SUMMARY AND CONCLUSIONS

It may be noted that use of methods like high pressure homogenization for scaling up of solid lipid nanoparticles, involves plausibly large number of formulation parameters that need to be considered before arriving at an optimized formula and formulation. In the present investigation scale up of the microemulsification technique to produce SLNs proved to be relatively easy, may be because the former is a spontaneous process and once the constituents (surfactant, lipid and water) are in appropriate proportion a stable microemulsion will almost always result. 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).

SLN preparation process was herein successfully scaled-up to a 100X batch on laboratory scale and showed an easy performance and permitted to obtain reproducible SLN dispersions in terms of particle size, drug content, and entrapment efficiency. Latter coupled with a confirmation of stability (3 months; proposed to extend further) of the formulation, in accordance to the ICH guidelines, validates robustness of the scale-up process. Developed SLNs did not show any crystal growth, increase in size or gelling upon storage, commonly reported for SLN formulations. This was attributed to a high viscosity imparted by the concentrated SLN dispersion including high tween 80 concentrations (small dilution of the microemulsion results in > 20% tween 80 concentration). Latter may also facilitate the nanoparticles to remain in a dispersed form without any aggregate formation. Another promising finding of the study was a significant increase (p<0.05) in the cumulative amount released for the 1X batch, from 68% till 24 h to > 90% at 24 h for the 100X batch. This difference was allocated to a higher % of surfactant both on the surface of the formed nanoparticles and/or in the surrounding aqueous phase, of the scale-up batch, helping the drug to diffuse out more rapidly especially the drug entrapped in the inner lipidic core to the outer aqueous phase. It is now planned to reach the second step of the scale up design to result in batches greater than 20 L and subsequently 200 L.

Second part of the study involved development of a highly validated, sensitive and specific HPLC technique for the quantitation of sesamol in plasma as well as the
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Brain tissue. The method was validated with a routine sensitivity limit of 0.5 μg/ml in 0.15 ml rat plasma. To our knowledge, this is the first report on a method which is rapid, sensitive, precise and accurate for measuring sesamol both in plasma and brain. Further to the above, excellent long term (-20°C for 5 days), bench top, processed sample and auto-injector stability of sesamol in plasma as well as brain samples was established, for the developed method.

The method was then applied to determine the concentration of sesamol in the plasma and brain homogenates of the mice administered S-SLNs and S upto 24 h after administration. **S-SLNs were developed with intent to improve the BA of sesamol especially in the brain tissue.** Sesamol is metabolized to sulfates and glucuronides in the plasma and it shows a low oral bioavailability of 35.5±8.5%. It is postulated that sesamol is incorporated into the liver first and then transported to the other tissues (lung, kidneys, and brain). Free sesamol can cross the BBB but its metabolic conjugates are majorly distributed to plasma and show a poor permeability across the BBB. Similar observations were made in the present study, where the concentration of free drug in plasma and brain was fairly low (as is expected because of its conversion to conjugated metabolites).

One of the most challenging problems, in drug development is to manage to distribute drugs to the CNS across the blood–brain barrier (BBB). The BBB is a system of vascular cellular structures, mainly represented by tight junctions between endothelial cells, and an ensemble of enzymes, receptors, transporters, and efflux pumps of the multidrug resistance (MDR) pathways that control and limit the access of molecules to the brain, either by paracellular or transcellular pathways. Since the vascular density in the brain is very high, once molecules have penetrated the BBB, they distribute rapidly to the whole brain tissue. Thus, an ideal theoretical therapeutics-delivery system for brain would be one that transports drugs across the cerebral vasculature and delivers them to their target, that is, the brain cells.

S-SLNs were developed in the present study to suitably deliver sesamol across the BBB. The concentration of sesamol in plasma upon administration of similar dose of S-SLNs was 1.22 times higher than that achieved with free drug. Further, a higher t\text{max} (8h) indicating a sustained effect and an enhanced BA were observed in terms of higher AUC\text{0–}\text{t} values. Achievement of higher BA can be assigned to the exhibition of
bioadhesion of S-SLNs to the gastrointestinal tract wall and/or their convenient passage into the intervillar spaces, thus increasing their residence time in the gastrointestinal tract because of the nanoparticulate nature of S-SLNs. Further, the use of tween 80 as a surfactant may also contribute to an increase in the permeability of the intestinal membrane as a result of improved affinity between lipidic particles and the intestinal membrane. Additionally, some of the particles may be taken up directly into the lymphatic organs and eventually enter the systemic circulation, overcoming the first pass effect and metabolism of sesamol to its conjugates. P-glycoprotein transporter located majorly in the intestinal gut, blood–brain barrier, liver and kidney, plays an important role in the membrane transportation monitoring ADME of a drug molecule. Presence of tween 80, which moderately inhibits the P-glycoprotein efflux system, in our S-SLN formulation may thus be responsible for an improved oral absorption of sesamol, achieved in the present study.

Additionally, administering SLN formulations tend to protect the entrapped drug from physiological as well as enzymatic degradation, thereby helping to put off the in-vivo metabolism. Protection of sesamol from metabolism (to form conjugates) due to its incorporation into SLNs combined with the intrinsic nature of the lipidic particles to cross the BBB may result in the transport of a higher amount (1.56 times) of sesamol into brain. The exponential increase in the brain concentration of sesamol post S-SLN administration may be due to (i) RES bypass by SLNs due to small particle size, hence prolonged circulation, (ii) inhibition of P-glycoprotein by polysorbate 80 present on the surface or within S-SLNs, (iii) adsorption of apolipoprotein-E on the surface of S-SLNs because of the presence of, again, tween 80 which shows a preferential pathway across the BBB. Results of the pharmacokinetic studies confirmed the successful targeting of sesamol to brain.

This bioavailable and stable solid lipid nanoparticulate formulation of sesamol would be highly effective for the treatment of various disorders of the brain, which is very well documented in our recent study on usefulness of S-SLNs in controlling oxidative stress, anxiety and memory loss associated with menopause (using ovariectomised animal model) (Kakkar et al., 2011a).