Colorectal cancer is the second leading cause of cancer deaths in the United States, and more than 66,000 cases of colon cancer are reported to occur in the Indian subcontinent every year. Conventional cancer chemotherapy is not very effective for treatment of colorectal cancer, as the drug molecule does not reach the target site in the required therapeutic concentration. Therefore, effective treatment of colon cancer by conventional therapy requires relatively large doses to compensate for drug loss during passage through the upper gastrointestinal (GI) tract. These large doses may be associated with undue side effects. This can be overcome by site-specific delivery of the drug molecule to colon. 5-Fluorouracil (5-FU) is one of the most commonly used chemotherapy agents in clinical oncology practice. 5-FU acts primarily as a cytotoxic drug affecting rapidly dividing cells. The use of high doses not only affects the tumor cells, but also cells of the gastrointestinal mucosa and the hematopoietic system. It has been assumed that 5-FU is immunosuppressive because of the inhibitory effects seen at these high doses. Therefore, colon specific release of 5-FU could minimise the side effects and enhance the efficacy during the treatment of colon cancer. However, 5-FU is water soluble and has low lipid solubility. In order to deliver 5-FU to the colon, the standard approach involves the use of enteric polymers. However, failure of these polymeric systems due to variation in dietary constituents, variable gastric emptying time etc. cannot be ruled out. Therefore, the use of polymers exhibiting pH-independent solubility seems to offer an attractive alterative approach for colon delivery of 5-FU. Among the approaches used, a microbially triggered approach that involves the use of biodegradable modified gums was explored for the effective delivery of 5-FU to colon.

Colon microflora is increasingly being recognized as a preferable triggering component in the design of colonic drug delivery systems since the great increase in bacterial population and corresponding activities in the colon represent an event independent of GI transit time and of fed or fasted state. A large number of polysaccharides such as pectin, chitosan, cyclodextrin, dextrans have been investigated as promising drug carriers in a biomimetic approach, for colon-specific drug delivery. As these polymers are usually soluble in water, they must be made water insoluble by cross-linking or hydrophobic

derivatization. Physical or chemical cross-linking of the polymers can reduce the water/acid solubility of natural polysaccharides to form swellable hydrogels.

In light of above facts, it can be postulated that the physico-chemical modification of the natural gums (*Cassia* species) could yield water insoluble complexes. Further, these ionic or covalent cross-linked polymeric complexes are expected to prevent drug release in the acidic pH of the stomach and deliver drugs to the colon. Therefore, gums obtained from Cassia species were chemically modified and cross linked with other biodegradable polymers. These polymeric blends were used for coating 5-FU tablets. These dosage forms were evaluated for *in vitro* drug release in presence or absence of rat caecal contents and for *in vivo* drug release in rats. Hence, the aim of the present investigation was to explore the potential of natural gums obtained from *Cassia* species for use as a carrier for colon delivery of 5-FU.

Cassia fistula Linn gum was extracted and characterized employing FTIR-ATR and viscoelastic analysis. The pure gum was chemically modified for preparing its two derivatives Carboxymethyl *Cassia fistula* gum *(CCG)* and Carbomoylethylated *Cassia fistula* gum (CEG). The purification of both the derivatives was performed by re-precipitation method using 80% methanol. The precipitates were freeze dried to remove water. The porous dried samples were characterized using FTIR-ATR method.

Preliminary investigations were conducted for optimizing the composition of Chitosan-CCG (CH-CCG) or Chitosan-CEG (CH-CEG) films possessing high mechanical strength, adhesive strength, cross linking density and lowest cohesive strength, swelling index, etc. These optimized CH-CCG or CH-CEG film compositions were coated on 5-FU tablets with the hypothesis that the films would protect the release of 5-FU in the gastric pH and would release it in the alkaline pH of the colon.

Chitosan is a natural carbohydrate polymer derived by alkaline deacetylation from chitin. It is insoluble in water. However, it is solublized by forming salts with organic acids like acetic acid, citric acid etc. The chitosan acetate solution is in protonated form because of protonation of $-NH_2$ moieties present in chitosan. Both CEG and CCG contain $-COO^-$ moieties. Hence, CEG or CCG could be expected to interact with CH during preparation of films. The $-NH_3^+$ moieties of chitosan were reacted with $-COO^-$ moieties of CEG/CCG leading to

the formation of CH-CEG or CH-CCG complex, which were found to be insoluble in water. The jelly like consistency material obtained after mixing CH and CEG or CCG, if dried in oven did not produce acceptable film. Therefore, ammonium acetate (5M) was added to stop the ionic reaction between chitosan acetate and CEG/CCG in solution. Therefore, solution of chitosan was prepared in acetic acid and ammonium acetate (5M) was added. CCG or CEG solution was prepared separately and added to chitosan solution. The clear solution was dried in oven at 37º C for 72 h. The drying process slowly evaporated the ammonium acetate and excess acetic acid along with water. This leads to formation of brown, colored, smooth, flexible film.

The prepared films were evaluated for physical (swelling index, contact angle, etc), chemical (FTIR and DSC), mechanical performance (tensile strength, resilience, etc) and electrical properties. The results suggested that the films prepared with CH-CCG::30:70 were resistant to buffer 1.2, 7.4 or 6.8 due to their lipophillic character. Further, the physical nature of films changed with increase in the proportion of CCG. Among all the films (FCG4-FCG7 and FEG4-FEG7) tested for mechanical performance, FCG6 was found to exhibit maximum tensile strength, extensibility and burst strength suggesting increased toughness of this film. Further, the enhancement in percentage resilience of FCG6 (CH-CCG, 30:70) films indicated enhanced recovery of this film after deformation both in terms of speed and force. This additionally supported the candidature of this film (FCG6) for coating on tablets. Interestingly, the increase in zeta potential towards negative was found to be associated with lowest conductivity as well as high negative mobility of electrons. Hence, the FCG6 film could be considered to be optimum for coating on 5-FU tablets that could possible form coat with high adhesive force strength needed to bind with tablet surface. Further, preliminary investigations aimed at checking the integrity of the films suggested that only CH-CCG films were intact for 24 hr in pH 1.2 for 2hr, buffer pH 7.4 for 3 hr and buffer pH 6.8 for 19 hr. The CH-CEG films lost their integrity in pH 1.2 within 2 hr. Analysis of the data revealed FCG6 (CH-CCG) films prepared using 30:70::CH:CCG had an ability to control the release of 5-FU. The *in vitro* flux in buffer pH 6.8 followed the order FCG6 > FCG5 > FCG4 > FCG7 FCG6.

The FTIR spectra of films prepared with CCG:CH 70:30 showed peaks at 1545 cm^{-1} of $–NH_3^+$ moieties, 1404 cm^{-1} of COO^- moieties and 1626 cm^{-1} of amide group suggesting strong cross-linking between CCG and CH when mixed in 70:30 proportion. Interestingly, a peak at 1292 cm^{-1} corresponding to -COOH moieties was absent that further supported lowest swelling of this film. This could be attributed to maximum cross-linking between CH and CCG in the films. Interestingly, a comparison of interaction strength in films containing CEG-CH or CCG-CH combination revealed appearance of strong interaction peak at 1516 cm^{-1} for $-NH_3^+$ and at 1404 cm^{-1} for $-COO^-$ in CCG-CH combination as compared to weak interaction peak at 1525cm^{-1} for $-NH^{3+}$ and 1425 cm^{-1} in CEG-CH combination. Similar attributes were observed for FCG6 and FEG6 films having maximum extent of cross-linking. These findings could be attributed to the lower degree of substitution of CEG as compared to CCG and increased solubility of CEG with respect to CCG. Hence, high degree of swelling in films prepared with CEG-CH composition was rather justifiable.

The ΔH of endothermic transition at 206ºC characteristic of CCG-CH electrostatic interaction followed the order FCG6 > FCG5 > FCG4 = FCG7. This suggested the presence of strong cross-linking density in FCG6 films containing CCG:CH in the proportion of 70:30. On the basis of overall findings obtained during film evaluation, FCG6 containing CCG:CH:: 70:30 was selected for coating 5-FU tablets.

The 5-FU tablets were prepared and the results revealed that batches containing spray dried lactose as diluent, of 4% w/v concentration of Eudragit L-100, 1.5 %w/v concentration of CH solution or 1.5% w/v concentration of CH-CCG (30:70) as binder were optimum because maximum yield and minimum fines were obtained. However, with MCC as diluent, 2 %w/v concentration of CH solution or 2% w/v concentration of CH-CCG (30:70) as binder were found to be optimum as they provided maximum yield and minimum fines. The batches containing 2-5% w/v Eudragit L-100 and MCC as diluents were found to provide less than 90% w/v yield. Hence, the batches B3, B6, B9, B17 and B20 were selected for further study as they provided more than 95% yield of granules. Further, estimation of the flow properties was made on the basis of angle of repose, Hausner's ratio and Carr's index. It was concluded that the granules were ideal for the formation of tablets.

Interestingly, the yield of tablet batch prepared with MCC was found to be 50% as compared to 95% with spray dried lactose. It was observed that a film-like structure was formed by the movement of upper punch into die cavity when MCC was used as diluent. This film formation could have contributed to decrease in yield of the batch when MCC was used as diluent. Hence, tablets were prepared using spray dried lactose as diluent for further investigations.

The *in vitro* release of 5-FU from different selected uncoated tablet batches (B3, B6 or B9) was investigated for identifying the best combination of binder and coating system, that could successfully deliver 5-FU to large intestine by crossing the upper GIT environment. The release of 5-FU from tablets containing CCG-CH (70:30; 1.5% w/v) as binder was higher as compared to tablets prepared with Eudragit L-100 (4% w/v) as binder (Table-1). When batches B3, B6 or B9 were coated with Eudragit L-100 (2% w/v), 5-FU release was retarded in pH 1.2. However, the tablets of batch B3 where Eudragit L100 (4%w/v) was used as binder exhibited lowest release of 5-FU. Interestingly, 5-FU released from batch EU-B9 was found to be high in pH6.8 with rat caecal content as compared to batch EU-B3 and EU-B6 (Fig-B). This was due to inherent ability of CCG-CH as binder to retard the release of 5-FU in pH 1.2 and pH 7.4 conditions. The release of 5-FU in pH 6.8 containing rat caecal content was due to activity of enzymes present in the rat caecal that had broken the CCG-CH conjugate thereby releasing the drug. This effect was found to be more pronounced when Eudragit L-100 (2 %w/v) coating was replaced with CCG-CH coating to form CCG-CH B3, CCG-CH-B6 and CCH-CH-B9 batches. Further, among CCG-CH coated batches, batch CCG-CH-B9 containing CCG-CH as binder as well as coating system was found to release 68.91% 5-FU in pH 6.8 containing rat caecal contents. Hence, the results indicated CCG-CH conjugate to be an effective carrier for delivering 5-FU to colon..

The ideal coating material is one that displays highest binding of coating material with tablet surface. Secondly, after drying, the coat of the coated tablet should exhibit negligible stickiness. Hence, an attempt was made to estimate these two essential properties using texture analyser by determining the adhesive force strength and cohesive force strength of the coated film. The use of dusting powders for prevention of sticking as well as penetration of moisture

during aqueous tablet coating is not new. However, the non uniform spreading of dusting powders during aqueous coating process leads to inconsistent *in vitro* release of drugs, specifically if the coating is for modified release tablets. The developed aqueous coating formulation could be suggested to possess a combination of adhesive force enhancing property [CCG-CH (70:30)] and cohesive force reduction property [CCG-CH (70:30) containing magnesium silicate]. The results indicated that coating the tablets with adhesive system [CCG-CH (70:30)] was not suitable for delivering 5-FU in the colon, probably due to its hydrophilic nature. The coating of cohesive force strength reduction system over these tablets significantly reduced the release of 5-FU from these tablets to 6% in acidic pH, thus complying with the requirement of enteric release tablets. FTIR, SEM and DSC analysis strongly indicated the role of hydrogen bonding between primary alcoholic ($-CH_2OH$) groups of carboxy methylated *cassia fistula* gum and Si-O-Si or Mg-O moieties of magnesium silicate in reducing the release of 5-FU from coated tablets in acidic pH.

The results of pharmacokinetic investigations showed no significant difference in peak plasma concentration (C_{max}) of both uncoated as well as CCG:CH coated 5-FU tablet batches. The time when first 5-FU content was observed in the blood was 4h and 0h, respectively, for coated and uncoated 5-FU tablets. Further, the results revealed that pharmacokinetic parameters K_a and K_e of coated tablets as compared to uncoated 5-FU tablets were decreased respectively by 9.1-fold and 5.7- fold. This suggests that 5-FU was present in the body for longer duration when administered in CCG-CH coated tablets. The greater MRT of 5.93-fold of coated tablets as compared to uncoated tablets further supported CCG-CH coated tablets to enhance the residence time of 5-FU. As a corollary, the delivery of 5-FU from these tablets could be expected to be more useful for local effect in the colon as the drug would be released at the site of effected tissues. The disintegration of these tablets in the colon was also ascertained by X-ray imaging studies.

Additional work was conducted to explore super disintegration potential of calcium salt of CCG (CaCCG) or calcium salt of CEG (CaCEG). The results revealed that the fast disintegration tablets (FDTs) containing pure *Cassia fistula* gum, CCG or CEG were neither directly compressible nor did they pass

the content uniformly test and friability test as per USP XXXNF XXV. However, FDTs prepared by using CaCCG or CaCEG (5%w/v) were directly compressible and passed the test as per USPXXXNFXXV. It is note worthy that the FDTs containing CaCCG could be prepared at high mechanical strength of 8.2 kg/cm^2. Evaluation of the mechanism of super disintegration suggested decreased WST without effecting SI and $R_{eff.p}$ to be the operative for rapid disintegration of the tablets. In addition, presence of Ca^{+2} that builds intra-bond/or inter-cross linked bridges in the CaCEG or CaCEG could have supported the water transporting system in the tablets even when the aqueous channels in the FDTs were blocked. Overall, the findings suggested CaCCG or CaCEG to hold a great potential for use as superdisintegrants that could provide FDTs with sufficient mechanical strength with quick disintegration.

The results of the present investigation revealed that CCG-CH (70:30) containing magnesium silicate (0.2% w/w) as coating film composition possessed the desired attributes of high adhesive force strength and low cohesive force strength. This film composition was observed to release 5-FU in the alkaline pH of colon while preventing its release in acidic pH as required for an enteric coated tablet. Further investigations involving the use of calcium salts of either CEG (CaCEG) or CCG (CaCCG) revealed their potential as superdisintegrants in FDTs because both these derivatives produced disintegration of FDTs within 10 seconds as required by USP standards.