CHAPTER 5

SUMMARY AND CONCLUSION

The main objective of the present study was to develop and characterize the camptothecin loaded microemulsions, for passive/active targeted delivery of the camptothecin to breast cancer tissues and to achieve improvement in treatment. To fulfill this objective, camptothecin loaded microemulsions and magnetic microemulsions composed of benzyl alcohol: captex 300 (3:1), TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate): Tween 80 (1:2) and camptothecin-loaded- polymer stabilized microemulsion composed of capmul MCM: poloxamer 407 (4:1), solutol HS 15: simulsol P23 (1:2) and water were developed and evaluated.

In order to develop camptothecin microemulsions, the solubility of CPT in various oils and surfactants was determined. Among these the mixtures of benzyl alcohol: Captex 300(3/1) posses the higher solubility of CPT than other medium chain triglyceride oils and chosen as oil phase. It is has also good miscibility with Vitamin E TPGS, Tween 80 and thus provides an isotropic stable system.

To develop camptothecin polymer stabilized microemulsions, the miscibility and solubility of CPT- poloxamer mixture in various oils were analyzed. Capmul MCM showed good miscibility and high solubility of CPT polymer mixture than other tested oils. Since the Capmul MCM as oil phase and Solutol HS 15 and Simulsol P 23 (1:2) as Smix were used for the development of PSMEs.
Pseudoternary phase diagrams were constructed to identify the ME forming region. Vitamin E TPGS: Tween80 at the ratio of 1:2 showed more monophasic area of microemulsion region with benzyl alcohol: Captex 300(3/1) and water. While, Solutol HS 15 and Simulsol P 23 at the ratio of 1:2 showed more monophasic area of microemulsion region with Capmul MCM and water. It had greater solubilization capacity for CPT and also acceptable for parenteral route.

The selected compositions from the phase diagrams were subjected to thermodynamic stability stresses. The compositions that withstand the thermodynamic stress were used to prepare stable camptothecin ME, MME and PSME. The formulated microemulsions were characterized for the globule size and its distribution, pH, refractive index, surface morphology, magnetic susceptibility and, effect of droplet size in plasma and evaluated for \textit{in-vitro} and \textit{in-vivo} targeting potential, \textit{in-vitro} drug release, \textit{in-vitro} haemolytic potential, cytotoxicity, genotoxicity, \textit{in-vivo} biodistribution and lactone ring stability.

The optimized microemulsions possess the mean globule size range of 39–58 nm and 158 - 206 nm for ME and MME. The zeta potential of the formulations varied between -0.1 and –2.95 mV. The mean globule size of the optimized polymer stabilized microemulsions possess the range of 12–31 nm and zeta potential ranges from -3.39 to 3.91 mV. All the CPT MEs and CPT PSMEs showed the pH about 5.5 and 4.71 - 4.8, which is within the acceptable pH range (4.5–8.0) for intravenous injections. The acidic nature of these formulations helped the existence of camptothecin in its active lactone form during storage. The mean values of the refractive indices of all the formulations were found to be similar (1.46 to 1.52) indicating isotropic nature of the MEs. The viscosity of the camptothecin MEs and CPT PSMEs were found to be in the range of 20–22 cP and 21–23 cP. There was no
significant change in viscosity even after the addition of magnetite in the ME and by changing the oil/surfactant concentration in ME and PSME. The amount of camptothecin present in the MEs, MMEs and PSMEs were evaluated by RP–HPLC and found to be in the range of 331–419 µg/mL for ME and MME and 114.55 – 193.81 µg/mL for PSME.

Photomicrograph of transmission electron microscope of ME, MME and PSME showed uniform distribution of spherical shaped oil droplets in continuous aqueous phase. The magnetic oil droplets were also more or less spherical in shape and appeared as dark particle with bright halo. Observation of camptothecin loaded MEs under fluorescent microscope showed a uniform distribution of oil globules and drug in ME and MME formulations.

The magnetic susceptibility of the camptothecin MMEs was in the range of $52–56 \times 10^{-6}$ indicating the formulated MMEs possess superparamagnetic properties. In-vitro targeting potential of the camptothecin MME study shows most of the magnetite containing oil droplets dispersed in water were attracted towards where the permanent magnet (1000 gauss) was placed. The incubation of MEs, MMEs and PSMEs with rabbit plasma for 3 h did not cause any significant change in its droplet size indicating poor adherence of plasma proteins over the surfaces of the nanodroplets.

*In-vitro* drug release of MEs, MMEs and PSMEs formulations were carried out by dialysis bag method and the mean (n=3) cumulative percentages of drug released were found to be range of 49.41 to 65.31 %, and 71.41 % to 76.07 % and 72.05 to 76.59 % at 24 h for ME, MMEs and PSMEs. All the formulations showed a rapid release of the drug at initial stage, and then exhibited an extended release over the period of time.
The developed ME, MME and PSMEs showed slight haemolytic activity (≤ 20%) but within the acceptable levels, also highly significant when compared with positive control (p<0.05). Camptothecin-loaded ME, MME and PSMEs showed significantly (p<0.05) higher levels of cytotoxicity to MCF-7 cells than that of camptothecin solution. The blank ME, blank MME and blank PSMEs were showed no significant cytotoxicity against MCF-7 cancer cells whereas blank MME at used concentrations showed some inhibition of cell growth. The result of the AO/EB assays showed that the camptothecin loaded ME, MME and PSMEs caused an increase in number apoptotic cells (MCF-7) than blank microemulsions. Alkaline comet assay confirms that the formulation and its components of the developed microemulsions did not produce any noticeable DNA damage in lymphocytes.

Camptothecin-loaded ME and PSMEs showed passive targeting of CPT into breast cancer tissues than other tissues. The active targeting studies on CPT MME confirmed the more accumulation of CPT in breast cancer tissues. We have also demonstrated that by using simple external magnetic field, MME can be directed for delivery of a drug to the target tissue within the body.

The in-vivo biodistribution studies revealed that PSME, MME and ME can able to efficiently target the breast tissue with maximum concentrations of CPT internalization and accumulation. Lactone ring stability study showed around 80% of the CPT in bioactive lactone form, which indicates that the incorporation of the CPT in the ME, MME and PSMEs may be the lucrative for the protection of the active lactone form. Hence, we conclude that camptothecin-loaded ME, MME and PSME developed in this study may act as a promising nanocarrier system for efficient parenteral targeted delivery of the CPT to the cancer tissues with better circulatory effect.
5.1 LIMITATIONS AND FUTURE PERSPECTIVE

The developed microemulsion systems has to be evaluated for *in-vivo* anticancer efficacy using murine mice models and long term stability as per ICH guidelines to explore the possibilities of utilizing the camptothecin loaded ME, MME and PSMEs for the treatment of cancer. On successful clinical trials, camptothecin may be utilized as therapeutic molecule for the treatment of different types of cancer.