6. SUMMARY AND CONCLUSION

Site specific drug delivery systems has riveted the attention of drug delivery, scientists as they can deliver the drug molecule in the vicinity of the target sites as well as they have potential of providing the patient with therapeutic levels of an adequate active principal as required. Different routes of administration have been studied for site specific delivery of the drugs. Out of which oral route is most preferred route because of several advantages associated with it such as ease of administration, avoidance of pain and discomfort leading to high patient compliance, elimination of infections and the ability to formulate more than one dosage form.

In early nineties the small intestine was contemplated as major site for drug absorption, while large intestine (colon) was considered as inconsequential for the delivery of orally administered drug molecules. However, recent advances in the technologies and fifteen years of research have turned this into one of the alternative site for the release of drugs. The colon targeted drug delivery has assimilated an increased prominence, as adverse effects associated with the treatment of local pathologies (IBD, IBS, motility disorders, cancer and infections) can be minimized by releasing the drug in the vicinity of desired sites. The site has been found suitable for the localized delivery of proteins and peptides as it have better susceptible to the absorption intensifiers and lesser enzymatic activity as compared to the small intestine. Prolong residence time renders it more suitable site for the delivery of the molecules which were liable to effectively get absorbed from this site. Recent studies indicated that most of the orally administered compounds have possible inhibitory action in colitis. The most effective class of these anti-inflammatory agents are amino salicylates, which blocks cyclooxygenases and inhibits prostaglandin production. This inhibiting effect of anti-inflammatory agents on colitis appears to increase with the duration of the drug use. Therefore, the object of the ongoing study was to formulate an advanced novel drug delivery systems to deliver anti-inflammatory agent, specifically targeted at the colonic site for the management of IBD.
It may be noted that use of methods like hot 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 hot homogenization technique to produce solid lipid nanoparticles (SLNPs) prove to be relatively easy, may be because the former was a spontaneous process and once the constituents (lipids, surfactants and water) were in appropriate proportion a stable nanoparticle will almost always result. Furthermore, the final particle size of the SLNPs was majorly monitored in the nano range.

Mesalamine is a drug candidate selected for the present study. It is a poorly water soluble amino salicylate known to exhibit multifactor mechanism of action. Mesalamine is formulated in order to assist an eminently adequate anti-inflammatory therapy with reduced side effects, notably unwanted GI events that are frequently accomplished with other anti-inflammatory agents. It is proved to be more effective than balsalazide, olsalazine and sulfasalazine in patients with UC and crohn’s disease. Mesalamine emerges to be exceptionally well endured amongst the amino salicylates, with a minimal prevalence of GI adverse effects. This exceptional tolerability profile results in a declined retraction rate and hence better consent with medication.

The objective of the present study was to design colon targeted drug delivery systems of mesalamine solid lipid nanoparticles. For successful colonic delivery of the drug, the delivery system should not release the drug in upper GIT and intestinal region in stipulated period. The lag time in the drug release of 8-9 hrs was required for successful colonic delivery as the transit time of stomach was 2-2.5hrs, small intestine was 3-4hrs and that of proximal colon was 3-3.5hrs.

The study commenced with the preformulation studies on the selected drug candidate mesalamine. Drug was identified by U.V spectroscopy, FTIR, Melting point etc. Various preformulation studies includes estimation of drug, solubility, partition coefficient, drug excipient compatibility study like FTIR, DSC and XRD analysis. The $\lambda_{\text{max}}$ value of the drug was found to be 232 nm. The UV spectra were recorded in
different media viz. 0.1 N HCl, phosphate buffer (pH 6.8) and phosphate buffer (pH 7.2). Melting point of drug was found to be 282°C, which was concurrent with the reported value of 280-285°C. Solubility studies of mesalamine in water, 0.1 N HCl, methanol, DMSO, DMF and phosphate buffer (pH 6.8) revealed higher solubility in acidic medium and lower solubility in water. The drug displayed pH dependent solubility, and solubility was decreased on increasing the pH. DSC studies showed no physical interaction between drug and excipients as peak were the depiction of melting point of the drug was seen in thermograms. The DSC of mesalamine exhibited endothermic peak at 282°C analogous to the melting point of mesalamine, which were in accordance with literature value.

Analysis of the drug is an important step in the formulation of any dosage form. As per International Conference on Harmonization (ICH) guidelines, it is necessary to fully evaluate the formulation’s stability pattern. Stability is a key element of the product attributes and the stability is represented as “a practice which manage identification of a particular drug substance in the presence of its debase products”. The cardinal objective of examining the stability of the drug is to persuade the shelf-life of the drug. The RP-HPLC approach was confirmed with consideration to linearity 0.9897 μg/ml and retention time of drug was found to be 2.8 min.

Various approaches were used for designing and development of colon specific delivery systems. The first approach was based on embedding the drug in biodegradable polysaccharide matrices in solid lipid nanoparticles. The release from such systems can be triggered in colon due to the presence of specific enzymes secreted by colonic microflora. Another system developed was based on probiotic, spirulina loaded biodegradable polysaccharide SLNPs for the purpose of site specific delivery. Presence of polysaccharide in both mesalamine and spirulina systems make it susceptible to enzymes secreted by microflora that exist in the colon, which can trigger the drug delivery at the specific site. Nanoparticulate system in the form of solid lipid nanoparticles was prepared for mesalamine as a model drug and spirulina as a probiotic using hot homogenization technique.
For successful colonic delivery of the drug, the delivery system should not release the drug in initial hours in the dissolution studies. The lag time in the drug release of 8-9 hrs was an acceptance criteria for colonic delivery. In the first approach drug embedded in solid lipid nanoparticles without polysaccharide and additional trials with polysaccharide i.e. sodium alginate in various concentrations were conducted. In the present study, stearic acid as fatty acid, tryglycerol monostearate as lipid, polysorbate 80 as a surfactant and sodium alginate as a release retarding polysaccharide were selected for development of mesalamine loaded SLNPs. SLNPs were prepared by using different formulation variables (ratio of SA: TGMS and surfactant concentration) and process variables i.e. rotation speed of homogenizer. SLNPs without polysaccharide of mesalamine comprising different ratio of SA: TGMS (70:30, 50: 50, 30: 70), surfactant concentration (0.5 to 1.5% w/v) on assorted rotation speed of homogenizer (10,000, 12,000 and 14,000) were prepared by hot homogenization technique. Further polysaccharide loaded drug SLNPs were formulated using different concentrations of sodium alginate (0.2 to 0.6% w/v) i.e. with 50: 50 ratio of SA: TGMS, 1% polysorbate 80 and 12,000 rpm rotational speed of homogenizer. Formulations without polysaccharide failed to retard the drug release for specified lag time. Hence, it was decided to add the release retarding agent, polysaccharide to prevent/minimize the drug release in the upper part of GIT to fit to the acceptance criteria.

SLNPs development approach was herein favorably scaled-up to laboratory scale exhibited an easy enforcement and formulation evaluation for reproducibility of SLNPs dispersion in terms of particle size, encapsulation efficiency and polydispersity index. The particle size, zeta potential and PDI of the SLNPs were determined by Malvern Zetasizer. The shape and surface morphology of selected formulations were performed by TEM (fig. 5.18) and SEM (fig.5.19). The particle size was found within the range of 127.3- 135.1 nm. The PDI was found to be in the range of 0.152- 0.253. The results of TEM and SEM was spherical in shape, with uniform size and having smooth surface. In vitro drug release studies of SLNPs without sodium alginate was carried out in dialysis bag method using 0.1N HCl for 2 hrs and continued in PBS pH 6.8 upto 24 hrs. In vitro
drug release studies shows a cumulative percentage release in the range of 84.3% to 90.8%.

The formulations F1, F2, ------ and F7 displayed more drug release in buffers (0.1N HCl for 2hrs and PBS upto 24 hrs) no retarding of drug release due to absence of polysaccharide sodium alginate was recorded. Which showed that the formulations without polysaccharide, were not effective for localization of drug at specific targeted site. Among the seven formulations F2 formulation comprising of 50: 50 ratio of SA: TGMS and 1% polysorbate 80 depicted optimum release. Hence, in order to retard the initial drug release, sodium alginate (as a polysaccharide) was used composition of selected F2 formulation with various concentrations (0.2% w/v, 0.3% w/v, -- and 0.6% w/v). However the sodium alginate containing SLNPs formulations [F2 (a) - F2 (e)] only 0.6% of drug release was recorded for 1st phase (2hrs) in 0.1N HCl. Thereafter exponential increase in drug release was found upto 6hrs followed by slow and constant drug release upto 9hrs. The release was gradually increased upto 24hrs. While in case of faecal medium and caecal medium, the drug release was poor upto 6hrs. After that significant increment in drug release was reported upto 24hrs. Whereas the release pattern from SLNPs without sodium alginate was exponential and consistent upto 24 hrs.

It was clearly depicted from dissolution profile of all sodium alginate containing SLNPs, that percentage of drug release was significantly higher in rat caecal media as compared to other media (human faecal medium and buffer medium) within the 3rd phase. This was due to degradation of sodium alginate in the existence of rat caecal content. As the human colon contains much higher concentration of the microflora as it contained in caecal contents in the dissolution media will ensure complete drug release. Among all the sodium alginate containing SLNPs formulations, 0.45% w/v sodium alginate showed a release of about 58% in the localized area of colon compared to others in 3rd phase (9-24 hrs).
In vitro drug release studies reveals that F2 (c) formulation displaying maximum percentage of drug release within the 3rd phase in rat caecal medium. This type of release pattern was required for localization of drug to the colon, Then F2 (c) formulation was selected for further studies such as in vitro release kinetics, stability studies and in vivo studies.

In vitro release kinetics studied on formulation containing 0.4% sodium alginate, showed Korse-Meyer-Peppas as a best fit model and follows non-fickian release. Studies coupled with a confirmation of stability (2 months; proposed to extend further) of the formulation, in accordance to the ICH guidelines, validates the robustness of the formulation and its development process. Developed SLNPs did not show any crystal growth, increased in size or gelling upon storage under opted conditions, commonly reported for SLNPs formulations. This was attributed to a suitable viscosity imparted by the concentrated SLNPs dispersion including lipids, fatty acids and surfactant concentrations. The developed formulation’s viscosity also facilitated the nanoparticle to remain in a dispersed form without aggregate formation.

In vivo studies of formulation containing 0.4% sodium alginate conducted on guinea pigs were performed as per approved protocol by animal ethical committee of M. M. College of Pharmacy, M. M. University, Mullana, Ambala. In vivo studies includes change in body weight, diarrhoea assessment studies, caecal bleeding assessment, assessment of caecal content, colon length measurement and histopathological studies. Five groups of animal were treated as diseased control, mesalamine standard, mesalamine SLNPs and combination mesalamine SLNPs and spirulina SLNPs and normal control as 1st, 2nd, 3rd, 4th and 5th group respectively. All the studies showed acceptable results of mesalamine loaded sodium alginate (0.4% w/v) containing SLNPs in correlation with mesalamine standard.

Formulation composition was suitably retarding the release of drug. The study revealed the possible involvement of mesalamine SLNPs in inhibition of the colitis in guinea pigs in dependent manner. The number of lesions was significantly reduced than that of
the mesalamine standard. The mesalamine SLNPs treated greatly compared with mesalamine standard. Similar results was also seen in probiotic, spirulina SLNPs.

The results of in vivo studies revealed that polysaccharide based sodium alginate comprising (0.4%w/v) solid lipid nanoparticles were suitable for colon specific drug delivery of mesalamine.

On the basis of results of various in vitro and in vivo studies, following conclusions can be drawn

- Preformulation studies of mesalamine identified drug, detected its purity, designed calibration curves, analytical method estimation.
- FTIR, DSC and PXRD studies ensured acceptable quality of mesalamine to be used in development.
- The RP-HPLC method was used for the determination of mesalamine in pharmaceutical formulation effectively.
- Drug excipient interactions was studied and evaluated using FTIR, PXRD and DSC. The study conclude selection of excipients and ensured no interactions of drug with those excipients to be used in the formulation composition.
- Formulation development of SLNPs without sodium alginate was unable to hinder the drug release in the upper part of GIT. However SLNPs containing sodium alginate as polysaccharide were successfully release the drug at specific targeted site colon.
  - The formulations containing equal ratio of lipids: fatty acids and 1% polysorbate 80 was selected for good sustainability.
  - The SLNPs formulations containing sodium alginate (0.4%w/v) exhibited acceptable release in rat caecal media and found suitable for localization of drug in the colon.
Characterization of sodium alginate (0.4%w/v) containing solid lipid nanoparticles for particle size, encapsulation efficiency, loading efficiency, polydispersity index, zeta potential and surface morphology by SEM and TEM showed no crystal growth and geometrically figured.

The in vitro release kinetics provide controlled release pattern.

In vivo research concludes that polysaccharide sodium alginate (0.4%w/v) containing solid lipid nanoparticles were acceptable for colonic drug delivery and targeting colitis. It also displayed that probiotic activity of spirulina enhance the mesalamine activity.

The developed polysaccharide based SLNPs containing 0.4% sodium alginate and 50:50 ratio of SA: TGMS could be used as a robust platform for delivery of poorly soluble drugs to colon.

Thus the developed polysaccharide based SLNPs containing 0.4% w/v of sodium alginate along with equal ratio of SA: TGMS was found to be an efficient drug delivery for localization of mesalamine to the colonic region.

Way Forward

Small sized polysaccharide based SLNPs containing sodium alginate (0.4%w/v) emanate in an enriched pharmaceutical profile of this drug, connected with their biocompatible and biodegradable disposition with competent safety, was a fascinating quality of the study.

However, additional skillful extensive studies are designed to explore and extend full possibilities of these recommendations.