Rationally designed drug delivery system enables us to precisely control drug release rates for prolonged duration and sometimes help targeting the drugs such as anti cancer agents to specific body sites. Only in recent years, the idea of development of such systems became practical. In a short time, new drug delivery systems have had an impact on nearly every branch of medicine. With this knowledge, we successfully applied liposome formulation technology for the anticancer drug, Irinotecan. The design and development of liposome formulations posed various challenges involving optimization of drug entrapment, release from the liposome vesicles, stability evaluation, pharmacokinetic and toxicokinetic evaluation and utility as a safer alternative to conventional i.v. administration. The basic idea was to reduce the toxicity of the cytotoxic anticancer drug by making use of relatively low doses and retaining the parent drug in the circulation for longer duration of time, without compromising the clinical efficacy. Liposome formulation demonstrated a great potential in this regard. However, some major issues limiting their utility are short circulation half-life of vesicles, lack of long term stability, batch-to-batch reproducibility, suitable sterilization method, particle size control, and difficulty in large scale production, most of which are acknowledged by our research. These are the problems seem to be limiting the manufacture and development of the liposomes.

The research envisaged in this project, encapsulation of Irinotecan in liposomes employing a lipid hydration procedure resulted in high trapping efficiency and excellent drug retention properties. The flexibility of liposome generation and loading procedure used here has allowed new insights to be gained into various parameters like liposome size, lipid composition and drug to lipid ratio on entrapment efficiency.

Pharmacokinetic and toxicokinetic evaluation along with toxicological assessment is an essential part of development of a novel drug delivery system. The study involved meticulously designed methodology comprised of development and validation of a sensitive, specific, robust bioanalytical method for the determination of Irinotecan and its metabolite, SN-38 by LC- ESI-MS/MS technique, appropriate grouping of animals for pharmacokinetic and toxicokinetic experiments and safety evaluation comprising
Conclusion

Body weight assessment, diarrhea grading, hematological evaluation, serum biochemistry along with histopathological evaluation.

The successful hyphenation of LC and MS has had an enormous impact on the field of quantitative analysis of drugs in biological samples for pharmacokinetic and toxicokinetic evaluation. MS detection provided far better sensitivity and selectivity as compared to other method of detection. Hyphenated Liquid Chromatography and Mass Spectrometry was the most suitable application for identification and quantification of parent drug (Irinotecan) and its metabolite (SN 38) simultaneously.

The LC-MS/MS method developed was simple, robust, specific, selective, sensitive and accurate for Irinotecan and SN-38. It was successfully applied to in vitro and in vivo evaluation of liposome. The assay method found to be suitable for routine analysis of Irinotecan and its metabolite SN-38 in pharmacokinetic and toxicokinetic studies. The simplicity and specificity of the extraction method made it an attractive procedure in high-throughput bioanalysis of Irinotecan and SN-38.

The pharmacokinetic and toxicokinetic evaluation was performed with the help of software, Winnonlin version 5.0.1. This software is the most accepted tool by the industry and regulatory agency for performing pharmacokinetic and toxicokinetic evaluations. The pharmacokinetics of liposomal Irinotecan differed significantly from those of Irinotecan i.v. formulation. The kinetic variables derived from toxicokinetic study correlated with the toxicity findings. The results revealed that the reduction in body weight, reduction in blood counts, severe diarrhea and histopathological changes of selected organs in conventional i.v. formulations were positively correlating predominantly with plasma concentration of SN-38. The same was not observed with liposome formulation.

The study demonstrated that the methodology employed for the formulation of liposome had given promising results in terms of simplicity in preparation, optimum drug entrapment and release, relatively increased stability and yet not contributing to the toxicity. We could demonstrate that our formulation is a promising candidate for further development and clinical utility as the results of in vitro characterization were completely supported by in vivo pharmacokinetic study. In addition to this to strengthen
the argument of safety of formulation, there was a positive correlation between the toxicity observed and toxicokinetic profile when compared with the conventional *i.v.* formulation.

In conclusion, liposome encapsulation of Irinotecan results in a potent drug formulation for the treatment of models of colorectal cancer as a result of increased drug longevity, protection of the active lactone species, maintains an effective plasma concentration and brings significant reduction in toxicity as compared to conventional *i.v.* formulation. The continuing studies focusing on optimizing the lipid formulation as well as studies with second agents that combine well with Irinotecan could result in a chemotherapeutic strategy that will improve survival for colorectal cancer patients.