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Research Article

Single-step and low-energy method to prepare solid lipid nanoparticles and nanostructured lipid carriers using biocompatible solvents

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Abstract

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLCs) are widely being explored for improving dermal/transdermal and oral delivery of drugs, neutraceuticals and cosmeceuticals. High-pressure homogenization (HPH) is the most commonly used preparation method for SLN/NLCs. SLN/NLCs preparation by the HPH requires high energy input and longer duration. Here, we describe a simple yet innovative low-energy method to prepare SLN/NLCs in a single-step using biocompatible solvents. We first show that biocompatible solvents such as Transcutol P, Soluphor P, N-methyl pyrrolidone, and glycofurol can solubilize glyceryl monostearate, glyceryl behenate, and glyceryl distearate to a variable degree. Our pre-formulation studies showed that only GMS could be transformed into SLN or NLCs despite high solubility of the lipids investigated indicating the importance of solvent-lipid interaction parameter in our preparation method. Finally, we show that SLN and NLCs of glyceryl monostearate with size < 150 nm and acceptable polydispersity index can be easily developed using Transcutol P as a biocompatible solvent and polyoxyl-40-stearate (MYS-40) as a stabilizer. As the Transcutol P has excellent acceptability for dermal/transdermal and oral route, there is no need to remove the residual Transcutol P (5% v/v) from the prepared glyceryl monostearate SLN/NLCs. Thus, our method offers a simple yet innovative way to prepare GMS SLN/NLCs suitable for dermal/transdermal and oral applications.

Keywords:

Transcutol, SLN, NLC, GMS, glycofurol, Capmul MCM, Capryol 90.

1. Introduction

Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLCs) have been and are being widely explored to enhance delivery and efficacy of drugs, nutraceuticals, and cosmeceuticals. SLN are composed of low-cost, generally regarded as safe (GRAS) biodegradable solid lipids such as highly purified triglycerides, monoglycerides, hard fats, complex glyceride mixtures or even waxes whereas NLCs are composed of a mixture of liquid lipid (oil) and solid lipid in appropriate proportion (Date, Joshi, & Patravale, 2007; Muchow, Maincent, & Müller, 2008; Müller, Radtke, & Wissing, 2002; Pardeike, Hommoss, & Müller, 2009; Uner & Yener,

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2007). The ability of the SLN and/or NLCs to improve dermal/transdermal and oral bioavailability of therapeutic agents has been very well established (Abhijit A Date et al., 2007; Muchow et al., 2008; Müller et al., 2002; Pardeike et al., 2009; Uner & Yener, 2007). Furthermore, the use of commercially viable techniques such as high pressure homogenization allows for ease of manufacture and scale-up of SLN and NLCs. To date, several dermatological products based on SLN and/or NLCs have been successfully commercialized in the European Market (Pardeike et al., 2009).

High-pressure homogenization (HPH), the most widely used technique for fabrication of SLN/NLCs, involves the use of high temperature and high homogenization pressure and typically the SLN/NLCs dispersion needs to undergo multiple homogenization cycles to achieve desired particle size and polydispersity index (Mehnert & Mäder, 2001). Thus, HPH is a relatively time-consuming process and it requires high energy input. Furthermore, HPH technique is not suitable for thermolabile drugs and/or peptides (Martins, Sarmento, Ferreira, & Souto, 2007). Hence, alternate preparation methods such as microemulsion template (Martins et al., 2007; Mehnert & Mäder, 2001; Uner & Yener, 2007), emulsion-solvent diffusion (Trotta, Debernardi, & Caputo, 2003), nanoprecipitation (Dong, Ng, Shen, Kim, & Tan, 2012; Schubert & Müller-Goymann, 2003), and emulsion-solvent evaporation (Martins et al., 2007; Mehnert & Mäder, 2001; Uner & Yener, 2007) have been explored for the development of SLN/NLCs. Many of these alternate methods use volatile organic solvents such as acetone, chloroform, dichloromethane, ethanol, and isopropanol (Dong et al., 2012; Schubert & Müller-Goymann, 2003). Hence, in these preparation methods, additional steps are required to remove the organic solvent from SLN/NLCs dispersion.

To our knowledge, no attempts have been made to use biocompatible solvents for the preparation of SLN/NLCs. Here, we show that SLN/NLCs can be prepared in a single step with the help of biocompatible amphiphilic solvents like Transcutol P. Furthermore, due to excellent biocompatibility of solvents, the step of solvent removal is not required. Our approach requires very low-energy for the preparation of SLN/NLCs and can yield glyceryl monostearate (GMS) SLN/NLCs with < 150 nm size and acceptable polydispersity index.

2. Materials and Methods

2.1. Chemicals

Poloxamer 188, Cremphor EL, Kolliphor HS 15, Soluphor P (BASF India Ltd., Mumbai, India), Cithrol GMS 0400 (Croda India Ltd., Mumbai, India), Precirol ATO 5, Compritol ATO 888, Transcutol P, Capryol 90, Lauroglycol 90, Labrafac WL1349, (Gattefosse India Ltd., Mumbai, India), Dynasan 114, Dynasan 118, Miglyol 812 (S. Zhaveri Co. Ltd., Mumbai, India), Capmul MCM (Abitec Corp., OH, USA), Cetyl Palmitate (Subhash Chemicals, Pune, India), N-methyl pyrrolidone (Anshul India Pvt. Ltd., Mumbai, India), MYS-40 (Nikkon Chemicals, Japan), were received as gift samples. Tween 80, Tween 20 (s.d. Fine Chemicals, Mumbai, India), and Glycofurol (Merck India Ltd., Mumbai, India) were purchased for the present investigation. All the chemicals used for the study were of analytical grade. Double distilled water was freshly prepared whenever required.

2.2. Determination of solubility of solid lipids in biocompatible solvents

The biocompatible solvents selected for the experiment were Transcutol P, N-methyl pyrrolidone (NMP), 2-Pyrrolidone (Soluphor P) and glycofurol. The ability of the biocompatible solvents to solubilize various solid lipids was evaluated by the method suggested by Devani et al. (Devani, Ashford, & Craig, 2004), with suitable modifications. Briefly, the selected solid lipid in 50 mg increments was added to a transparent glass vial containing 1ml of the biocompatible solvent and the vial was shaken at 800 rpm at 75°C in a water-bath shaker (Remi Instruments, Mumbai, India). The experiment was continued until turbidity was observed visually. All the experiments were performed in triplicate to confirm the results of solubility. The solid lipids evaluated were glyceryl behenate (Compritol ATO), glyceryl distearate (Precirol ATO), tristearin (Dynasan 118), trimyristin (Dynasan 114), cetyl palmiate and glyceryl monostearate (Cithrol 0400).

2.3. Feasibility studies on the formation of solid lipid nanoparticles with the help of biocompatible solvents

It was important to establish the feasibility of lipid nanocarriers fabrication using biocompatible solvents. Briefly, solid lipid (200 mg) was dissolved in 0.5 ml of biocompatible solvent maintained at 70°C. To this solution, 9.5 ml of 2% w/v Tween 80 solution (maintained at 70°C) was added at once under vortexing (~1200 rpm). The vortexing was continued until uniform lipid dispersion is formed. The lipid dispersion was cooled to room temperature. The solid lipids investigated in this investigation were glyceryl monostearate (GMS, Cithrol 0400), glyceryl behenate (Compritol ATO 888) and glyceryl distearate (Precirol ATO 5). The biocompatible solvents used for the preliminary study were Transcutol P and Soluphor P. All experiments were carried out in triplicate.

2.4. Development of GMS nanoparticles using biocompatible solvents

2.4.1 Evaluation of various surfactants for the preparation of GMS nanoparticles

Various surfactants were evaluated for the preparation of GMS nanoparticles. Transcutol P was selected as a biocompatible solvent. Briefly, GMS, 200 mg was dissolved in 0.5 ml of Transcutol P maintained at 70°C. To this solution, 9.5 ml of 2% w/v surfactant solution (maintained at 70°C) was added at once under vortexing

Table 1: Solubility of various solid lipids in biocompatible solvents (n=3). The lipids, in 50 mg increments, were added to the biocompatible solvent maintained at 75°C and the addition was continued until turbidity is observed.

	Solubility (mg/mL)			
	Soluphor P	NMP	Transcutol P	Glycofurol
Glyceryl behenate (Compritol®)	< 600	< 600	< 600	< 600
Glyceryl distearate (Precirol®)	< 1050	< 1050	< 1050	< 1050
Tristearin (Dynasan® 118)	Insoluble	Insoluble	Insoluble	Insoluble
Glyceryl monostearate (Cithrol® GMS 0400)	< 1250	< 1250	< 1250	< 1250
Trimyristin (Dynasan® 114)	Insoluble	< 600	Insoluble	Insoluble
Cetyl palmitate	Insoluble	Insoluble	Insoluble	Insoluble

(~1200 rpm). The vortexing was continued until uniform lipid dispersion is formed. The lipid dispersion was cooled to room temperature. The surfactants evaluated were Tween 80, Tween 20, Poloxamer 188 (Kolliphor P188), Kolliphor HS 15, Cremophor EL and MYS-40. All experiments were carried out in triplicate. The particle size and polydispersity of GMS nanoparticles were evaluated by the photon correlation spectroscopy (PCS; Beckman Coulter N4 plus, Wipro, India).

2.4.2 Effect of type of biocompatible solvent

In this study, the effect of the various biocompatible solvents on the particle size of the blank GMS nanoparticles was evaluated. The biocompatible solvents evaluated in this investigation were Transcutol P, NMP, Soluphor P and Glycofurol. Briefly, GMS, 200 mg was dissolved in 0.5 ml of the selected biocompatible solvent by heating at 70°C. To this solution, 9.5 ml of aqueous solution containing 2% w/v MYS-40 (maintained at 70°C) was added at once under vortexing (~1200 rpm). The vortexing was continued until uniform lipid dispersion is formed. The lipid nanodispersion was cooled to room temperature. The particle size of the GMS nanoparticles was measured by the PCS. All experiments were carried out in triplicate.

2.4.3 Effect of increase in the GMS concentration

The effect of variation in GMS concentration on the particle size of the GMS nanoparticles was evaluated. Briefly, GMS at various concentrations (200 mg, 300 mg, 400 mg and, 500 mg) was dissolved in 0.5 ml of Transcutol P maintained at 70°C. To this solution, 9.5 ml of aqueous solution of 2% w/v MYS-40 (maintained at 70°C) was added at once under vortexing (~1200 rpm). The vortexing was continued until uniform lipid dispersion is formed. The lipid nanodispersion was cooled to room temperature. The particle size of the GMS nanoparticles was measured by the PCS. All experiments were carried out in triplicate.

2.4.4 Effect of decrease in the surfactant concentration

The effect of the decrease of surfactant concentration on the particle size of the GMS nanoparticles was evaluated. Briefly, GMS, 200 mg was dissolved in 0.5 ml of Transcutol P maintained at 70°C. To this solution, 9.5 ml of aqueous solution containing 0.5% w/v, 1% w/v, 1.5% w/v and 2% w/v MYS-40 (maintained at 70°C) was added at once under vortexing (~1200 rpm). The vortexing was continued till the uniform lipid dispersion is formed. The lipid nanodispersion was cooled to room temperature. The particle size of the GMS nanoparticles was measured by the PCS. All experiments were carried out in triplicate.

2.5. Fabrication of nanostructured lipid carriers (NLCs) based on GMS

The feasibility of preparing NLCs based on GMS was evaluated. Transcutol P was selected as a biocompatible solvent for this purpose. Briefly, GMS (150 mg) and 50 mg of liquid lipid (oil) were dissolved in 0.5 ml of Transcutol P maintained at 70°C. To this solution, 9.5 ml of aqueous solution containing 2% w/v MYS-40 (maintained at 70°C) was added at once under vortexing (~1200 rpm). The vortexing was continued until uniform lipid dispersion is formed. The lipid nanodispersion was cooled to room temperature. The liquid lipids (oils) evaluated were Miglyol 812, Labrafil WL 1349, Capmul MCM, Lauroglycol 90 and Capryol 90. All experiments were carried out in triplicate. The particle size and polydispersity of NLCs were evaluated by the PCS.

3. Results

3.1. Solubility of solid lipids in the biocompatible solvents

Our solubility studies showed that tristearin and cetyl palmitate formed a separate layer indicating lack of solubility in all the amphiphilic biocompatible solvents whereas trimyristin (Dynasan 114) was soluble in only NMP. Glyceryl behenate (Compritol ATO), glyceryl distearate (Precirol) and GMS were highly soluble in the

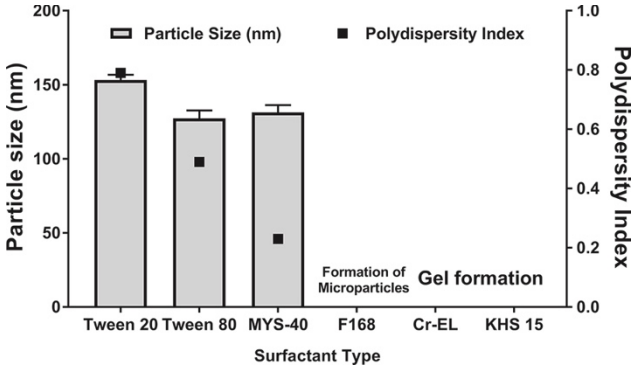

Figure 1: Type of surfactant affects the particle size and polydispersity of glyceryl monostearate (GMS) nanoparticles. The concentration of all surfactants was 2% w/v and that of GMS was 200 mg. 0.5 ml Transcutol P was used for preparing GMS nanoparticles. Trials with Cremophor EL (Cr-EL) and Kolliphor HS 15 (KHS 15) resulted in the formation of gel. Pluronic F168 resulted in formation of GMS microparticles. PEG-40-stearate (MYS-40) resulted in GMS nanoparticles with small size (Avg: 131.4 nm) and low polydispersity index (Avg: 0.23). Data expressed as mean ± S.D (n=3).

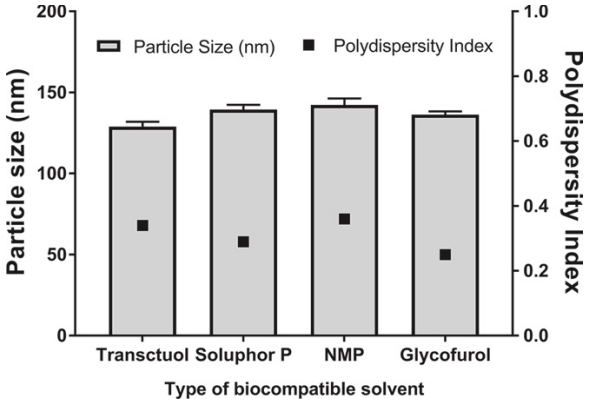

Figure 2: The type of biocompatible solvent used for the preparation of GMS nanoparticles does not significantly affect the size and polydispersity index of GMS nanoparticles. The concentration of MYS-40 was 2% w/v and that of GMS was 200 mg. Various biocompatible solvents such as Transcutol, Soluphor P (2-pyrrplidone), N-methyl pyrrolidone (NMP) and glycofurol (quantity: 0.5 mL) were evaluated. The type of biocompatible solvent used for the preparation of GMS nanoparticles did not significantly affect the size as well as polydispersity index of GMS nanoparticles. Data expressed as mean ± S.D (n=3).

biocompatible solvents (Table 1).

3.2. Feasibility studies on the formation of solid lipid nanoparticles with the help of biocompatible solvents

In this study, solid lipids with different molecular weight, hydrophobicity and chemical nature were evaluated for their ability to form lipid nanoparticles. It was observed that glyceryl behenate (Compritol ATO 888) showed the formation of lipid aggregates and glyceryl distearate (Precirol ATO 5) resulted in the formation of lipid microparticles with size > 1 µm. Only GMS, as per the conditions used in this investigation, resulted in the formation of lipid nanoparticles. These observations were constant irrespective of the type of biocompatible solvent used (data not shown).

3.3. Development of blank GMS nanoparticles using biocompatible solvents

We evaluated various FDA approved surfactants to identify the surfactant that would yield GMS nanoparticles with smallest size and polydispersity index. We observed that GMS nanoparticles fabricated with Cremophor EL and Kolliphor HS 15 showed gel formation after cooling to the room temperature whereas the Kolliphor P188 (poloxamer 188) yielded GMS particles with size > 1 µm. It was observed that tween 80, tween 20 and MYS-40 resulted in GMS nanoparticles with size < 200 nm and acceptable polydispersity index (Figure 1). Tween 80 and Tween 20 stabilized GMS nanoparticles showed aggregation on standing for 48 h at the room temperature whereas MYS-40 stabilized GMS nanoparticles did not show any signs of aggregation or significant change in particle size even after 7 days of storage at the room temperature.

Our studies showed that the GMS nanoparticles prepared using Transcutol P had lowest particle size compared to the other solvents (Figure 2). However, the GMS nanoparticles prepared using the other solvents (Soluphor P, NMP and glycofurol) were not significantly different from the GMS nanoparticles prepared from Transcutol P. We studied the effect of increase of the GMS concentration in a biocompatible solvent (Transcutol P) on the size of GMS nanoparticles (Figure 3A). The particle size and polydispersity of the GMS nanoparticles increased when the concentration of GMS in the biocompatible solvent was increased from 200 mg up to 400 mg. However, when 500 mg of GMS was used for the preparation, there was a formation of GMS microparticles instead of nanoparticles. We also evaluated the impact of concentration of surfactant (MYS-40) on the size and polydispersity of GMS nanoparticles. It was observed that decreasing the MYS-40 concentration from 2% w/v to 1% w/v increased the size and polydispersity of GMS nanoparticles (Figure 3B). When 0.5% w/v of MYS-40 was used for the preparation, the formation of GMS microparticles was observed.

3.4. Fabrication of nanostructured lipid carriers (NLCs) based on GMS

We observed that the type of the liquid lipid blended with GMS for the formation of NLCs had a considerable impact on the size and polydispersity of GMS NLCs (Figure 4). The Miglyol 812 (M812) and Labrafac WL 1349 (WL 1349) resulted in NLC with greatest particle size and higher polydispersity index whereas Capmul MCM, Lauroglycol 90, and Capryol 90 yielded NLCs with smaller particle size and polydispersity index.

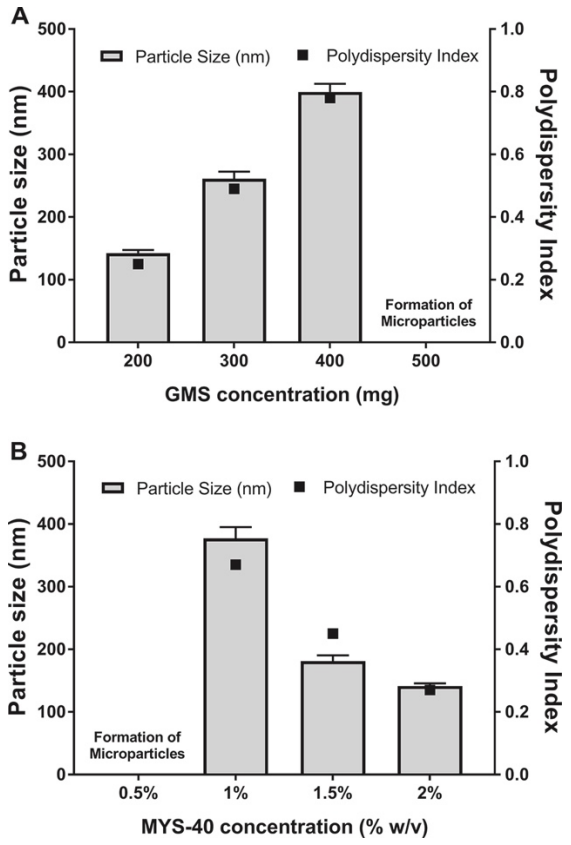

Figure 3: The concentration of GMS and MYS-40 affect the size and polydispersity of nanoparticles. Effect of variation of lipid (GMS) concentration and surfactant (MYS-40) concentration on the size and polydispersity index of GMS nanoparticles was evaluated. The particle size as well as polydispersity index increased with (A) increase in GMS concentration and (B) decrease in MYS-40 concentration. Data expressed as mean ± S.D (n=3).

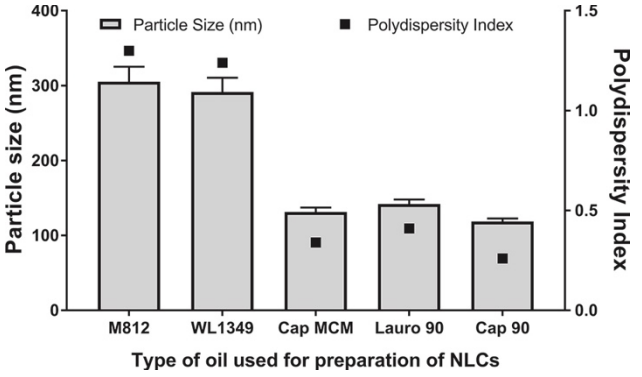

Figure 4: The chemical nature of the oil used for the preparation of GMS nanostructured lipid carriers (NLCs) affects the size and polydispersity index of GMS NLCs. The concentration of MYS-40 was 2% w/v and that of GMS was 200 mg. 0.5 ml of Transcutol was used for the preparation of NLCs. The ratio of GMS to oil was 1:1. Long and medium chain triglycerides such as Labrafil WL 1349 and Miglyol showed higher particle size and high polydispersity index. However, medium chain mono- and diglycerides (Capmul MCM) and propylene glycol easters such as Lauroglycol 90 (Lauro 90) and Capryol 90 (Cap 90) yielded small particle size and low polydispersity index of NLCs. Data expressed as mean ± S.D (n=3).

4. Discussion

SLN and NLCs have demonstrated a great potential in improving dermal/transdermal and oral delivery of a variety of drugs, nutraceuticals, and cosmeceuticals (Pardeike et al., 2009; Date, Vador, Jagtap, & Nagarsenker, 2011; Shah, Date, Joshi, & Patravale, 2007). Moreover, several dermatological products such as Nanorepair Q10® and Nanovital Q10® cream are available in the European market that contain SLN or NLCs (Pardeike et al., 2009). While high-pressure homogenization is the most commonly used method to prepare SLN/NLCs, it requires very high energy input and longer processing time. Furthermore, the high-pressure homogenization is not suitable for incorporating heat sensitive drugs into SLN/NLCs. Low-energy preparation methods such as "microemulsion templating" have been investigated for the preparation of SLN/NLCs (Chirio et al., 2018; Dudhipala & Puchchakayala, 2018; Zhao et al., 2018). However, it is well known that the microemulsion template technique requires a high amount of surfactant and it is necessary to considerably dilute the microemulsion to obtain lipid nanoparticles. This yields SLN/NLCs with very low lipid concentration. The nanoprecipitation process is another low-energy preparation method to obtain nanoparticles (Dong et al., 2012; Schubert & Müller-Goymann, 2003). However, the investigations described till date, use volatile organic solvents such as acetone, ethanol and, isopropanol for the preparation of SLN/NLCs (Dong et al., 2012; Schubert & Müller-Goymann, 2003). The removal of these solvents from the SLN/NLCs dispersion requires energy input and it also adds another step in the preparation of SLN/NLCs.

We have previously shown that amphiphilic biocompatible solvents such as Transcutol P, NMP, Soluphor P, glycofurol, Labrasol can be used for the single-step preparation of polymeric and phospholipid-based nanoparticles (Date, Jain, Khachane, & Nagarsenker, 2010; Date, Srivastava, et al., 2011). This low-energy and single step method allowed for preparation of concentrated nanoparticles without the need for removal of solvent used for the preparation of nanoparticles. In the present investigation, we extended the application of our approach for the preparation of SLN/NLCs. We envisaged that amphiphilic biocompatible solvents such as Transcutol P, NMP, Soluphor P and glycofurol could be a viable alternative for organic solvents such as acetone and ethanol. As these biocompatible solvents have good acceptability for dermal/oral/parenteral route of administration, there would be no need to remove them from the SLN/NLCs dispersion provided that the residual concentration of biocompatible solvents is within acceptable limits.

We did not attempt to determine the equilibrium solubility of the solid lipids in the biocompatible solvents instead we approximately checked the solubility of lipids with 50 mg increment until turbidity appeared. We observed that tristearin and cetyl palmitate formed a separate layer and were completely insoluble with the amphiphilic solvents whereas trimyristin (Dynasan 114) was soluble in only NMP. Monoglycerides of long-chain fatty acids such as glyceryl behenate, glyceryl distearate and, GMS showed good solubility in the amphiphilic solvents. We correlated the molecular weight and reported solubility parameters of the lipids (Jensen et al., 2010) with their solubility in the biocompatible solvents. The solubility of solid lipids did not correlate with the molecular weight (or molecular volume) of the solid lipids as highest molecular weight glyceryl behenate had good solubility in all biocompatible solvents. Interestingly, a good correlation was observed between the solubility parameter of solid lipids and their tendency of getting solubilized in biocompatible solvents. It is noteworthy that the solubility parameters were in the order cetyl palmitate < tristearin < trimyristin < glyceryl behenate < glyceryl distearate < glyceryl monostearate. The glyceryl monostearate (GMS) with the highest solubility parameter showed the highest solubility in all the biocompatible solvents.

The process of the nanocarrier fabrication by nanoprecipitation method is dependent on the properties of the solvent, properties of the surfactant, properties of the material to be nanosized and temperature used for fabrication. We observed that despite high solubility in the amphiphilic solvents, that glyceryl behenate (Compritol® ATO 888) and glyceryl distearate (Precirol® ATO 5) could not yield lipid nanoparticles whereas glyceryl monostearate (GMS, Cithrol® 0400) yielded lipid nanoparticles. We tried to use different surfactants or different biocompatible solvents, but in all the cases, Compritol showed signs of lipid aggregates and slight phase separation whereas Precirol yielded uniform lipid dispersions with particle size > 1 µm (data not shown). This clearly indicated the influence of the properties of the solid lipid to be nanosized via the nanoprecipitation process. Our observation also delineated the importance of the solvent-lipid interaction on the process of the nanoparticle fabrication as all the other parameters were kept the same. The solvent-lipid interaction was calculated by the following equation (Abhijit A Date, Srivastava, et al., 2011; Galindo-Rodriguez, Allémann, Fessi, & Doelker, 2004):

$$\chi_{solvent-lipid} = \frac{V_{solvent}}{RT}\left(\delta_{solvent} - \delta_{lipid}\right)^2$$

We used the reported solubility parameter (δ) values for the solvent and solid lipids to calculate the solvent-lipid interaction parameter and the values of the transcutol-lipid parameter obtained for the Compritol, Precirol and, GMS were 7.3, 7 and 5.86 respectively. This indicated that Compritol and Precirol have more affinity for the solvent as compared to that of the GMS and hence, the diffusion of these lipids in the aqueous phase is likely to be lesser than that of the GMS. Thus, it can be concluded that the lesser the lipid-solvent interaction, better are the chances of formation of particles with lesser size. This observation has already been established for the polymeric nanoparticles.

Furthermore, Compritol and Precirol have higher molecular volume and hydrophobicity than that of GMS. Hence, they have lesser 'ease of emulsification' as compared to that of the GMS. Hence, they would require more surfactant and greater energy for the interfacial stabilization as compared to that of GMS. It may be possible that the amount of the surfactant and energy employed in the investigation is too less to give nanoparticles of these lipids. Finally, unlike a considerably high amount of organic solvent used in the conventional nanoprecipitation process, we use only 0.5 mL of biocompatible solvents. The ratio of the organic to aqueous phase also influences the nanoprecipitation process (Malkani, Date, & Hegde, 2014) and could also be a contributing factor for the results obtained with Compritol and Precirol. We decided to focus our further studies on the development and optimization of GMS SLN and NLCs using biocompatible solvents.

Our studies on GMS nanoparticles showed that surfactants Cremophor EL (Polyoxyl-35-castor oil; HLB value: 13.5) and Kolliphor HS 15 (PEG-660-12 hydroxystearate; HLB value: 15), when used for the preparation of GMS nanoparticles showed the formation of a gel. The polymorphic transitions in the lipids after cooling to the room temperature and the interaction between surfactant and lipid are known to cause gel formation in SLN dispersions. When poloxamer 188 (HLB value: 29) was used as a stabilizer, we could not achieve the formation of GMS nanoparticles indicating that polymeric emulsifiers are unsuitable for stabilization when using our method of preparation. Tween 80 (Polyoxyl-20-sorbitan monooleate; HLB value: 15), Tween 20 (Polyoxyl-20-sorbitan monolaurate; HLB value: 16.9) and MYS-40 (Polyoxyl-40-monostearate; HLB value: 16.9), when used as stabilizers, were able to yield GMS nanoparticles. However, only MYS-40 (also known as Myrj®52) resulted in GMS nanoparticles with low polydispersity index. These studies showed that the presence of stearate backbone in the surfactant structure and HLB > 16 were necessary parameters to achieve formation of stable GMS nanoparticles.

The type, viscosity and chemical nature of the solvent used for the fabrication of the nanoparticles can have considerable influence on the particle size as reported earlier for the polymeric nanoparticles. We used 4 chemically different biocompatible solvents for the preparation of GMS nanoparticles. However, unlike earlier observations (Date, Srivastava, et al., 2011; Malkani et al., 2014), we did not see a significant change in the size of the GMS nanoparticles as a function of the change in the biocompatible solvent. This may be in part due to equivalent solubility of GMS in all the biocompatible solvents which may have overcome the contribution of solvent-water interaction parameter while preparing GMS nanoparticles.

Our studies showed that the size of the GMS nanoparticles increased with the increase in the concentration of GMS in the

biocompatible solvent. Similar results have been observed by Quintanar-Guerrero et al., and Schubert and Mueller-Goymann for the lipid nanoparticles fabricated using nanoprecipitation and emulsification-solvent diffusion process (Quintanar-Guerrero, Tamayo-Esquivel, Ganem-Quintanar, Allémann, & Doelker, 2005; Schubert & Müller-Goymann, 2003). For this study, we dissolved a high amount of lipid in the fixed amount of the biocompatible solvent. Hence, with the increase in the lipid concentration, there would be an increase in the viscosity of the biocompatible solvent phase. The increase in the viscosity would decrease the rate of the diffusion of the biocompatible solvent phase in the aqueous phase leading to the formation of nanoparticles with higher size or lipid aggregates (when 500 mg of GMS was used for preparation). The concentration of MYS-40 also had a clear influence on the size of the GMS nanoparticles. It is obvious that the decrease in the surfactant concentration would result in the decrease in the surfactant available for the interfacial stabilization during the nanoprecipitation process leading to the formation of nanoparticles with higher size or lipid aggregates due to insufficient stabilization (observed when 0.5% of MYS-40 was used). Similar observations have been noted by Quintanar-Guerrero et al., for the Gelucire 44/14 nanoparticles (Quintanar-Guerrero et al., 2005).

5. Conclusion

Finally, we observed that the chemical nature, molecular volume and relative lipophilicity of liquid lipids (oils) had a considerable impact on the size and polydispersity of GMS NLCs. When long and medium chain triglycerides such as Labrafil WL 1349 (HLB: 1) medium chain triglycerides (Miglyol 812) were used, the size and polydispersity of GMS NLCs were higher. On the contrary, when high HLB, low molecular volume liquid lipids viz. Capmul MCM (Mixture of mono and di-glycerides of caprylic acid; HLB: 3-4), Lauroglycol 90 (propylene glycol monolaurate; HLB-4) and Capryol 90 (Propylene glycol monocaprylate; HLB: 6) were used, GMS NLCs with lower particle size and polydispersity were obtained. To conclude, we successfully developed a single-step and low energy method to develop SLN and NLCs using biocompatible solvent.

6. Acknowledgments

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