PART 2

SCALE-UP FEASIBILITY OF SESAMOL LOADED SOLID LIPID NANOPARTICLES
CHAPTER I

PREPARATION, CHARACTERIZATION AND SCALE-UP OF SESAMOL LOADED SOLID LIPID NANOPARTICLES
1.0 INTRODUCTION

Solid lipid nanoparticles (SLNs) consist of spherical solid lipid particles in the nanometer range, dispersed in a solution of surfactant, which facilitates their passage across the biological membranes. Although there are numerous methods which discuss the preparation of SLNs, only few of them have the capability to be easily scaled-up.

The high pressure homogenization (HPH) technique which is being used since 1950s for the production of parenteral emulsions is the most common technique being used to produce large volumes of SLN dispersions. However, this manufacturing process requires large energy. Moreover, the process is not suitable for the production of water soluble drugs, which may partition towards the water phase as a result of the homogenization pressure, resulting in low entrapment efficiencies (Shegokar et al., 2010).

Still another efficient and rugged method of producing SLNs is the microemulsification method. In this method SLNs are prepared by the dispersion of warm oil-in-water (o/w) microemulsion in cold water (0-4°C); solid lipids with high melting points forming the internal phase of the microemulsions (Gasco, 1997). Although scaling up of this method for parenterals, was reported by Marengo et al. in 2000, but they scaled-up the process for 1 ml of microemulsion to only 100 ml using specially designed apparatus. In the apparatus the warm liquid microemulsion was sterilized through a 0.22 µm membrane filter as the nanodroplet size of warm o/w microemulsion is lower than 100 nm. After passing through the filter, the warm microemulsion was made to flow through the interchangeable needle and drop directly into a stirred cold aqueous medium (Marengo et al., 2000).

Small batches of 5 ml microemulsion being poured into 50 ml of cold water (1:10 dilution) were earlier being produced in our lab. However, in order to test the commercial viability of the process we planned to develop the scale-up batches of ≥1 litre (L) and characterize the developed SLN formulation for various in vitro parameters, including particle size, % entrapment efficiency (%EE), transmission electron microscopy (TEM), infra-red spectroscopy (IR), differential scanning calorimetry (DSC), powder X-ray diffraction studies (PXRD) and in vitro release. We
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proposed to produce three, 1L scale-up batches of sesamol SLNs, using microemulsification method with a dilution factor of 1:1, and characterize them suitably. A small dilution of 1:1 was the other intent of the study, so as to result in a concentrated dispersion of nanoparticles. Production of a very dilute SLN dispersion is one of the major limitation of microemulsification method. The method thus requires subsequent step(s) like diafiltration or lyophilisation to result in a stable concentrated product, followed by reconstitution for compliant usage. Both lyophilisation and reconstitution can result in aggregation of particles to a micron size. So the other very important consideration of the study was to try and develop a one step, concentrated and a ready to use dosage form, without any further treatment.

2.0 MATERIALS AND METHODS

2.1 Materials

Sesamol was obtained from Jubilant Life Sciences (Noida, Uttar Pradesh, India); Soy Lecithin (Hi Media, India); Tween 80 (S.D. Fine Chemicals Ltd., India); and Compritol® 888 ATO (Glycerol Behenate, gift sample from Gattefosse, USA) were also used in the study.

All other chemicals and reagents were of analytical grade and were used without further purification.

2.2 Preparation of scale-up batches of sesamol loaded solid lipid nanoparticles (S-SLNs)

Small batches (1X) of S-SLNs were produced using 10 ml glass beakers for maintaining the lipidic and aqueous phases at temperature of 80-85°C. Suitable quantities were taken to result in a 5 ml of microemulsion which was poured into ice-cold (0-4°C) water under magnetic stirring for the formation of dilute (10-100 times) solid lipid nanoparticulate dispersions.

For production of 100X batch of S-SLNs, 500 ml of microemulsion was produced, which was poured into equivalent amount of ice-cold water (500 ml) to result in a concentrated dispersion. All the ingredients were weighed proportionally to result in a 100X batch. The process involved, placing of polysorbate 80 (P-80)(45.45%), soy lecithin (0.58%), and water together in a beaker which was heated to the lipid melt...
Glyceryl behenate (7.27%) was melted separately at 82-85°C. Sesamol (1:10 with respect to lipid) was added to the aqueous phase, following which the hot aqueous emulsifier mix, was dropped at once into the lipid melt, under magnetic stirring to obtain a clear microemulsion. The hot microemulsion thus formed, was poured slowly in a streamline, into an equivalent amount of cold water (~2°C), under continuous mechanical stirring (5000 rpm) for 2.0 h. In the aqueous medium, SLNs are formed by crystallization of high melting point lipid droplets present as the oil phase of the microemulsion (Manjunath et al., 2005). The prepared SLNs were stored in refrigerator until further analysis. Three scale-up batches were produced to check reproducibility of the selected method. All the three were characterized using suitable parameters to ensure complete in vitro characterization and the results were also compared for uniformity of the parameters with the 1X lab scale batch.

2.3 Characterization of S-SLNs

2.3.1 Particle size analysis

The mean diameter of SLNs in the dispersion (with appropriate dilutions with TDW) was determined using photon correlation spectroscopy (Zeta sizer 2000, Malvern Instruments, UK).

2.3.2 Transmission electron microscopy (TEM)

The morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-100; Japan).

2.3.3 Differential scanning calorimetry (DSC)

DSC was performed with a Perkin-Elmer differential calorimeter. DSC is a tool to investigate the melting and crystalline behaviour of crystalline materials like SLNs (Orecchioni et al., 2003; Venkateshwarlu and Reddy, 2004). The breakdown or fusion of the crystal lattice by heating or cooling the sample yields information about the internal polymorphism, crystal ordering, or glass transition processes (Uner, 2006). It uses the fact that different lipid modifications possess different melting points and enthalpies. The thermal analysis of the pure drug, lipid and SLNs were done to observe for any significant changes in the pattern of the peaks. Samples were placed in a conventional aluminum pan and heated from 10°C to 250°C at a scan speed of 10 °C/min.
2.3.4 Infrared spectroscopy (IR)

The IR spectroscopy is used to elaborate the structure and stereochemistry of the bulk material and the nanoparticles (Muller, 1996; Saupe et al., 2006). Analysis of the drug, the lipid, and the formulated SLNs were done. The peaks obtained for the free drug and lipid and their physical mixture was compared with the lyophilized SLN formulation to observe for any significant changes.

2.3.5 Powder X-ray diffraction (PXRD)

The crystalline/amorphous nature of formulated nanoparticles was confirmed by X-ray diffraction measurements carried out with an X-ray diffractometer (XPERT-PRO, PANalytical, Netherlands). PXRD studies were performed by exposing the samples to CuKα radiation (45 kV, 40 mA) and scanning from 5° to 50°, 2θ at a step size of 0.017° and scan step time of 25 s. Samples used for PXRD analysis were same as those of DSC analysis. The instrument measures interlayer spacing d which is calculated from the scattering angle θ, using Bragg's equation nλ = 2d sin θ where λ is the wavelength of the incident X-ray beam and n is the order of the interference. Obtained XRD patterns were compared for characteristic drug peak intensity.

2.3.6 Total drug content (TDC)

TDC was estimated spectrophotometrically at λmax of 294 nm by disrupting 1 ml of the SLN dispersion using an appropriate volume of chloroform: methanol (1:1).

2.3.7 Entrapment efficiency (EE)

For estimating the EE, dialysis bag with a cut off of 12KDa (Hi Media) was used. 1 ml of S-SLN dispersion (3.72 mg/ml) was poured in the dialysis bag, both ends of which were tied hard to prevent any leakage. The bag was dipped in 100 ml of water stirred magnetically at 150 rpm. Dialysate was withdrawn after 15 min and analyzed spectrophotometrically (time was optimized using an equivalent amount of free sesamol to be released in water; this was done assuming if no amount of sesamol is entrapped within the SLNs).

\[
\% \text{ EE} = \left( \frac{\text{Total drug content} - \text{Amount of drug/ml of dialysate}}{\text{Total drug incorporated}} \right) \times 100
\]

Absorbance value obtained for blank SLNs treated in a similar manner was used as the control value to compensate for any interference of the ingredients. All the
determinations were performed in triplicate for all the three batches produced. Amount of drug in the dispersion retained inside the dialysis bag gave a direct measure of the extent of drug entrapped.

Values obtained for the amount of drug in the supernatant and that retained in the dispersion inside the dialysis bag were added to confirm the mass balance.

2.4 In vitro drug release

The in vitro release studies were carried out by the dialysis membrane method for studying the release of sesamol from the developed SLNs (Uner, 2006). The receptor media comprising 100 ml of phosphate buffer, pH 7.4, pre-equilibrated at 37±0.5°C was used for the studies. Aqueous SLN dispersion (1 ml containing 3.72 mg of sesamol as per the drug content studies) was placed in the dialysis tubing, which was then sealed from both the ends, and dipped into the receptor media, maintained at 37±0.5°C and stirred continuously at 150 rpm. 3 ml samples were withdrawn from the receptor medium with replacement at various time intervals and analyzed spectrophotometrically at 294 nm. All the measurements were performed in triplicate for the three scale-up batches and also for the 1X lab-scale batch. Results obtained were analysed statistically to confirm the reproducibility of the large scale batches produced using microemulsification technique.

2.5 Stability study

S-SLNs were stored in vials at 5±3°C for 3 months, and the samples were withdrawn at 0, 1 and 3 months, as per ICH guidelines. The average size, total drug content and the entrapment efficiency were determined at each time point.

3.0 RESULTS

3.1 Characterization of S-SLNs

3.1.1 Particle size analysis

Average particle size of all the three 100X batches was found to be ≤ 106.6 nm (Figure 1) with the actual size being 95.35, 98.11 and 106.6 nm for batch I, II and III. Respective PI for the batches was 0.432, 0.482 and 0.303. Particle size and the PI obtained for 1X batch was 122 nm and 0.255, respectively (Zetasizer 2000, Malvern Instruments, UK).
Size Distribution Report by Intensity

Sample Details

Sample Name: vanlife 1.30 h S-SLN BATVH 21
SOP Name: manu settings.nano
General Notes:

File Name: carbon nano tubes dt 21-
Dispersant Name: Water
Record Number: 37
Dispersant RT: 1.330
Material RT: 1.50
Viscosity (cP): 0.0137
Material Absorption: 0.01
Measurement Date and Time: Thursday, April 21, 2011 2:42...

System

Temperature (°C): 25.0
Duration Used (s): 60
Count Rate (kcps): 359.5
Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette
Attenuator: 5

Results

<table>
<thead>
<tr>
<th>Diam. (nm)</th>
<th>% Intensity</th>
<th>Width (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>166.5</td>
<td>96.9</td>
<td>99.44</td>
</tr>
<tr>
<td>0.303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.962</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Result quality: Good

Figure 1. Particle size report (100X; batch III) as obtained after analysis using PCS
3.1.2 TEM

When observed under TEM, SLNs were found to be spherical in shape (Figure 2). The size of the lipidic nanoparticles observed under TEM (40-90 nm) was close (though smaller) to the results obtained using PCS.

Figure 2. TEM micrograph of S-SLN

3.1.3 DSC

DSC is a thermoanalytical technique in which the difference in the amount of heat required to maintain the sample and reference at same temperature is measured as a function of temperature and time. The basic principle underlying this technique is that, when the sample undergoes a physical transformation (such as melting, desolvation), some amount of heat is required to flow through it, depending on whether the process is exothermic or endothermic to maintain both reference and sample at the same temperature. DSC measures this heat flow into or from the sample when it is heated or cooled. These measurements provide qualitative and quantitative information about physicochemical changes (i.e. endothermic, exothermic processes or changes in heat capacity). Figure 3 depicts DSC thermograms of pure sesamol, Compritol® 888 ATO and the formed SLNs. The DSC
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curve for pure sesamol showed a fusion endotherm at 65.43°C, corresponding to melting point of sesamol (60-65°C). While the physical mixture shows peaks 71.13°C (corresponding to the lipid) and another peak at 162.28°C (corresponding degradation peak of sesamol). Pure Compritol® 888 ATO shows an endotherm peak at 73.06°C. It also showed a degradation peak at >160°C. However, DSC developed SLNs did not show peak corresponding to sesamol confirming successful incorporation of drug into SLNs. A broad endotherm starting from 95.17°C and showing two distinct peaks at 99.97°C and 112.36°C was observed for SLNs, although a small shoulder was observed near the lipid melting point 73.06°C. Observation of broad peaks indicate the amorphous nature of developed SLNs. DSC for the lab scale (1X) batch and the 100X batch were more less similar.

Figure 3. DSC thermograms of pure sesamol, Compritol® 888 ATO, the physical mixture and the formed SLNs batches (1X and three 1(1 batches)

3.1.4 IR spectroscopy

The IR peaks obtained with the developed formulation of S-SLNs revealed an infrared molecular stretching of the –OH group (3400-3200 cm⁻¹) of the drug, when compared...
with the peak of pure sesamol (Figure 4). This is a direct indication of the forma
do of SLNs as the stretching could not be observed when the physical mixture (Fig
e 4) of the same components were analyzed. Thus the formation of SLNs coul
c confirmed by IR analysis. Similar studies have been reported by Mehnert

![Figure 4. IR Analysis of Sesamol, Compritol® 888 ATO, Physical Mixture i
Compritol® 888 ATO SLNs](image)

3.1.5 PXRD

Overlaid PXRD patterns of Sesamol, Compritol® 888 ATO, lyophilized blank SLN
SLN) and S-SLN are shown in Figure 5. PXRD pattern of sesamol exhibited sh
peaks at 2θ scattered angles 7.12, 15.19, 18.08 and 18.36 which indicated
crystalline nature. However, no characteristic peaks for sesamol in lyophilized
SLNs were observed. Latter indicated the amorphous nature of the formed SL
PXRD pattern of Compritol® 888 ATO shows sharp peaks at 2θ scattered angles 21.16, 23.37, 23.52 and 35.76; indicating crystalline state of Compritol® 888 ATO.

Figure 5. Overlaid PXRD patterns of Compritol® 888 ATO, Sesamol, BSLN and S-SLNs

3.1.6 TDC and EE

TDC and EE of S-SLNs (100X batch) was estimated to be 94.26±2.71% and 72.57±5.20% (n=9) respectively, and there was no significant difference (p≤ 0.05) between the TDC and EE of the three batches produced and the small scale batch (1X) (TDC-91.28±3.28%; EE- 67.28±3.86%). High (EE) values (~70%) indicate the efficiency of the method for preparation of sesamol loaded SLNs and TDC of > 90% confirms insignificant drug losses during formulation. A significant EE also shows the suitability of the components and their relative proportion in the formulated SLNs.

3.2 In vitro drug release

The drug release from S-SLNs at 37±0.5°C is shown in Figure 6. The release of sesamol from S-SLNs was fitted to a first order kinetics model and occurs through diffusion. The release was prolonged up to 24 h with approximately 50% of the drug being released before 4 h (Figure 6). Almost 90% of the drug was released in less than 16 h. Latter may be due to the water soluble nature of sesamol and hence
Part 2: Clincorporation into the outer phospholipid layer of the lipidic nanoparticles. Hna prolonged release was observed in the later stage (upto 24 h) and this iattributed to the diffusion of the remaining drug from the lipidic core. Ove system followed a non-fickian drug release. In case of 1X batch, slower releas obtained and a total release of ≥ 68% was achieved at the end of 24 h.

![Graph showing in vitro release profile of sesamol, from S-SLNs, by dialysis method](image)

**Figure 6.** In vitro release profile of sesamol, from S-SLNs, by dialysis method.

### 3.3 Stability study

After 3 months of storage at 5±3°C the S-SLNs were found to be staccordance to ICH guidelines for stability without any significant increase (p< the particles size (Table 1). Change in EE (5.2%) and TDC (2.2%) at 3 monalso insignificant (p< 0.05) with respect to the 0 time samples indicating a formulation.

**Table 1.** Stability study parameters during storage of S-SLNs at 5±3°C

<table>
<thead>
<tr>
<th>Time Points</th>
<th>Av. Particle Size (nm)</th>
<th>Total Drug Content (%)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time</td>
<td>106±19.6</td>
<td>94.26±2.71</td>
<td>72.57±5.20</td>
</tr>
<tr>
<td>1 month</td>
<td>95±22.0</td>
<td>92.70±4.16</td>
<td>71.60±1.49</td>
</tr>
<tr>
<td>3 month</td>
<td>109±15.6</td>
<td>92.30±1.58</td>
<td>68.30±2.15</td>
</tr>
</tbody>
</table>
4.0 DISCUSSION

Main aim of this research was to scale up the preparation process of SLN so as to result in a highly stable concentrated dispersion. Further, it was desired to design a process which can be carried out with utmost ease and if possible with a minimum sophistication of equipment so that there is a scope for its commercial launch even by conventional companies who are specializing in simple pharmaceutical products like emulsions or by the phytochemical industry which is ready to venture into developing “phytopharmaceuticals” with minimum investment in sophisticated equipments.

A stable microemulsion was formed even when it was prepared in a 100-fold (500 ml) batch volume in comparison to the small 1X lab batch. The formed microemulsion needs to be stable for a sufficient period of time to allow its slow and streamline pouring into the cold water, under mechanical stirring (5000 rpm). Latter process could now be completed in 30 min, with 5-10 min of pouring time, and 15 min of continued mechanical stirring even after the complete addition of microemulsion. In the small scale (1X) batch we were following stirring for long periods of time (3-4 h) post transfer of the microemulsion to achieve a small particle size.

In our previous experiments o/w microemulsion was dispersed using a 18 gauge needle attached suitably to a glass syringe. The same is recommended and reported (even for scale-up batches) by other workers (Gasco 1993, Marengo et al., 2000). Even though the process always resulted in a small particle size (less than 200 nm), but it is tedious and difficult to use a hot syringe (as the emulsion constitutes a high melting lipid, so any cool surface results in congealing of the lipidic phase); nor is the process suitable to be scaled up with ease on laboratory scale. To overcome the problem we tried to directly pour the microemulsion into cold water. We could successfully produce SLNs with required particle size and a low polydispersity index, and the process could be suitably scaled up to result in a concentrated (1:1 dilution) SLN dispersion (1 L), with the average particle size as small as 95 nm (PI of 0.3) for one of the batches. However, PI is not very important issue for the formulations which are administered per orally (Muchow et al., 2008).
SLNs are formed by rapid quenching of the warm o/w microemulsion and, the temperature difference between the warm microemulsion and the cold dispersing water plays an important role on the size of SLN (Cavalli et al., 1996). A rapid crystallization of the oil droplets of the warm microemulsion during quenching favours the formation of small SLNs, avoiding the coalescence among the oil nanodroplets.

Presently, the aqueous system constituted of sesamol in P-80, and soy lecithin, while Compritol® 888 ATO was chosen as the lipid component as it results in dispersions with small particle size (Kaur et al., 2008; Blasi et al., 2008). The main reason of selection of Compritol® 888 ATO as lipidic phase is its unique composition comprising of glycerol tribehenate (28-32%), glycerol dibehenate (52-54%) and glycerol monobehenate (12-18%). The most abundant fatty acid is behenic acid (> 85%) but other fatty acids (C16-C20) are also present. Due to the presence of partial acylglycerols, Compritol® 888 ATO has an amphiphilic character. Its hydrophilic lipophilic balance (HLB) is approximately 2, having a melting point between 69°C and 74°C and a density value of 0.94 g/cm³. Compritol® 888 ATO has a peroxide value lower than 6 meq O₂/kg, indicating a high chemical stability. It is purported that a lipid containing a major portion of diglycerides would have a better capability to hold an amphiphilic drug and would thus result in better entrapment efficiencies. Sesamol shows a solubility of 38.8 mg/ml and a log P of 1.29, favouring octanol phase thus indicating it to be an amphiphilic molecule.

The results obtained with the scale-up batches of S-SLNs, were found to be reproducible and further, there was no difference between the characterization parameters of 1X and 100X batch. **No significant difference (p≤0.05) between the particle size, drug content, and entrapment efficiency, both within the scale-up batches and 1X batch, are indicative of the robustness of the developed method.** However, a significant difference (p≤0.05) was observed for the cumulative amount released for the 1X batch, which was only 68% till 24 h and the 100X batch (> 90% at 24 h). This difference may be assigned to the amount of surfactant left unused after the formulation of SLNs. Scale up batches may accumulate higher % of surfactant both on the surface of the formed nanoparticles and/ or in the surrounding
aqueous phase helping the drug to diffuse out more rapidly especially the drug entrapped in the inner lipidic core to the outer aqueous phase.

A high reproducibility of results with respect to the characterization of SLNs may be assigned to an optimised formula for producing the microemulsion. Final composition of the microemulsion resulted in its spontaneous formation with an average particle size of less than 40 nm (Zeta sizer 2000, Malvern Instruments, UK). Latter when poured in ice cold water under high speed stirring was anticipated to result in solid particles in nanometric range. Stability of the formulation upto 3 months was in accordance to the ICH guidelines. It has been reported in several cases that SLNs show an inadvertent increase in particle size upon keeping and storage under refrigerated conditions, due to the gelling of the lipid phase. We however, did not observe any such gelling phenomenon, which may be due to the high viscosity imparted by the concentrated SLN dispersion. A high concentration of P-80 (small dilution of the microemulsion containing > 20% P-80) may also contribute to the high viscosity. Latter may also facilitate the nanoparticles to remain in a dispersed form without any aggregate formation.

It may also be noted that in other methods used for scaling up of solid lipid nanoparticles, especially high pressure homogenization, in general, plausibly large number of formulation parameters are required to be considered before reaching to an optimized formulation. Based on the obtained data, scale up using microemulsification technique proved to be relatively easy, mainly because latter is a spontaneous process and once the constituents (surfactant, lipid and water) are in appropriate proportion they will always result in a microemulsion. Furthermore, the final particle size of the formed SLNs is majorly monitored by the particle size of the microemulsion (which will form only when the particle size is in the nano range).

5.0 CONCLUSION

In conclusion, the SLN preparation process was successfully scaled-up to a 100X batch on laboratory scale and showed an easy performance and permitted to obtain reproducible SLN dispersions. Research is in progress to reach the second step of the scale up design to result in batches greater than 20 L and subsequently 200 L.