8. SUMMARY

Oral drug delivery system is the most commonly used route for drug delivery due to its ease of administration, better patient compliance, and flexibility in design and development of formulation. The drug delivery to the colon has attracted a lot of attention of the scientist working on oral drug delivery system which is mainly due to the fact that colon is a site where both local and systemic drug delivery can take place. Many approaches have been attempted for the development of colon-specific delivery systems that rely on gastrointestinal pH, transit times, enterobacteria and luminal pressure for site-specific delivery. It appears that microbially-controlled systems based on natural polysaccharides have the greatest potential for colonic delivery, particularly in terms of site-specificity and safety because of the presence of the biodegradable enzymes only in the colon.

In recognition of this, it was thought to design microbially triggered matrices of an inexpensive and abundantly available okra gum (OG), obtained from the pods of *Albemoschus esculentus*, could be exploited as novel carrier for colon specific drug delivery. Okra is a polysaccharide consisting of D-galactose, L-rhamnose and L-galacturonic acid. OG has been investigated as binding agent in tablet dosage forms and has been shown to produce tablets with good hardness and desired controlled drug release profile.

Advantageously colonic drug delivery system would be valuable when a delay in absorption is therapeutically desirable in treatment of chronic medical conditions like nocturnal asthma and rheumatoid arthritis had apparent circadian rhythm and peak symptoms in the early morning. Nocturnal worsening of asthma has become a recognized and important problem that must be considered in the management of this disorder. The treatment of nocturnal asthma requires a chronopharmacologic approach, in which more intense therapy is targeted to coincide with the time that the disease is the worst.
Theophylline is a bronchodilator used in the symptomatic treatment of mild bronchial asthma and reversible bronchospasm acts by inhibiting cyclic nucleotide phosphodiesterase and hence it is selected as a drug candidate for the treatment of nocturnal asthma considering its short half life (7-9h), good oral bioavailability (96%) and good colonic absorption. It is required to develop such formulation containing Theophylline, when administered in the evening should release the drug after 4-5h to achieve an elevated Theophylline level overnight when the risk of asthma is found to be maximum.

Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by joint synovial inflammation and progressive cartilage and bone destruction resulting in gradual immobility which contribute to clinical stiffness of the joints that is most pronounced in the morning. Diclofenac sodium is a non-steroidal anti-inflammatory drug that considered as first line agent for the treatment of RA. With orally administering conventional Diclofenac sodium formulation, it is difficult to achieve the desired clinical effect, because it elicits patients’ noncompliance of administration in the early morning to coordinate the rhythm of these diseases, due to rapid absorption of the conventional formulation. It is imperative to design a drug delivery system that administered at bedtime but releasing drug during morning hours would be ideal in this case. However, in comparison to the conventional formulation, colon specific drug delivery is very effective and more convenient for administration. Thus in present investigation orally-administered tablets of natural polysaccharide okra gum were prepared that would ensures chronotherapeutic delivery of drugs in colon for the treatment of diseases like asthma and rheumatoid arthritis.

Isolation of Okra gum (OG)

Three different methods (Method A, Method B and Method C) were used for the isolation of OG to obtain better yield from the okra pods. It was found that Method C was relatively economical, gave better yield with required
characteristics of products. Due to these reasons method C was selected for further study.

Obtained okra gum powder was subjected to various phytochemical tests, physicochemical tests and instrumental analysis. It was found that OG powder was pure and has good quality and excellent thermal stability. Biodegradation study of okra gum was performed by pH change study. It was found that the pH of dissolution medium containing rat caecal content rapidly decreases by the addition of OG which might be attributed to the fermentation of OG by colonic bacteria in the caecal contents to generate an organic acid thereby reducing the pH.

Theophylline

Identification of Theophylline was performed by melting point study and IR spectroscopy study. Preformulation study was performed by Differential Scanning Calorimetry that showed drug, polymer and excipients does not reacted adversely in the formation of tablets.

The tableting properties (granulation, swelling, erosion and dissolution characteristics) of OG were compared with other extensively used natural polymers (GG, XG, LBG and chitosan) and semi synthetic polymer (HPMC). The tableting properties of extracted okra gum were found satisfactory and were comparable with tableting properties of extensively used semi synthetic HPMC.

Preliminary intact study of matrix tablet

The successful delivery of drug to colon requires the protection of drug from being released in the stomach and small intestine (upper GIT). In present section, matrix tablets using okra gum and other gums like guar gum, locust bean gum and chitosan were prepared by using direct compression method (IS01-IS18) and wet granulation method (IS19-IS39). Preliminary intact study
for these tablets was carried out in the physiological environment of stomach and small intestine to check the tablet intactness. During the preliminary intact study it was found that direct compression method for matrix tablets of Theophylline leads to faster disintegration in the dissolution medium because of poor binding property. Hence, further batches of tablets containing 200mg of Theophylline were prepared by using wet granulation method.

**Preparation of matrix tablets by wet granulation method for colon specific drug delivery**

Preparation of matrix tablets by wet granulation method using PVPK30

To prepare colon specific drug delivery system using natural gums it is the prime requirement of formulation that it should not release the drug in upper GIT i.e. stomach and small intestine and when it reaches to colon it should abruptly release the drug. Tablet formulations (OG1-OG13) were prepared using increasing concentration of OG with increasing concentration of PVPK30 (3% to 3.75%). Further tablet formulations OG14 to OG23 were prepared to check the effect of okra gum alone and its combination with other polymers like HPMC/pectin/amylose/ethyl cellulose. Prepared formulations were evaluated for weight variation, hardness, friability, drug content uniformity and drug release for 8h according to methodology section 4.2.5. Dissolution study in presence or absence of rat caecal content revealed that drug released in presence of rat caecal content was increased to a great extent as compared to drug release in absence of rat caecal content (control) for the above said formulations except (OG7 and OG11). Among prepared tablets, formulation (OG23) containing higher amount of OG (400mg) and lower amount of HPMC (75.5mg) showed restricted drug release in upper GIT (Rel5h=29.14%). This may be due to synergistic effect of HPMC (high viscosity and stable gel forming property) and OG (lower solubility in 0.1N HCl, pH 1.2). Thus further study was carried out by using simplex lattice design to study the synergistic effect of OG and HPMC on tablet properties.
Simplex lattice design

As per the mixture design the total amount of excipients was maintained for total weight of 400mg (OG, HPMC and MCC) and the Theophylline content was 200mg so that the total weight of tablet was adjusted to 600mg. Based on design, fourteen tablet formulations (SD1-SD14) were prepared and evaluated similarly as above. As per the ANOVA report for Rel₅h, a quadratic design model appears that did not show significant lack of fit thus the used model was statistically accepted because of high F value (20.45) and low probability values (less than 0.0500). The "Lack of Fit F-value" of 41.90 implies the Lack of Fit is significant. In this case Linear Mixture Components, AB, AC are significant model terms (A=OG, B= HPMC, C= MCC).

The below equation was generated from the data.

\[
\text{Rel}_{5h} = 35.06A + 30.11B + 23.90C + 66.72AB + 46.56AC + 12.84BC
\]

R-Square= 0.9191

The results of dissolution study showed that the rate of drug release from these matrix tablets was mainly influenced by OG concentration. As the amount of the OG increases the extent of drug release decreases. This could be attributed to an increase in the density of the gum matrix and the diffusional path length that the drug has to traverse. Among prepared formulations, duplicate formulations SD03 (Rel₅h=38.98%) and SD12 (Rel₅h=38.44%) containing higher amount of OG (300mg), relative amount of HPMC (100mg) and no MCC gave restricted drug release in upper GIT. Formulations SD03 and SD12 showed fast drug release within 3h of dissolution in presence of rat caecal content. This may be due to anaerobic bacteria present in rat caecal content which might get acted upon by enzymatic action on matrix tablet containing natural polysaccharide (OG) and leads to degradation of OG with fast drug release.
Preparation of matrix tablets by wet granulation method using 5% starch paste

Tablet formulations OG24 to OG27 and OG28 to OG29 containing increasing concentrations of OG and combinations of OG with GG, respectively were prepared by using 5% starch paste as binder. Formulation (OG27) containing higher concentration of OG (400mg) showed restricted drug release in upper GIT (12.21%). This is due to higher density of gum matrix at higher concentration in the formulation resulted in an increased diffusional path length that controls the drug release in upper GIT. Dissolution study in presence or absence of rat caecal content revealed that drug release in presence of rat caecal content was increased to a great extent as compared to drug release in absence of rat caecal content (control) for the above said formulations. Hence further study was performed based on the combination of okra gum and guar gum as per factorial design.

Preparation of matrix tablets by wet granulation method using Factorial design

To know the best combination of okra gum and guar gum, \(3^2\) factorial design approach was used and according to which nine batches of tablets (FS1-FS9) were prepared using independent variables (\(X_1\), amount of okra gum and \(X_2\), amount of guar gum) to check their effect on dependent parameters (\(R_{5h}\) and \(R_{8h}\)). The statistical analysis of the factorial batches was performed by multiple regression analysis using Microsoft Excel followed by ANOVA.

To elucidate the influence of okra gum and guar gum on drug release, following polynomial equations were evolved for \(R_{5h}\) and \(R_{8h}\).

\[
R_{5h} = 30.83 - 11.05X_1 - 3.35X_2 - 1.85X_1X_1 - 0.5X_2X_2 - 0.02X_1X_2, R^2 = 0.987746
\]

\[
R_{8h} = 86.08 - 11.05X_1 - 3.38X_2 - 5.91X_1X_1 - 1.22X_2X_2 - 1.29X_1X_2, R^2 = 0.988177
\]

The \(R_{5h}\) and \(R_{8h}\) for all the batches FS1 to FS9 varied from 13.24% to 43.09% and 62.88% to 92.55%, respectively with good correlation coefficient as 0.994 and 0.993, respectively. The dissolution profiles indicate that the drug release is proportional to the amount of okra gum and guar gum present in matrices. It was found that with increasing concentration of gums in the formulations drug...
release was decreased. Restricted drug release in upper GIT was found for the formulation containing higher amount of OG. Drug release study for above said formulations in presence of rat caecal content showed higher drug release. This may be due to anaerobic bacteria present in rat caecal content which might get acted upon by enzymatic action on matrix tablet containing natural polysaccharides (OG and GG) and leads to degradation of OG and GG with fast drug release. To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixon crowell, peppas and matrix type. It was found that all formulations FS1 and FS9 follows zero order release kinetics with the \( n \)-values in the range of 0.45-0.89, signifying anomalous transport.

**In vivo pharmacokinetic study in rabbits**

Optimized Formulations FS9, OG10 and OG27 containing 200mg Theophylline were chosen on the basis of in-vitro drug release study (restricted drug release in upper GIT and abrupt drug release on arriving colon) as dosage forms to carry out in vivo pharmacokinetic study. Theo-SR a marketed formulation containing 200mg Theophylline was also selected as dosage form for comparison of pharmacokinetic parameters with above said optimized formulations.

The values of \( C_{\text{max}} \) were found to be 20.12 ± 2.34\( \mu \)g/mL, 16.45 ±1.97\( \mu \)g/mL, 19.24 ±1.96\( \mu \)g/mL and 20.31 ±1.50\( \mu \)g/mL for the formulations TheoSR, OG10, OG27 and FS9, respectively. The values of \( T_{\text{max}} \) were found to be 4.2h, 6h, 5.2h and 5.1h for TheoSR, OG10, OG27 and FS9, respectively. The half-life were found to be 7.0h, 11.55h, 15.4h and 15.75h with the elimination rate constant of 0.099h\(^{-1}\), 0.061h\(^{-1}\), 0.045h\(^{-1}\) and 0.044h\(^{-1}\) for TheoSR, OG10, OG27 and FS9, respectively. These results showed that the drug absorption was relatively rapid with marketed formulation (TheoSR) as compared to prepared formulations (OG10, OG27 and FS9) as well as the drug absorption from the prepared formulations was most likely occurred in the lower GI tract of the
rabbit. Moreover, the Analysis of variance (ANOVA) for data of AUC\textsubscript{0-t} showed no statistically significant difference ($p<0.05$) between the prepared formulations (OG10, OG27 and FS9) when compared with marketed formulation (TheoSR) that suggest the prepared formulations have similar rate and extent of drug absorption. These results indicates the good control over drug release in upper GIT with abrupt drug release as the tablet formulations reaches the lower part of GIT.

**Stability study**

In view of the potential utility of the formulations OG10, OG27 and FS9 for colonic release of Theophylline, stability study was carried out by storing the formulations at 40\textdegree C/75\% RH for 6 months to access the long term stability. There was no change in the physical properties of these formulations at the end of storage period which indicate the formulations could have a minimum shelf life of 2 years.

**DICLOFENAC SODIUM**

Identification of Diclofenac sodium was performed by melting point study and IR spectroscopy study. Preformulation study was performed by Differential scanning calorimetry that showed drug, polymer and excipients does not reacted adversely in the formation of tablets.

**Formulation of fast disintegrating core tablets**

Fast disintegrating core tablets of Diclofenac sodium were prepared by using direct compression method that would allow the core tablets to disintegrate rapidly once the coat material is digested by the resident microflora of the colon. Tablet formulations (C01-C13) were prepared by using various disintegrating agents like chitosan, starch, pectin, lactose and MCC with magnesium stearate as lubricant for different tablet formulations. The compression force was adjusted to give core tablets with hardness of 2 to 3kg/cm\textsuperscript{2}. Prepared formulations were evaluated for weight variation, hardness
drug content and disintegration time. Average weight of the core tablets was fixed at the lowest possible level (155mg) to accommodate maximum amount of coat material over the core tablet. The core tablet formulation (C05) was disintegrated within 18sec showing required fast disintegration characteristics of core tablets. Diclofenac sodium core tablet formulation (C05) contains fast disintegrating additives like microcrystalline cellulose (50mg) and starch (50mg) which might have contributed for such a fast disintegration of core tablets (disintegration time=18sec) that would ensures abrupt and complete release of drug when the dosage form reaches the colon. The fast disintegrating characteristics of the core tablets prevent it from being the rate limiting factor. Hence, core tablet formulation (C05) containing starch along with MCC was selected for further study.

**Compression coating of fast disintegrating core tablets**

Wet granulation method for the formulation of compression coat

Formulations CC14 to CC17 and CC18 to CC21 containing various concentration of OG were prepared by using 5% starch paste and 4% PVPK30 as binders, respectively. These tablets were evaluated for weight variation, friability, hardness and Rel5h. Values of Rel5h were found to be decreased (25.34% to 5.14%) with increasing concentration of OG in the formulations. This may be due to increased thickness of coat with increase OG concentration that act as resistance and thus retarded the drug release. Drug release study was carried out with PBS pH 6.8 in presence or absence of rat caecal content for the formulations (CC17 and CC21) because they showed restricted drug release in upper GIT. During in vitro drug release study, the percentage drug released was found to be increased from 5h onwards indicating the commencement of breaking of gum coats in presence of rat caecal content. So it was concluded that in presence of rat caecal contents the drug release from the formulation was increased significantly. PVPK30 as binder was found to restrict the drug release in upper GIT and thus further batches were prepared using PVPK30.
To study synergistic action of OG and GG, compression coats were given using combinations of OG:GG viz. 25:75, 50:50, 75:25 to prepare core coat tablet formulations (CC22-CC24). These formulations were evaluated for weight variation, friability, hardness and Rel$_{5h}$. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (20.14% to 16.24%). It was found that with formulations (CC24) containing OG (300mg) and GG (100mg) could control the drug release in upper GIT (16.24%). This may be due to increased thickness of coat with increase OG concentration that act as resistance and thus retarded the drug release.

Direct compression method for the formulation of compression coat

A direct compression method was used to prepare coat which is to be given on core tablet with the aim of controlling further drug release in upper GIT. Tablet formulations (CC25-CC28) containing increasing concentration of OG were prepared where coat was given by direct compression method. These prepared tablets were evaluated for weight variation, friability, hardness and Rel$_{5h}$. Values of Rel$_{5h}$ were found to be decreased (11.14% to 3.11%) with increasing concentration of OG in the formulations. This was attributed to the increased thickness with increase OG concentration that act as resistance and thus retarded the drug release. Drug release study in presence or absence of rat caecal content was performed for the formulation (CC28) that restricted drug release in upper GIT. During drug release study the percentage drug released from tablets coated with coat formulation CC28 (500mg OG) was found to increase from 5h onwards indicating the commencement of breaking of gum coats in presence of rat caecal content. The percentage drug released after 8h was 78.24% in presence of rat caecal content as compared to 27.27% in absence of rat caecal content. So it was concluded that in presence of rat caecal contents the drug release from the formulation was increased significantly.

Further batches of tablets (CC29-CC40) were prepared using combinations of OG and other polymers (HPMC/ethyl cellulose/pectin/guar gum) to check their synergistic effect on tablet properties. These tablets were evaluated for weight
variation, friability, hardness and Rel$_{5h}$. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (36.45% to 11.64%). During dissolution study some tablet formulations containing combinations of OG with HPMC and EC were broken down in two halves and thus their percentage drug release was not mentioned and these combinations were not used for further study. Formulations containing combination of OG and pectin were found to release the drug relatively faster in upper GIT and hence not included for further study. Formulations containing combination of OG and GG were effective to retard the drug release in upper GIT and gave faster drug release when it reaches to the colon due to synergistic effect of OG (low water solubility) and GG (tableting properties).

Based on this preliminary study it was found that directly compressed formulations containing combinations of OG:GG as coat were able to control the drug release in upper GIT to a great extent and hence further study was employed using direct compression method.

Factorial design

A factorial design was employed to find out the best combination of OG and GG that controls further drug release in upper GIT and upon arrival to colon that releases the drug as quickly as possible. Tablet formulations (FC1-FC9) were prepared using $3^2$ factorial design wherein $X_1$, ratio of OG to GG (25:75, 50:50, 75:25) and $X_2$, total weight of coat (250mg, 350mg, 450mg) were selected as independent variables and their effect on Rel$_{5h}$ as dependent variable was studied. These tablets were evaluated for weight variation, friability, hardness, and Rel$_{5h}$. Values of Rel$_{5h}$ were found to be decreased with increasing concentration of OG in the formulations (32.12% to 5.45%). The data clearly indicates that the dependent variable is strongly dependent on the independent variables.
The equation of the response Rel_{5h} is given below:

\[
\text{Rel}_{5h} = 18.57 - 5.518X_1 - 7.7066X_2 - 2.705X_1X_1 + 0.53X_2X_2 + 1.6725X_1X_2
\]

R square= 0.9543

Percentage drug released for fifth hour (Rel_{5h}) for the 9 batches showed wide variation in the response ranging from a minimum 5.45% to a maximum of 32.12%. This large difference between the minimum and maximum value indicates that the ratio of OG to GG and coating thickness of the formulation (total weight of coat) plays an important role in controlling drug release in upper GIT.

Drug release study in presence or absence of rat caecal content was performed for the formulation (FC9) that restricted the drug release in upper GIT. The percent drug released from tablets coated with coat formulation FC9 (X_1=75:25 and X_2=450mg) was found to increase from 5h onwards indicating the commencement of breaking of gum coats in presence of rat caecal content. The percentage drug released after 8h was 97.64% in presence of rat caecal content as compared to 35.24% in absence of rat caecal content. Moreover, similarity factor (f_2) was used to compare dissolution profiles for the formulation (FC9) in presence and absence of rat caecal content. The f_2 value for comparison of dissolution data of tablet formulation was found to be less than 50 indicating that drug release profiles of the formulations in presence and absence of rat caecal content are dissimilar. So it was concluded that in presence of rat caecal contents the drug release from the formulation was increased significantly.

Drug release kinetics

To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixon crowell, peppas and matrix type using software PCP Disso V 3.0. In present section of investigation best fit model was found to be Korsmeyer-Peppas (R^2=0.9983) for the formulation FC9. The n value was found to be
0.756 indicating anomalous transport. In this formulation, ratio of OG to GG \( X_1 \) decreases the hydration of matrix and retards the release owing to its relative hydrophobic property where as total weight of coat \( X_2 \) retards the release by diffusion mechanism.

**Scanning electron microscopy (SEM)**

During dissolution study, formulation (FC9) showed controlled drug release in upper GIT with subsequent rapid drug release in colonic fluid was subjected to scanning electron microscopy (SEM) to check the surface integrity during different phases of dissolution. Images were taken at different time intervals viz. 0h, 2h, 5h and 6h. SEM image of fresh tablet formulation (FC9) at 0h showed smooth surface. SEM image of same tablet at 2h exhibited less porosity, after 5h showed increased porosity. The low porosity in 0.1N HCl (pH 1.2) may be due low solubility of OG in 0.1N HCl (pH 1.2). The higher porosity of tablet surface in PBS (pH 7.4) may be due to its solubility at near to the neutral pH (pH 6.8). SEM image of same tablet after 6h in PBS pH 6.8 containing rat caecal content showed larger pore formation. The microbiota of rat caecal content might act upon the natural polysaccharides and stimulates microbial degradation that resulted in the larger pore size. The increased porosity of the surface promoted the increase of drug diffusion and the opening of the channels with abrupt release of drug. These pictures clearly show that pore formation is the most important mechanism of drug release.

**In vivo pharmacokinetic study**

Formulations CC28 and FC9 containing 50mg Diclofenac sodium were chosen on the basis of in-vitro drug release study (restricted drug release in upper GIT and abrupt drug release on arriving the colon) as dosage forms for administration. Voveran, a marketed conventional formulation containing 50mg Diclofenac sodium was also selected as dosage form for administration for comparison purpose. The tablet formulations containing drug were administered to rabbits with sufficient flush of water in fasting conditions.
which was assisted by a local veterinary doctor. Blood samples (1mL) were collected from marginal ear vein at before dosing (zero hour) and at predetermined time intervals after dosing and quantitative determination of drug in plasma was performed by HPLC assay to generate plasma concentration time profiles for above said formulations.

The values of $C_{\text{max}}$ were found to be 23.64 ± 2.11μg/mL, 21.21 ± 2.24μg/mL and 22.31±2.64μg/mL for the formulations Voveran (marketed formulation), CC28 and FC9, respectively. The $T_{\text{max}}$ values were found to be 1.9h, 6.2h and 6.0h for Voveran (marketed formulation), CC28 and FC9, respectively. The half-life were found to be 3.01h, 6.3h and 8.15h with the elimination rate constant of 0.23h$^{-1}$, 0.11h$^{-1}$ and 0.085h$^{-1}$ for Voveran (marketed formulation), CC28 and FC9. These results showed that the drug absorption was relatively rapid with Voveran (marketed formulation) as compared to prepared formulations (CC28 and FC9) as well as the drug absorption from the prepared formulations was most likely occurred in the lower GI tract of the rabbit. Moreover, the Analysis of variance (ANOVA) for data of AUC$_{0-t}$ showed no statistically significant difference ($p<0.05$) between the prepared formulations (CC28 and FC9) when compared with Voveran (marketed formulation) that suggest the prepared formulations have similar rate and extent of drug absorption. These results indicates the good control over drug release in upper GIT with a lag time of about 4h and abrupt drug release as tablet formulations reaches the lower part of GIT.

**Stability study**

In view of the potential utility of the formulations CC28 and FC9 for colonic release of Diclofenac sodium, stability studies were carried out by storing the formulation at 40$^o$C/75% RH for 6 months to access the long term stability. There was no change in the physical properties of these formulations at the end of storage period which indicate the formulation could have a minimum shelf life of 2 years.