used to elaborate the Compritol® ATO 888, Geleol®, and Gelucire 44/14 SLNs.

Statistical analysis. An analysis of variance (ANOVA) with comparisons between means by Duncan test at 0.05 significance level was used to evaluate the influence of the different preparative variables on the particle size.

Results and discussion

The first step in the elaboration of SLNs by the emulsification–solvent displacement method consisted in determining the solubility of the four model lipids in methyl ethyl ketone through a qualitative visual trial, to find out whether temperature was required for the solubilization of the model lipids to be used. The results obtained for the solubility of the four model lipids and their minimum solubilization temperature are shown in Table 1. The results obtained for lipids, Compritol® ATO 888, Geleol®, Gelucire® 44/14, are consistent with those obtained in a previous work performed by Quintanar-Guerrero[15], where it was found that the only lipid that is soluble at room temperature is Gelucire 44/14.

In the formation of SLNs, the solvent is extracted first from the saturated external phase, which causes the diffusion of the solvent from the globules to the external phase, generating a nonsolvent system for the lipid, which will aggregate in the form of particles. If the stabilizer is capable of preventing lipid coalescence during this stage, submicron lipid particles will be obtained.

In the diffusion, a lipid crystallization process takes place, which consists of two stages (nucleation and growth). There are two types of nucleation: primary nucleation, which can be homogeneous or heterogeneous, and secondary nucleation. In homogeneous nucleation, the environment surrounding the nuclear substance is free of impurities. In fact, the most common type of nucleation for lipids is heterogeneous nucleation because they contain impurities such as mono- and diglycerides or because of the presence of extraneous entities. Once nuclei have been formed, they grow and become formal crystals. In our case, most batches were prepared at controlled temperature with gradual cooling until reaching room temperature. High temperatures are known to facilitate rapid lipid crystallization, and therefore, its aggregation in large particles is prevented. In the process proposed, solubilization and the diffusion stage were carried out at controlled temperature, with the aim of promoting lipid aggregation mediated by solvent displacement and gradual temperature reduction until reaching room temperature. It is suggested that the solvent flux will form the first nuclei within an adjacent region rich in lipid molecules whose stability and growth will be controlled by temperature and by the presence of the stabilizing agent. Considering the conditions proposed in this process, it is possible to anticipate the obtainment of submicron size particles.

Table 1. Qualitative lipid solubility of the four model lipids (400 mg) in water-saturated methyl ethyl ketone (20 mL).

Lipid (m.p, °C)	Temperature (°C)
Compritol® ATO 888 (69.0–74.0)	65
Geleol® (54.5–58.5)	40
Gelucire® 44/14 (42.5–47.5)	25
Stearic acid (69)	60

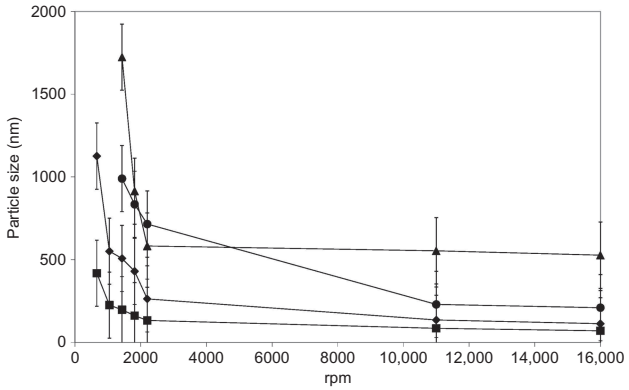

Figure 1. Effect of stirring rate on particle size average, for the four model lipids using a variable speed stirrer and an Ultra-Turrax apparatus. Compritol® ATO 888/Poloxamer-127 (♦); Gelucire 44/14/Poloxamer-127 (■); stearic acid/poly(vinyl alcohol) (▲); and Geleol®/Poloxamer-127 (●).

The results obtained for stirring rate are shown in Figure 1. As expected, stirring rate and particle size have an inversely proportional relationship, that is, the higher the stirring rate used, the smaller will be the particle size, with a biphasic behavior being observed, a significant effect between 500 and 2200 rpm (micro- to nanometer transition zone) followed by a change in slope where no significant reduction in particle size is seen. These batches are interesting from the technological point of view, as it was feasible to obtain nanometric sizes for the four model lipids using a variable speed stirrer with a stirring rate of 2200 rpm, without homogenization processes being required. The smaller particle size was obtained for Compritol (113.33 nm), Gelucire 44/14 (70.53 nm), Geleol (210.03 nm), and stearic acid (527.33 nm), using homogenization at 16,000 rpm. When the stirring rate is increased to 24,000 rpm, there is no important reduction in particle size, that is, particle size remains constant. This is consistent with a previous study carried out by Know[17]. For poly(lactic-co-glycolic acid) (PLGA) NPs with estrogen stabilized with PVAL by the emulsification–diffusion method, our results are also in agreement with those obtained in a previous work by Quintanar-Guerrero[15], for lipidic nanospheres by the emulsification–diffusion method. These authors found that, with an increase in the stirring rate, there was a reduction in the size of the emulsion globule, originating in NPs of smaller size up to the limit of approximately 100 nm.

The ANOVA analysis ($\alpha < 0.5$) demonstrates significant differences in stirring rate, when the variable speed stirrer and the rotor/stator homogenizer are used ($F = 2.81$; $P < 0.05$), and lipid type ($F = 3.29$; $P < 0.05$); therefore, it is not feasible to prepare SLNs at low stirring rates (664 rpm). The minimum speed to obtain nanometric size dispersions is 1800 rpm for the four lipids evaluated. Because the two P-values are less than 0.05, these factors have a statistically significant effect on particle size at the 95% confidence level. The Duncan test was used to comparatively analyze the obtained means. The differences of averages appear at the speed of 664 and 1048 rpm. The polydispersity index was not significantly affected by the stirring rate (ANOVA $\alpha < 0.5$).

A series of batches were prepared with the aim of evaluating the influence of the proportion of phases (oily/aqueous) and finding the ideal ratio. The results obtained are shown in Figure 2. As can be observed, lipid aggregation is significantly affected by two effects, lipid concentration and system viscosity, and both will determine the thickness of the saturated layer from which NPs are formed. The smaller sizes were obtained for the 1:2 oily/aqueous ratio. If the proportion of organic phase is low, a high amount of lipid is found, which causes the saturated layer formed during diffusion to be very thick; crystallization will occur within a region rich in lipid molecules, therefore, the particles formed will have a greater size. A similar behavior is observed when the proportion of phases allows the formation of viscous emulsions (high proportions of organic phase), because viscosity limits the diffusion rate; hence, the adjacent saturated layer will also be thick. This behavior can be explained from the viewpoint of the formation mechanism proposed for the emulsification–diffusion process[15,16,18,19]. NP formation is highly dependent on the diffusion rate of the solvent from the internal phase to the external phase and on the capability of the stabilizer to prevent lipid aggregation. Solvent diffusion brings lipid to the nonsolvent phase, forming local supersaturation regions in the globule–aqueous phase interface, which originate the first lipid nuclei. Based on the lipid crystallization theory, nucleation occurs because of rapid local fluctuations at molecular scale in a homogeneous phase that is in metastable equilibrium state. The metastable form nucleates first, before the more stable form, when it is induced by kinetic factors such as supersaturation or supercooling. When there are other factors involved

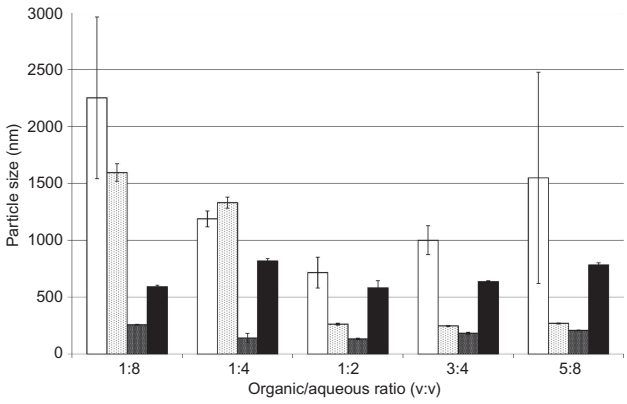

Figure 2. Effect of proportion of phases on particle size average. Geleol®/Poloxamer-127 (□); Compritol® ATO 888/Poloxamer-127 (▒); Gelucire 44/14/Poloxamer-127 (▓); and stearic acid/poly(vinyl alcohol) (■).

(temperature, pressure, and so on), the more stable form nucleates first. Nucleus formation occurs first, with a subsequent gradual growth of the nucleus. Crystal growth is a layer-by-layer process, and it can only occur on the crystal face. Diffusion resistance to molecule displacement toward the growing crystal face and resistance of integration of these molecules onto the crystal face should be considered because crystallization rates may be different.

If the protective effect by the stabilizer is adequate during the diffusion step, NPs will be formed. Our results are in agreement with those reported by Choi[20], where rapid solvent diffusion from the internal phase within the external phase causes aggregation of small particles. The ANOVA analysis ($\alpha = 0.5$) demonstrates nonsignificant differences because of the use of different phase proportions ($F = 2.37$; $P > 0.05$), with the optimal proportion for the four model lipids used being 1:2 (oily/aqueous). The results of ANOVA also showed significant differences because of the use of different lipid types ($F = 7.45$; $P < 0.05$). Duncan test shows differences in the proportion phase 1:8 and 1:4 (oily/aqueous).

A series of batches were subsequently prepared to evaluate the amount of stabilizer and lipid that can be transformed into NPs. It has been established that in NP formation processes, particle size diminishes with the increase in the amount of stabilizer up to a certain limit[15,16], that is, when the globule reaches its smallest size, an excess stabilizer is no longer important for globule stabilization or NP formation. As previously explained, the amount of lipid determines the trend of the aggregates generated during diffusion to coalesce, and therefore, the size of the particle obtained. The behavior due to the effect of the amount of stabilizer and lipid is similar for the four types used, a marked reduction in size between 0.5% and 2.5% (w/v) of stabilizer, followed by a lower effect at high concentrations. High lipid concentrations result in large particle sizes, especially when low stabilizer concentrations are used. It is important to point out that even at high lipid concentrations, it is possible to achieve nanometric sizes if an adequate stabilizer concentration is used. Figure 3 shows the results obtained by effect of stabilizer concentration and the amount of lipid for Compritol® ATO 888. With the use of 0.5% (w/v) of P-127, particle sizes greater than 1 μm are obtained; nanometric sizes are obtained starting from 2.5% of P-127. Sizes under 500 nm were obtained at a 10% stabilizer concentration. Figure 4 shows the results for Gelucire® 44/14. In this case, a submicron size was obtained with only 0.5% of P-127 for all lipid amounts, except for 1000 mg. Particle sizes smaller than 100 nm were obtained when a concentration of 10% P-127 was used. The results for Geleol® are shown in Figure 5; for this lipid, the minimum amount with which nanometric sizes were obtained for all amounts assayed was 5% (w/v) of P-127. Sizes under 300 nm were obtained with 10% P-127. Figure 6 shows the results for

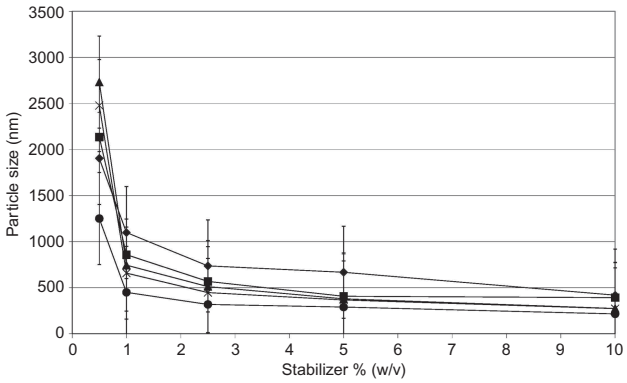

Figure 3. Effect of surfactant percentage (Poloxamer-127) and the amount of lipid on particle size average for Compritol® ATO 888. Compritol/1000 mg (♦); Compritol/800 mg (■); Compritol/600 mg (▲); Compritol/400 mg (×); and Compritol/200 mg (●).

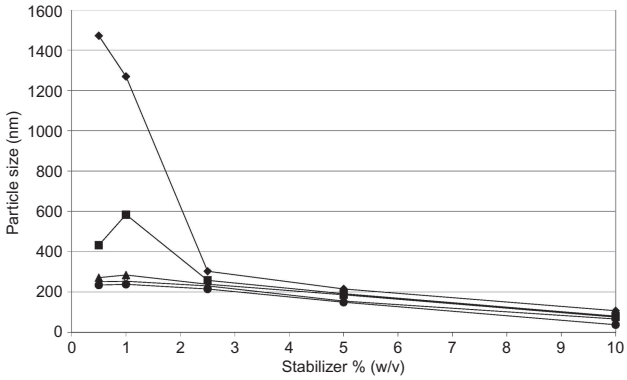

Figure 4. Effect of surfactant percentage (Poloxamer-127) and the amount of lipid on particle size average for Gelucire 44/14. Gelucire 44/14/1000 mg (♦); Gelucire 44/14/800 mg (■); Gelucire 44/14/600 mg (▲); Gelucire 44/14/400 mg (×); and Gelucire 44/14/200 mg (●).

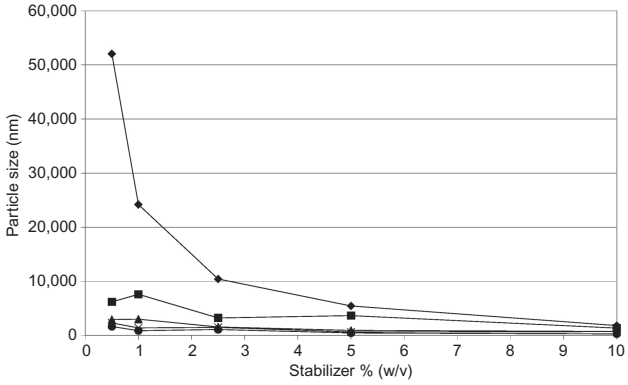

Figure 5. Effect of surfactant percentage (Poloxamer-127) and the amount of lipid on particle size average for Geleol®. Geleol/1000 mg (♦); Geleol/800 mg (■); Geleol/600 mg (▲); Geleol/400 mg (×); and Geleol/200 mg (●).

stearic acid; particles of submicron size were obtained starting from 2.5% of PVAL. For this lipid, no clear reduction in particle size is observed when high stabilizer concentrations are used. The sizes observed for all batches

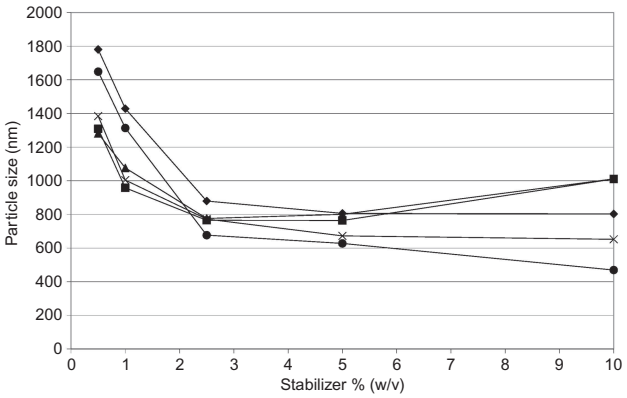

Figure 6. Effect of surfactant percentage [poly(vinyl alcohol)] and the amount of lipid on particle size average for stearic acid. Stearic acid/1000 mg (♦); stearic acid/800 mg (■); stearic acid/600 mg (▲); stearic acid/400 mg (×); and stearic acid/200 mg (●).

were greater than 500 nm. These results are consistent with the investigations by Trotta[21], who established that when the lipid content is increased above 5–10%, large particles are obtained, including microparticles and a wide range of size distribution. This is due to a reduction in the efficiency of the homogenization process and to an increase in the probability of particle agglomeration. Ahlin et al.[9] prepared polymethyl methacrylate (PMMA) NPs using PVAL as stabilizer by the emulsification–diffusion method, and they investigated the effect of PVAL concentration on PMMA NP size. They observed that the size of PMMA NPs increases or remains constant with the increase in PVAL concentration. This effect was attributed to an increase in the viscosity of the continuous phase. Another study[17] found that there is a concentration necessary for NP stabilization, and an excess concentration does not have an important role in the reduction of particle size. Song[22] prepared PLGA NPs and found that particle size is dependent on the stability of the emulsion globules after diffusion, and if the effect of the stabilizer (Poloxamer 127) is adequate, NPs are formed. Both PVAL and P-127 are nonionic stabilizers that exert a steric stabilization action on the globules dispersed in the internal phase[1].

When the stabilizer remains in the liquid–liquid interface during the diffusion process, and if the protective effect is adequate, NPs are formed. In addition, the mechanism of action of PVAL is by steric hydrance, indicating that when the stabilizer concentration increases, more molecules are absorbed on the interface of the emulsion globules, providing a greater protection to the emulsion globules, which results in small particle sizes.

The ANOVA analysis (α=0.5) demonstrates that there are significant differences; therefore, the amount of stabilizer and the amount of lipid are key factors in the determination of the average size of NPs. In this model, the results of ANOVA also showed significant differences because of the use of different amounts of lipid (F=4.24; P<0.05) and lipid type (F=7.49; P<0.05), whereas there are nonsignificant differences because of the percent of stabilizer (F=2.48; P<0.05). The interaction between amount of lipid and lipid type showed significant differences (F=2.48; P<0.05), but nonsignificant differences due to the interaction between the amount of lipid and the percent of surfactant (F = 1.08; P > 0.05) and lipid type and percent of surfactant (F = 1.34; P > 0.05). Duncan test shows differences in the amount of lipid of 1000 mg.

Conclusions

The results obtained demonstrate that the emulsification–solvent displacement technique represents an interesting alternative to prepare SLNs that allows increasing the lipid concentration up to ~20 mg/mL compared with other methods, such as HPH.

It was possible to optimize the emulsification–solvent displacement method to prepare Compritol 888 ATO, Gelucire 44/14, Geleol, and stearic acid SLNs; the following were the best conditions: stirring rate, 16,000 rpm; organic/aqueous phase ratio, 1:2; surfactant %, 5% (w/v); and amount of lipid, 200 mg. With this modification, the problems with the low efficiency achieved by the emulsification–diffusion method were corrected. The particle aggregation and crystal growth process is a layer-by-layer process, and it is dependent on the diffusion rate. This method is feasible for industrial upscaling because it is a robust and reproducible method and solvent recovery is achieved, and it may have pharmaceutical applications for the implementation of new formulas through loading with active substances with lipophilic characteristics.

Declaration of interest

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