CHAPTER-6

Summary and Conclusion

6.1 Summary

Colon cancer, also known as large bowel cancer or colorectal cancer refers to the cancerous growth in colon or rectum region. It is regarded as the fourth most common form of cancer and is liable to several pathological conditions such as infectious conditions, for instance, irritable bowel syndrome, inflammatory bowel disease etc. Chemotherapy and use of chemotherapeutic agents have become a practice as a first line defence against cancer. Presently, chemotherapy has advanced in terms of new drugs and modified chemotherapeutic regimens, however, the route of administration is still unchanged and remains adhered to the intravenous route which is painful and requires long term exposure of chemotherapeutic agents resulting into severe side effects. Thus, there is a need to improve the drug delivery. Route of administration for several drugs have been changed and among them, fluoropyrimidines are the best examples. 5-FU is extensively used drug against cancer but due to its poor oral absorption, it is preferred to be administered as intravenous bolus injection that rapidly distributes and eliminate quickly. Thus, to surmount the adversities associated with bolus injection a continuous infusion of 5-FU was prepared that showed the effective anticancer effect as well as the limited adverse effect. Although the continuous infusion of 5-FU has overcome the problems of bolus infusion of 5-FU, but the pain, sufferings and trauma caused due to the intravenous route of administration remain a serious concern during chemotherapy. Thus, to lessen these inconveniences to the patients caused while administration of chemotherapeutics, USFDA approved an oral prodrug of 5-FU Capecitabine (CAP) that is commercially available as Xeloda®. CAP during clinical trials has successfully mimicked the continuous effect of 5-FU and also exhibited excellent activity

against colon cancer. The conversion of CAP to 5-FU occurs rapidly. Due to the short elimination half-life of CAP (1-2 h) approximately within 6 h, the converted main active pharmaceutical ingredient (API) 5-FU is unnoticeable in plasma, therefore, it's approved morning and evening twice dosing regimen (1250 mg/m^2 twice daily) exhibits drug exposure gap of 6 h between two subsequent doses. Therefore, the present study is an effort towards the preparation of sustained/ controlled release dosage form of CAP for once / twice day medication with continuous maintenance of drug concentration in therapeutic range during the therapy.

In the field of drug delivery, biopolymers have been extensively used as valuable excipients. However, individually these polymers degrade early hence, to surmount the poor biological performance and to improve and enhance the mechanical strength properties a novel and new family of polymers have been introduced known as an interpenetrating polymeric network (IPN). They are the concoction of two or more than two polymers where at least one of the polymer is either synthesized or cross-linked independently in the immediate presence of each other. In this type of delivery vehicle each individual polymeric network exerts its own property in a synergistic way thus, results in advancements in the properties such as mechanical strength, morphology or toughness.

The present work embodies to design, optimize, characterize and evaluate natural polymers locust bean gum and sodium alginate based IPN microbeads encapsulating CAP for improved therapy of colon cancer with an attempt to circumvent in between exposure gap that appears in between two subsequent doses of CAP through IPN drug delivery vehicle by utilizing its property of swelling, mucoadhesion and buoyancy.

The cross-linked IPN beads carrying CAP were prepared by the polymeric blending of natural polymers LBG and NaAlg using ionotropic gelation method and optimized by QbD

approach that helped in screening the most important and desirable independent variables and responses in minimum experimental trials. LBG and NaAlg both are well known and established polymers in controlling and extending the drug release, however, alone they show instability and disintegration. Thus, the combination of both the polymers and their cross-linking with different cross-linkers i.e. divalent (Ca^{2+}) and trivalent (Al^{3+}) optimized by QbD aided in developing such optimized IPN microbeads that could deliver the CAP for a prolonged time in GIT.

The entire experimental work and their outcomes are described in Chapter 4 and 5 respectively. **Chapter 4** dealt with the material and methods comprising preformulation study of CAP, and optimization preparation of IPN microbeads loaded with CAP using different ratios of polymers LBG and NaAlg and the cross-linkers. The preformulation study involved detection of pure drug through the organoleptic property. The observed wavenumbers during FTIR study further confirmed presence of a same functional group that are reported for drug capecitabine in pharmacopoeias. Further, UV method was employed to determine the λ_{max} of CAP in buffers at different pH i.e. Simulated Gastric Fluid (SGF; pH 1.2) and Simulated Intestinal Fluid (SIF; pH 6.8) which was found to be 239 nm in both the cases. Also, an inexpensive and rapid HPLC method was developed and validated for the estimation of CAP in plasma. The obtained linearity, accuracy, precision, LOD and LOQ were found to comply with the ICH guidelines stating the method to be sufficient for the estimation of drug from the prepared formulation.

The optimization and preparation of CAP loaded IPN microbeads were further divided into three parts, Part I, Part II and Part III.

IPN microbeads were formed with two different cross-linkers of different valancies such as Ca^{2+} in Part I (F-1) and Al^{3+} in Part II (F-2) and Part III (F-3). Part I (F-1), the study was

initiated with the first step of QbD, risk assessment method named as 'Risk Ranking and Filtering' involving determination of all the possible factors that may affect development of microbeads and categorizing in terms of severity and probability which were multiplied to get RPN score. Those scoring 10 or > 10 i.e. polymer amount, cross-linker amount, stirring speed, dropping distance and needle gauze were considered as a high risk factor. Now, the selected high-risk factors were further subjected to the screening purpose 'Fractional Factorial Design' (FFD). Finally based on Pareto chart the high risk factors that crossed the reference line in the chart (polymer amount, cross-linker amount, stirring speed) were taken into consideration to check the influence of the above risk factors on the responses particle size, and % drug entrapment (selected on the basis of previous studies done). After screening the main factor from the above study, finally, optimization study was done using BBD method including independent variable as polymer ratio, cross-linker amount and stirring speed and responses as particle size and % drug entrapment. Finally based on the statistical analysis and response optimizer using Minitab-17® the best batch was obtained showing the predicted value of the independent variables to be used to get an optimized formulation.

In the second part, Part II (F-2) all the QbD steps were applied in the same way as Part I. However, the method of risk assessment and the cross-linkers used here were FMEA and Al^{3+} respectively. The independent variables selected for this study were polymer ratio, cross-linker amount and curing time whereas responses selected were particle size, % drug entrapment and % drug release. Likewise, Part I, here also BBD was utilized to get an optimized formulation.

In the final part, Part III (F-3) again same methods and procedure were followed with a change in the method of risk assessment named as risk estimation matrix method and slight modification during preparation of IPN microbeads. Here, to further sustain the release of CAP and to surmount its drug exposure gap an attempt was made to prepare a formulation

exhibiting dual mechanism of buoyancy and mucoadhesion. The formulation was prepared using the same method, ionotropic gelation method as employed in Part II, however, to make the system buoyant gas-forming agent, sodium bicarbonate was added during formulation preparation. The independent variables selected were polymer ratio, cross-linker amount and amount of sodium bicarbonate ($NaHCO_3$) whereas the responses were particle size, % drug entrapment and % buoyancy. Finally, after implementation of BBD, an optimized batch was obtained.

Further, the optimized batch obtained from the experiments of all three parts were subjected to several characterization techniques, for instance, optical microscopy, scanning electron microscopy from morphological aspects, DSC, FTIR and XRD studied for physical and chemical compatibility between the pure crystalline drug and all optimized formulations. *In vitro* studies including swelling study, drug release, mucoadhesion study, buoyancy, and SRB assay were also performed. For estimation of drug from the formulations and to compare its pharmacokinetic parameters (PK) with pure drug, *in vivo* pharmacokinetic study was performed. To check whether the formed IPN microbeads were safe for oral administration, oral toxicity study and histology was done. To confirm the stability of the prepared three formulations, stability study at three different temperatures i.e. refrigeration (5°C ± 3 °C), room temperature (25°C ± 2°C with 70 ± 5% RH) and controlled oven high temperature (40°C ± 2 °C with 75 ± 5%) with respect to particle size, drug entrapment and total drug content was performed following the ICH guideline Q1A(R2). And finally for determination of the gastroretention of the optimized buoyant IPN microbeads *in vivo* γ-scintigraphy study was done.

Chapter 5 dealt with the interpretations of the outcomes of the experiments performed in Chapter 4. In Part I (F-1), the IPN microbeads of LBG and NaAlg carrying water soluble CAP was successfully prepared by ionotropic gelation method. The prepared optimized batch

of microbeads cross-linked with divalent calcium chloride exhibited the particle size of 494.37 ± 1.4 µm and % drug entrapment of 81.39 ± 2.9 %, which was in close agreement with the predicted result by Minitab-17®. The results of in vitro cumulative % drug release of 92% for 12 h showed extended release of drug from the polymeric shell and followed Korsmeyer –Peppas model with an exponent n=0.62 exhibiting drug release mechanism to be the combined effect of swelling and diffusion. The comparative estimated PK parameters of optimized batch formulation (F-1) that showed $AUC_{(0-t)}$, MRT and $t_{1/2}$ 1.85 folds, 3.45 folds and 7.87 folds, respectively greater than the pure drug, thus exhibited good oral bioavailability of CAP encapsulated within microbeads and maintained plasma drug concentration for longer duration in comparison to pure drug in solution. SEM image showed spherical shape and regular surface of the microbeads. The EDX study showed the details of element involved during preparation and further presence of trace amount of Ca^{2+} in the formulation confirmed its cross-linking with the LBG and NaAlg microbeads. FTIR, DSC and XRD studies performed evidenced the compatibility of pure drug with all the excipients and the prepared formulation. These studies also confirmed about the successful conversion of crystalline pure drug into amorphous form. The swelling study showed a visible transfer of water molecules inside the microbeads confirmed by the swelling which was low in SGF but high in SIF. The property of swelling was found to be affected by factors such as polymer ratio, pH and amount of cross-linker. The SRB study performed showed growth inhibition (GI_{50}) at a minimum concentration of 10 µg/mL of 56.3% exhibiting high cytocompatibility than the pure drug which was 27.5% only at the same minimum concentration. To confirm the suitability of IPN microbeads through oral administration acute oral toxicity was done. The vital organs harvested showed no major lesions or any changes in comparison to the normal control.

In Part II (F-2), the developed optimized batch of trivalent cross-linked aluminum chloride IPN microbeads was studied for three different responses particle size, % drug entrapment and % drug release. For all three responses, the results obtained were 457.92 ± 1.6 µm, 74.11 ± 3.1% and 90.23 ± 2.1 %. In all aspects, the value of the responses was found to be lower than the results of Part I this may be due to the rigidity of polymeric shell when cross-linked with a trivalent ion in comparison to the cross-linking with divalent ion. However, the IPN microbeads loaded with CAP and cross- linked with aluminum chloride showed more improved PK parameters than the microbeads formed in Part I. The $AUC_{(0-t)}$, MRT and $t_{1/2}$ of the optimized batch were 3.456 folds, 5.974 folds and 12.53 folds higher respectively in comparison to pure drug . Further, the other studies performed such as SEM, FTIR, EDX, DSC, XRD, swelling and oral toxicity study revealed almost same result as obtained in Part I, however, the results of cytocompatibility study showed marginally better outcome than calcium ion cross-linked microbeads which was found to be 59.5% at the minimum concentration of 10 µg/mL.

In Part III (F-3), further to sustain the release of CAP from IPN microbeads composed of LBG and NaAlg the concept of a dual mechanism of buoyancy and mucoadhesion was employed. The property of buoyancy will make the formulation gastroretentive however, with the passage of time as the stomach empties the floating capacity of the dosage form also reduces when it moves to the other part of GIT. At that time, the property of mucoadhesion can help the formulation to remain in intimate contact with the mucosal wall of the GIT. Thus, prolong the drug release as well as the bioavailability of the model drug CAP from the delivery vehicle IPN. Based on the improved PK parameters of Part II, IPN microbeads of same composition and same method i.e. by ionotropic gelation method, buoyant IPN microbeads were prepared by adding effervescent or gas generating agent. The optimized batch of the formed buoyant IPN microbeads (F-3) showed a particle size of 402.12 ± 1.9

μm, % drug entrapment of 82.45 ± 2.5% and % buoyancy of 85.03 ± 1.1%. Cumulative % drug release was highest (95% till 20 h) in comparison to the other formulations (F-1 and F-2), mentioned in Part I and Part II, respectively. The buoyant IPN microbeads (F-3), initially at pH 1.2 for first 2 h showed a negligible release of 10% as found same in case of other formulations (F-1 and F-2) and continued releasing the drug approximately 44% till 8 h in the same pH 1.2. However, when the pH of the media was changed i.e. pH 6.8, the drug release was found to be 95% till 12 h. At pH 1.2 the release of drug was observed till 8 h may be due to the combined effect of swelling, diffusion and an additional effect, presence of pores in the microbeads. The ions present in the media at low pH (pH 1.2) undergoes protonation thus, less swelling resulted in slow drug release whereas presence of pores opened multiple entries for the media to enter inside the matrix that resulted into maximum but slow diffusion of drug from the polymeric matrix. On the other hand, high pH (pH 6.8), lead to deprotonation resulted into more swelling and increased ions in the buffer media, resulting in enhanced osmotic and electrostatic force that showed continuous notable extended release. Another important factor that extended the release of the drug from the formulation till 20 h was the inherent property pure drug CAP. With respect to CAP, it is reported that amorphous CAP possess low glass transition temperature thus turns into a gel that might also slowed down the drug release from the formulation. The result of *ex vivo* mucoadhesion testing performed by wash off method demonstrated excellent mucoadhesion in SGF than SIF. The rapid wash off in SIF was due to the presence of moisture resulted into slippy mucilage surface that was not able to retain the microbeads for a longer time. The *in vitro* buoyancy study performed in case of buoyant IPN microbeads showed total floating time of approximately >10 h. The *in vivo* PK studies showed excellent results as bioavailability ($AUC_{(0-t)}$) was enhanced by 6.346 folds, MRT by 10.40 folds and ($t_{1/2}$) by 24.044 folds as compared to F2- and F-1. Stability study performed for six months for all the three formulations following the ICH guideline

Q1A (R2) indicated that they were stable at all three temperatures i.e. refrigeration temperature (5 ± 3 °C), room temperature (25°C ± 2°C with 70 ± 5% RH) and controlled oven high temperature (40°C ± 2 °C with 75 ± 5% RH) and showed no significant change with respect to particle size, drug entrapment and total drug content. The *in vitro* cell cytotoxicity study performed on human colon cancer cell line HT-29 showed highest % growth inhibition of 61.6% by formulation F-3 in comparison to formulations F-1, F-2 and pure drug. The other studies performed such as SEM, EDX, FTIR, XRD and DSC study revealed the results in same way as the other two formulations. The oral toxicity study performed in case of F-3 proved also it to be safe to be administered orally.

The results of *in vivo* antitumor activity performed for all the three formulations in comparison to control concerning to relative tumor volume, survival and weight loss check showed that among all prepared formulations, the third buoyant IPN microbead formulation (F-3) significantly inhibited tumor size in comparison to other prepared formulations. Further, in support to gastroretention property of the formulation (F-3), gamma scintigraphy images showed that the formulation retained in stomach more than 6 h. This proves that formed IPN microbeads composed of natural polymers are capable of enhancing the gastric residence of formulation F-3, encapsulating capecitabine.

6.2 Conclusions

Conclusively, the optimization and formulation of CAP loaded IPN microbeads of LBG and NaAlg using QbD approach proved to be a simple and reproducible method. The developed HPLC validation method proved to be quick and easy for the routine analysis of a large number of samples. Application of FFD for screening purpose and BBD for optimization purpose generated reproducible and effective optimized batch within minimum experimental trials with a good understanding of relationship between the formulation variables and the

desired responses. The IPN microbeads prepared were easy to formulate and were suitable to be used for oral administration due to use of natural, mucoadhesive and biodegradable polymers sodium alginate and locust bean gum. The prepared IPN microbeads were morphologically spherical in shape without any aggregation. The solid-state characterization techniques performed, showed successful conversion of crystalline drug into an amorphous state. *In vitro* SRB assay performed in human cell line, HT-29 exhibited excellent % growth inhibition of cancerous cell in case of all optimized batch in comparison to pure drug. The results of a pharmacokinetic study performed in Albino Wistar rats following the oral administration of the optimized formulations showed good bioavailability (higher AUC) and prolonged retention of drug in blood as evidenced by higher MRT and $t_{1/2}$ values than the pure drug. The oral toxicity and histopathology study done in non pregnant Swiss Albino female mice proved that all the formulations are safe for oral administration purpose. The results of anticancer activity conducted for all three formulations proved them to be effective in the treatment of colon cancer. Further, in support to dual mechanism of buoyancy and mucoadhesion of the IPN microbeads, the result of *in vivo* gamma scintigraphy study revealed the gastroretention of formulated microbeads in GIT more than 6 h. The result of oral toxicity study proved the prepared formulation safe for oral administration. Thus, it can be concluded that all the prepared IPN microbeads could prove to be a promising and potential delivery vehicle in sustaining the release of CAP as well as may result in better treatment of colon cancer.