Pharmaceutical Division, University Department of Chemical Technology, Mumbai, India.

Study of Solid Lipid Nanoparticles with respect to particle size distribution and drug loading

V. B. PATRAVALE, A. V. AMBARKHANE

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Dr. (Mrs) Vandana Patravale, University Department of Chemical Technology, University of Mumbai, Nathalal Parikh Marg, Mumbai 400019, India
vbp@pharma.udct.ernet.in

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This work deals with the formulation and development of Solid Lipid Nanoparticles (SLN) using pressure homogenization technique, nimesulide being used as the model drug. Main emphasis of the work was to study the effect of individual process parameters (homogenization pressure and homogenization cycles) and formulation parameters (lipid concentration and surfactant concentration) on particle size distribution and drug loading. Particle size distribution data indicate that by optimizing the homogenization process and formulation parameters it is possible to produce SLN within a desired size range as required for carrier mediated drug targeting. Approaches to improve drug loading efficiency indicate that drug loading was higher in case of SLN prepared from glyceryl beheanate, palmitostearate and glyceryl tristearate + span 60 as compared to monoacid triglyceride (MAT) tristearate. Thermal analysis by differential scanning calorimetry of the drug loaded SLN indicates the solid nature of the lipid carrier as required for sustained drug release.

1. Introduction

The concept of SLN has emerged by combining the advantages of polymeric nanoparticles (solid matrix) with parenteral emulsions (bio-compatibility, bio-degradability). Melt homogenization method for the production of sterile, pyrogen free, i.v. administrable SLN formulation can be scaled up from bench to bulk manufacture [1, 2] Müller et al. who have done the pioneering work in this field have reported that SLN are 10–100 fold less cytotoxic to their polymeric counterparts [3–5]. Moreover, Freitas developed a three-year stable SLN formulation [6, 7], a factor with paramount importance with respect to colloidal drug carriers.

Although widely studied, detailed reports related to formulation of SLN with respect to particle size distribution are rare. In this work, SLN were characterized with respect to particle size by Photon Correlation Spectroscopy (PCS). Detailed effect of homogenization process and formulation parameters on particle size distribution was studied with blank SLN. Drug loading studies were performed using nimesulide as a model drug. Selection of the drug was based on its highly lipophilic nature and very low aqueous solubility. Various approaches to improve drug loading were studied. Developed formulations were analyzed by DSC to assess the sustained release potentials of SLN.

2. Investigations, results and discussion

2.1. Studies on blank SLN

Effect of the process parameters was studied with respect to homogenization pressure and number of cycles.

Homogenization pressure has a critical effect on particle size distribution. Mean particle size as well as polydispersity index (PI) values were reduced at increasing homogenization pressure from 250 bars to 600 bars (Table 1). Particle size distribution data (Fig. 1) indicates that percent particles in higher size ranges (450–1000 nm and 1000–3000 nm) are considerably lowered from 450 bars to 600 bars homogenization pressures. At the same time percent particles in 100–450 nm are considerably increased from 250 to 600 bars through 450 bars. The results indicate that homogenization efficiency increased with higher homogenization pressure. Homogenization at even higher pressure up to 1500 bars is possible with other models of APV Gaulin, further increasing the homogenization efficiency.

At 600 bars there was no significant difference in mean particle size and PI values (Table 2) between 5 and 7 passes but after 10 passes the values were considerably lowered. Particle size distribution data indicate (Fig. 2) that percent particles in the 100–450 nm range were increased at the expense of particles in higher size range for 10 homogenization cycles. It can be concluded that the dispersion of required particle size distribution and mean

Table 1: Effect of homogenization pressure

Homogenization pressure (Bars)	Polydispersity index	Mean particle size (nm)
250	0.489 ± 0.031	382.3 ± 2.24
450	0.394 ± 0.022	315.6 ± 1.07
600	0.318 ± 0.016	240.4 ± 1.14

± values indicate standard deviation

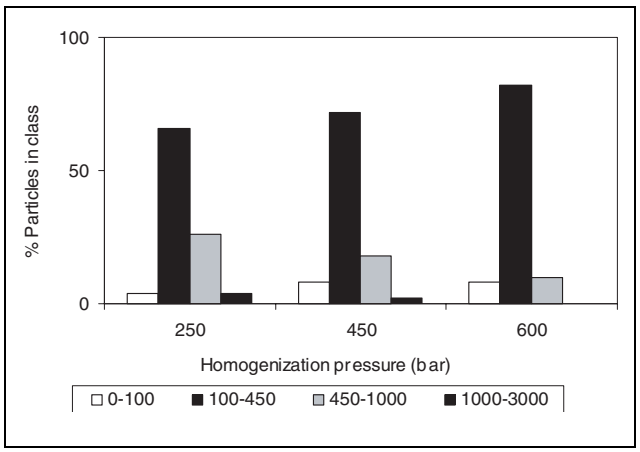

Fig. 1: Effect of homogenization pressure on particle size distribution

particle size can be produced by a combination of homogenization pressure and homogenization cycles.

The contribution of lipid concentration to the efficiency of homogenization process (Table 3, Fig. 3) indicates that for homogenization at the same pressure for the same number of cycles, the polydispersity values and mean particle size are slightly lowered for higher lipid concentrations.

The effect of surfactant concentration on particle size distribution and mean particle size was not significant (Table 4, Fig. 4) indicating less contribution of surfactants to the process of particle size reduction, hence surfactants contribute mainly to the stabilization of newly formed surfaces.

2.2. Studies on drug loaded SLN

Drug loading studies indicate that 90% of the total drug solubilized in triglyceride lipid melt (85 °C) was expelled from the solid lipid nanoparticles. High melt solubility but rapid expulsion within 24 h of storage at 4 °C by solidification of the dispersed particles can be attributed to the highly ordered crystalline lattice structure of the tristearin, a monoacid triglyceride (MAT) in solid crystalline state as reported by Westesen et al. [8] Garti and Sarig [9] have reported the detection of β tristearin in non-dispersed state after storage at room temperature for 24 h. Transformation into the β form (triclinic), the most stable with least vacancies for drug incorporation, could be the most logical reason for the drug expulsion. 10% of the drug loading might be associated with the coexistence of less ordered α (hexagonal) or β′ (orthorhombic) forms with the highly ordered β form.

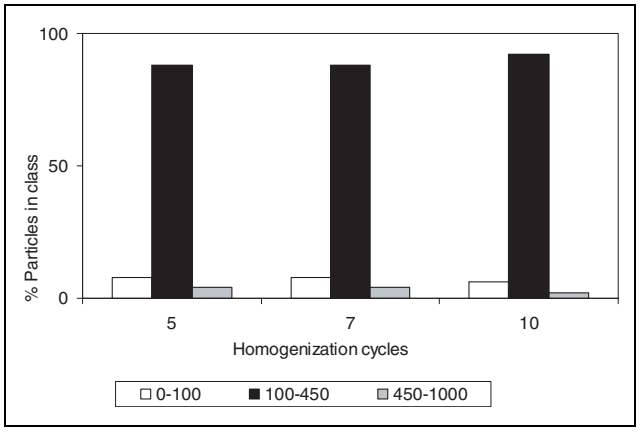

Fig. 2: Effect of homogenization cycles on particle size distribution

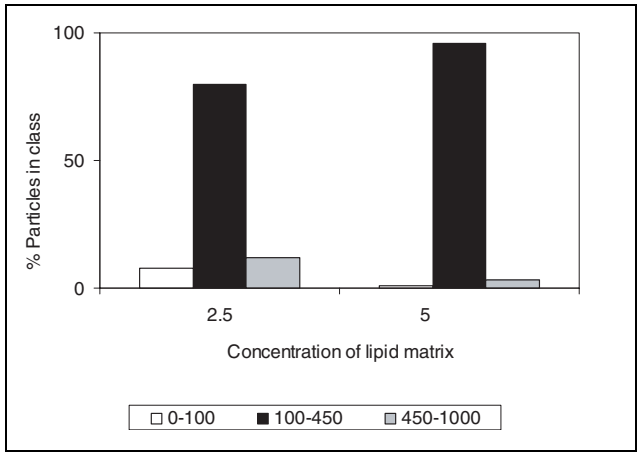

Fig. 3: Effect of lipid concentration on particle size distribution

Table 2: Effect of homogenization cycles

Homogenization cycles	Polydispersity index	Mean particle size (nm)
5	0.216 ± 0.033	225.3 ± 3.2
7	0.216 ± 0.029	229.1 ± 2.15
10	0.180 ± 0.15	193.6 ± 1.6

Table 3: Effect of lipid concentration

Lipid concentration	Polydispersity index	Mean particle size (nm)
2.5	0.318 ± 0.036	240.4 ± 4.72
5.0	0.216 ± 0.024	227.5 ± 1.68

Table 4: Effect of surfactant concentration

Lecithin	Polydispersity index	Mean particle size (nm)
α-Lecithin	0.224 ± 0.016	224.8 ± 1.67
Lipoid S-75	0.242 ± 0.012	241.7 ± 3.43
Lipoid S-100	0.221 ± 0.015	222.8 ± 2.64

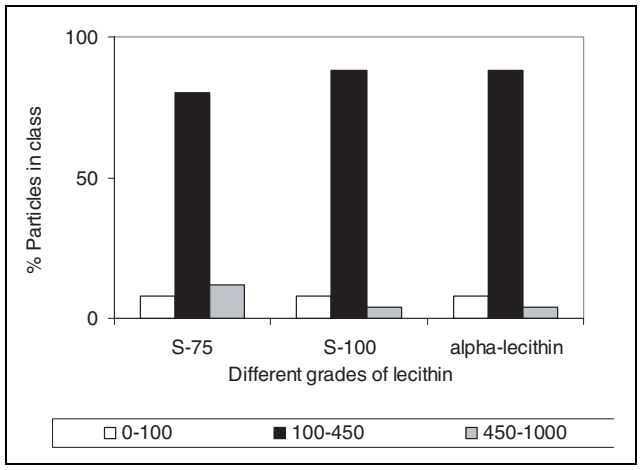

Fig. 4: Effect of surfactant concentration on particle size distribution

Table 5: Drug loading efficiency with different lipid matrices

Lipid	Loading efficiency after 24 h
Glyceryl tristearate	10.69 ± 1.34%
Glyceryl tristearate + SPAN 60	32.26 ± 2.26%
Glyceryl palmitostearate	27.57 ± 0.56%
Glyceryl behenate	25.68 ± 0.96%
Glyceryl behenate + SPAN 60	9.57 ± 3.26%

In order to increase the drug loading or drug carrying capacity of SLN, it is useful to prefer the α form and to prevent polymorphic transformation into the β form. In another study related to the kinetics of polymorphic transformation in monoacid saturated triglycerides and to the influence of certain solid surfactants, Garti and Aronhime [10] have reported the guidelines for selection of additive concerning their effect as dynamic controllers of polymorphic transformation by their capacity to hydrogen bond neighboring triglycerides, a phenomenon called 'button syndrome'. The criteria for the selection of the additives were as follows:

- They should be solid at room temperature, as liquid additives enhance the polymorphic transformation of triglycerides.
- They should posses a large hydrophobic head group, which can hydrogen bond with neighboring triglyceride molecules.
- A hydrophilic head group should be attached to a long saturated hydrocarbon side chain with a chain length perfectly equal to the hydrocarbon chain of triglycerides so that it can actually co-crystallize with the triglyceride and will interfere with polymorphic transformation.

Above all, the additive should allow i.v. administration. With all these constraints recommended additives for the final formulation with tristearate were glyceryl monostearate (GMO) and sorbitan monostearate (Span 60).

Formulating SLN of tristearin with Span 60, drug loading efficiency was increased from 10% to 34% indicating the increased presence of voids for the drug incorporation (Table 5). Mixed triglyceride-glyceryl palmitostearate increased drug loading to 28% as compared to 10% with tristearin, the MAT. With an asymmetric glyceride, glyceryl behenate, which is a mixture of mono-behenate, dibehenate and tri-behenate, drug loading efficiency was higher, 27% as compared to tristearate.

Characterization of solid lipid nanoparticles with respect to their physical state is of primary importance since the production technique (melt homogenization) utilizes dispersion of the molten lipid in a nanometric size range to form an O/W nano-emulsion and then solidification by rapid cooling to form solid lipid nanoparticles. Therefore it was required to ensure the complete solidification of the droplets emulsified. Complete solidification is required because of drug leakage (due to higher mobility of drug molecules in oil globules than in solid matrix particles) highly associated with liquid state of dispersed particles (high surface area/volume ratio) as observed in the case of o/w emulsions. Apart from preventing undesired drug leakage, solid matrix can provide prolonged drug release. There are reports by Westesen et al. [10] that SLN prepared from trimyristin (Dynasan 114) exist in the form of super-cooled melt and not solid nanoparticles.

Table 6: Results of thermal analysis of drug loaded SLN

Glyceride	Weight in pans (mg)	Lipid concentration	Reported	Melting peak			
				Start	End	M. P.	δH (J/g)
Tristearate	4.9	5%	67.1	62	76	71.27	2.06
Palmitostearate	3.8	2.5%	55.1	44	59	51.48	3.44
Behenate	3.4	2.5%	71.8	64	77	70.69	2.02

DSC analysis (Table 6) of tristearate SLN shows a single sharp peak at 71.3 °C indicating complete polymorphic transformation to the most stable form of tristearin. This must be the reason for low drug loading efficiency of tristearate SLN.

Occurrence of two melting peaks, at 51.5 °C and 63 °C indicates the presence of two polymorphic forms of palmitostearate, which needs to be characterized further. The broad peak indicates a less ordered structure or lack of uniformity in crystalline arrangements. This could explain the higher drug loading efficiency as compared to tristearate.

Glyceryl behenate shows two melting peaks, a major one at 70.7 °C characteristic of a stable polymorphic form, which needs to be characterized further.

3. Experimental

3.1. Materials

All tristearates (Compritol 888 ATO, Precifac) were gift samples from Gattefosse France; lecithins (Lipoid S75, Lipoid S100) were gift samples from Lipoid and poloxamer was a gift sample from BASF; Span 60, and GMO were of extra pure grade and obtained from ICI surfactants; All other chemicals were of AR grade and obtained locally.

3.2. Procedures

3.2.1. Effect of homogenization pressure

Formulations containing glyceryl tristearate (2.5% W), soya lecithin (Lipoid S 75 0.5% W), poloxamer 188 (1.0% W) were homogenized with a APV Gaulin LAB 60 homogenizer (APV Gaulin, Lubek, Germany) for 5 cycles at 250, 450, 600 bar pressure. The dispersion was characterized for particle size distribution.

3.2.2. Effect of homogenization cycles

Formulations containing glyceryl tristearate (5% W), soya lecithin (Lipoid S75 0.5% W), poloxamer 188 (1.0% W) were homogenized at 600 bar pressure for different homogenization cycles (5, 7, 10) and the dispersions were characterized for particle size distribution.

3.2.3. Effect of lipid concentrations

The formulations containing glyceryl tristearate (2.5% W and 5% W), soya lecithin (Lipoid S75 0.5–1% W), poloxamer 188 (1.0% W) were homogenized at 600 bars pressure for 5 homogenization cycles and the dispersions were characterized for particle size distribution.

3.2.4. Effect of surfactant concentration

Formulations containing glyceryl tristearate (2.5% W), different grades of soya lecithin Lipoid S75 (64% α-phosphatedyl cholin) Lipoid S100 (94% α-phosphatedyl cholin), α-phosphatedyl cholin in concentration 0.5% W, poloxamer 188 (1.0% W) were homogenized at 600 bars pressure for 5 homogenization cycles and the dispersions were characterized for particle size distribution.

3.2.5. Particle size measurement

Photon correlation spectroscopy (Dynamic light scattering) was the method of choice, which can be effectively used in the measurement of particles in the size range of 0.003–2 um. A zetasizer III (Malvern instruments UK) that works on photon correlation spectroscopy (PCS) was used for particle size analysis. This employs He, N_2 as a laser source and a Photo Multiplier Tube (PMT) as a laser detecting device.

For a single analysis the instrument performs three runs each of approx. 2 min duration. The values reported in the presented data are the average of three runs.

3.2.6. Drug loading studies

Lipid triglyceride (2.5% W), nimesulide (3.0% W of glyceride), α-lecithin (0.5% W), poloxamer 188 (1.0% W), glycerin (2.25% W) volume made

up to 650 ml with distilled water; homogenized at 600 bar for 10 cycles. Drug loading efficiency was determined after 24 h storage at 4 °C subsequent to production.

3.2.6.1. Determination of drug loading efficiency

Dug loading = total drug content (T) − expelled drug (E)
Drug loading efficiency was determined by the formula
Drug loading efficiency = drug loading · 100/total drug used.

3.2.6.2. Determination of expelled drug

The dispersion was uniformly mixed by gentle shaking, 0.2 ml of the drug loaded dispersion was taken and diluted to 25 ml with pH 9 phosphate buffer B.P. It was then ultrafiltered using Milipore membrane with the driving force being provided by centrifugal force at 4000 g (5000 rpm) for 45 min. The filtrate was then collected and diluted appropriately. Drug content was then determined by UV-spectroscopy interpolating the concentration from a standard curve prepared in buffer pH 9. This would give the amount of drug expelled in 0.2 ml SLN dispersion. The device was tested for any drug adsorption by centrifuging the known concentration of drug solution and then comparing the drug content of the filtrate and the original drug solution. There was no adsorption of drug on the filtration assembly of the centrifugal device.

3.2.6.3. Determination of total drug

The dispersion was uniformly mixed by gentle shaking, 0.2 ml of the drug loaded dispersion was solubilized in 4.8 ml of DCM : propanol (1 : 1). This was appropriately diluted with DCM : propanol (1 : 1) and analyzed for drug content using an UV-Visible spectrophotometer (CECIL 2021) at 295 nm. This would give the total amount of drug in 0.2 ml SLN dispersion.

3.2.7. Thermal analysis

Thermal analysis was performed in a Dupont 2100 calorimeter using accurately weighed samples. Heating curves were recorded with a scan rate of 5 °C/min.

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