CHAPTER II

FORMULATION AND CHARACTERIZATION OF SESAMOL AND CURCUMIN LOADED SLNS
1. INTRODUCTION

The success of liposome and polymer based nanoparticles to deliver drugs to liver coupled with their limitations like toxicity, stability, difficulty in scalability on the other hand, asks for alternative nanoparticulate drug delivery. In this regard solid lipid nanoparticles (SLNs) are being looked upon with great anticipation. SLNs have been utilized as a carrier for improving the oral bioavailability (BA) of plant based drugs, however, only a few reports indicate their use for hepatic delivery, especially for plant based drugs (He et al., 2007; Hu et al., 2013; Lu et al., 2008; Yang et al., 2012). Howsoever, the reports demonstrate the potential of SLNs to improve the pharmacokinetics of poorly bioavailable drugs undergoing fast metabolism and clearance by improving absorption, permeation, avoiding metabolism, prolonging release and achieving a sustained action with reduced toxicity. SLNs are reported to efficiently incorporate lipophilic drugs (Hu et al., 2004; Kakkar et al., 2011) because the latter can be incorporated easily within the lipidic core. However, they are also reported to be suitable for hydrophilic drug molecules (Bhandari and Kaur, 2013a; Dong et al., 2009). Presently SLNs of curcumin (lipophilic) and sesamol (amphiphilic) were prepared using the microemulsification method. Characterization of sesamol SLNs (S-SLNs) and curcumin SLNs (C-SLNs) was done in terms of particle size, zeta potential, shape, drug content and % entrapment.

2. MATERIALS AND METHODS

2.1 Materials: Sesamol was obtained from Jubilant Organosys Ltd (Noida, Uttar Pradesh, India); Curcumin was a gift sample from Sanat Products Ltd, New Delhi, India. The sample constituted a mixture of three curcuminoids, namely curcumin (95%), demethoxycurcumin, and bisdemethoxycurcumin (latter two constitute the remaining 5%). Tween 80 (S.D. Fine Chemicals Ltd., India); Soy Lecithin (Hi Media, India) and Compritol® 888 ATO (Glyceryl 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 S-SLNs/C-SLNs: Polysorbate 80 (45.45% w/v), soy lecithin (0.58% w/v) were added to water (aqueous phase) and heated to the lipid melt temperature (82-85°C). Compritol® 888 ATO was taken as the (7.27% w/v) lipid phase and was melted separately at 82-
85°C. Sesamol/curcumin 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 transferred into an equivalent amount of cold water (~2°C) under continuous mechanical stirring (5000 rpm) for 1.5 h. In the aqueous medium, SLNs are formed by crystallization of the oil droplets present in the microemulsion (Kakkar et al., 2011; Manjunath et al., 2005). The prepared SLNs were stored in a refrigerator until further analysis. Blank SLNs were prepared similarly without addition of any drug agent.

2.3 Characterization of S-SLNs/C-SLNs

2.3.1 Particle size analysis: The mean diameter and polydispersity index (PDI) of SLNs in the dispersion was determined using photon correlation spectroscopy (PCS) using DelsaNano C, Beckman Coulter, Inc.

2.3.2 Zeta potential: Zeta potential of SLNs in the dispersion was determined using DelsaNano C, Beckman Coulter, Inc.

2.3.3 Transmission electron microscopy (TEM): The morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-100; Japan). Samples were stained with phosphotungstic acid (PTA, 2%, 5 min and excess PTA removed), spread on a copper grid and examined.

2.3.4 Total drug content (TDC): TDC was estimated spectrophotometrically at λ\text{max} of 294 nm for S-SLNs and λ\text{max} of 425 nm for C-SLNs by disrupting 1 mL of the SLN dispersion using an appropriate volume of chloroform: methanol (1:1).

2.3.5 % Entrapment: The % entrapment of S-SLNs was estimated by dialysis bag method where 1 mL of S-SLNs dispersion was dialysed against 100 mL of water to remove the free sesamol, maintained at 37°C and stirred at 150 rpm, using dialysis bag (12 KDa, Hi Media, India). After 15 min SLN dispersion remaining in the dialysis bag, was disrupted with a suitable quantity of chloroform: methanol (1:1, v/v) and the clear solution was analyzed spectrophotometrically to give a direct measure of the entrapped sesamol/mL of the SLN dispersion.

Similarly, for estimation of % entrapment of C-SLNs dialysis bag method was used where in 1 mL of C-SLNs dispersion was dialysed against 50 mL of methanol, maintained at 37°C and stirred at 50 rpm, using the dialysis bag (12 KDa, Hi Media, India). After 45 min, contents of the
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dialysis bag i.e. C-SLNs (after disruption with suitable quantity of chloroform: methanol:: 1:1) were analyzed for curcumin spectrophotometrically to give a direct measure of the entrapped curcumin/mL of the SLN dispersion.

3. RESULTS

3.1 Characterization of S-SLNs

3.1.1 Particle size analysis: The S-SLNs formulation exhibited a small particle size below 200 nm (D 50 % was found to be 120.30 nm) with PDI of 0.111 (Figure 1).

3.1.2 Zeta potential: Zeta potential of S-SLNs in the dispersion (with appropriate dilution) was 51.31 mV (Figure 2).
3.1.3 TEM: When observed under TEM, S-SLNs appeared singly, with a spherical shape (Figure 3). The size of the nanoparticles under TEM was observed to be in the range of 60-140 nm confirming the results obtained using PCS.
3.1.4 TDC: Total drug content of the prepared S-SLNs was estimated to be $3.10\pm0.01$ mg/mL ($n=3$) and this value was used for all subsequent dose calculations for animal studies.

3.1.5 % Entrapment: % entrapment of sesamol in S-SLNs was estimated to be $73.92\pm2.49$ % ($n=3$).

3.2 Characterization of C-SLNs

3.2.1 Particle size analysis: The C-SLNs formulation exhibited a small particles size below 200 nm (D 50 % was found to be 174.4 nm) and PDI of 0.318 (Figure 4).

3.2.2 Zeta potential: Zeta potential of C-SLNs in the dispersion (with appropriate dilutions) was $-80.55$ mV (Figure 5) when determined using DelsaNano C, Beckman Coulter, Inc.

3.2.3 TEM: When observed under TEM, C-SLNs were found to be near spherical in shape (Figure 6) and the particle size matched with those obtained using PCS.
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3.2.4 TDC: Total drug content of the prepared C-SLNs was estimated to be 3.31±0.01 mg/mL (n=3) and was used for dose calculation in subsequent animal studies.

3.2.5 % Entrapment: % entrapment of sesamol in C-SLNs was estimated to be 83.92±0.49 % (n=3).

Figure 4: Particle size analysis of C-SLNs.
Figure 5: Zeta potential of C-SLNs.

Figure 6: TEM micrograph of C-SLNs.
4. DISCUSSION

Previously reported studies on SLNs encapsulating phytochemicals for hepatic delivery have used homogenization (He et al., 2007), film evaporation extrusion method (Hu et al., 2013; Lu et al., 2008) and emulsification ultrasonic dispersion methods (Yang et al., 2012) of preparation. However, all but one (He et al., 2007) reported the use of i.v route of administration for the developed SLN systems. Presently we propose the microemulsification method for oral hepatic delivery of the phytochemicals. Microemulsions are clear, thermodynamically stable and optically isotropic systems which require less energy and are obtained spontaneously by mixing surfactant, co-surfactant, lipid and water (Gasco, 1997). Microemulsification method provides efficient, easy, cost effective and rugged method of producing SLNs (Das and Chaudhury, 2011; Kakkar et al., 2011b) and further to it large-scale production of SLNs by the microemulsion technique is also feasible (Kakkar and Kaur, 2012). It may also be highlighted here that the method of preparation and the formula optimized in our laboratory (Kakkar et al., 2011b) uses dilution of hot microemulsion with a significantly small (1:1) quantity of cold water (usual recommended volume is 1:25 to 1:100) such that it results in a concentrated dispersion. Latter ensures (i) a higher drug content/volume of the dispersion such that suitable doses can be administered in smaller volumes and, (ii) overcomes the need to concentrate the dispersion by dialysis or lyophilisation. Both of which are time consuming and costly techniques.

Compritol® 888 ATO was chosen as the lipid component of the SLN formulations because its use is reported to result in stable dispersions with small particle size (Bhandari and Kaur, 2013b; Cavalli et al., 1997). Compritol® 888 ATO has a peroxide value lower than 6 meq O$_2$/kg, indicating a high chemical stability (Kakkar and Kaur, 2012). Tween 80 assigns both stability and improved BA to SLNs. Being a permeability enhancer, it tends to increase the permeability of the drug carrier across the intestinal membrane as it is reported to have inhibitory effects on intestinal efflux transporters (P glycoprotein efflux pumps) (Hu et al., 2010) which is regarded as major impediment leading to low or variable oral absorption and BA (Wacher et al., 2001) of various drug agents. Tween 80 being amphiphilic in nature also aid in minimizing the opsonization of the nanoparticles (hydrophobic surface are prone to clearance by opsonization) and prolong the circulation of SLNs in vivo (Muller et al., 1996; Singh and Lillard, 2009).
Size of nanoparticles monitors their uptake, into pathological and inflamed tissues by macrophages, or delivery across the fenestrae of the liver sinusoid (Hu et al., 2007; Li et al., 2010; Liang et al., 2005). It has been reported that nanoparticles with a diameter of less than 200 nm reach the parenchymal cell of the liver and generate higher efficacy (Hashida et al., 1998; Li et al., 2010; Yen et al., 2009). Thus, it is proposed in the present study that SLNs (<200 nm) will pass through the sinusoidal fenestrations and effectively build up a high concentration in the space of Disse, where diffusion to the various liver cell types will take place and the drug agent entrapped in SLNs will be released at the site of damage. In an early study authors (He et al., 2007) have reported 2.79 times improved oral BA of silymarin upon incorporation into SLNs (170.7 nm) which could result in a better drug targeting to the liver. Similarly, in a very recent study (Rao et al., 2014) authors prepared SLNs of bixin (135.5 to 352.8 nm) to improve its low BA and enhance its hepatoprotective property. The authors evaluated bixin SLNs in an acute model of paracetamol induced hepatotoxicity and reported better effects of SLNs in comparison to free drug (2.5 mg/kg) in terms of serum injury markers, oxidative stress markers and histology.

Furthermore, a PDI of ≤0.3 confirms uniform distribution of nanosized particles in the SLN dispersion with low incidence of micrometer particles (Figure 1 and Figure 4). The latter may also indirectly be taken to confirm that all particles are of nano size and there are no aggregates. The small size of the stable and uniform S-SLNs/C-SLNs dispersion is expected to result in cumulative uptake by the pathological liver over prolonged period due to repetitive filtration and passes through a probable facilitated transport across the fenestrae of the liver sinusoids and the same has been reported by us for C-SLNs in a post-induction sub-chronic model of hepatotoxicity (Singh et al., 2014). Nanoparticles having zeta potential > ±30 mV are stable dispersions with little or no chance of aggregation (Singh and Lillard, 2009). Thus, the SLNs prepared in the present study are expected to remain stable even when present as disperse systems and the same is confirmed by the TEM analysis (Figure 3 and Figure 6) wherein SLNs are depicted to occur as single or separate entities with no sign of aggregation. A stability of up to three months and one year for the respective S-SLN and C-SLN dispersion upon refrigeration (5±3°C) is reported by us earlier (Kakkar and Kaur, 2012; Kakkar et al., 2011). A high % entrapment of 73.92 and 83.92 was presently observed for S-SLNs and C-SLNs respectively.
5. CONCLUSION

The developed SLNs exhibited size <200 nm, high % entrapment and stability in dispersion. Further to it, the use of Tween 80 gives an edge to the prepared SLNs in terms of overcoming clearance by opsonization and thus preventing induction of significant aggregation with serum proteins self-aggregation. The above mentioned characteristics make the prepared formulations suitable for oral hepatic delivery and will aid in overcoming problems associated with these agents as highlighted in the ‘Aims & Objective’ section.