Formulation, Development and *In Vitro* Evaluation of Colon Targeted Drug Delivery System

Anup D Ganore, Atishkumar S Mundada[*]

SNJB's SSDJ College of Pharmacy, Neminagar, Chandwad, Nashik, India

ABSTRACT

Natural materials have been gaining lot of interest in the development of various drug delivery systems as they are readily available, cost effective, environment friendly, possibility of multitude of chemical modifications, biodegradable and biocompatible due to their natural origin. The present study was an attempt to evaluate the gum obtained from Delonix regia Seed Gum (DRSG) as a novel sustained release excipients in the development of tablet dosage form. The DRSG was evaluated for different physicochemical properties as per Indian Pharmacopoeia (IP) 2007. The water uptake was studied using, surface morphology and Fourier Transform Infrared (FTIR) spectroscopy study was carried out to find drug-polymer interaction. Tablets of diclofenac sodium, a model drug, were formulated using DRSG as matrix former by wet granulation method. Factorial design was employed considering amount of DRSG and core to coating ratio as independent variables while the percentage drug released and the lag time were the dependent variables. The developed tablets were assessed for thickness, diameter, weight variation, hardness, friability, content uniformity and in vitro drug release kinetics and were observed to have uniform appearance, average weight, drug content and adequate hardness. The inverse proportion was observed in the release rate and the DRSG concentration. It can be inferred from the present study that the desired drug release can be obtained by using DRSG as a novel sustained release excipients.

Keywords: DRSG, Diclofenac sodium, Factorial design, Sustained release

INTRODUCTION

Due to the lack of digestive enzymes and the long transit time, colon is considered as suitable site for the absorption of various drugs. Colon Drug Delivery System (CDDS) is helpful in administering drugs that are Gastrointestinal (GI) irritant such as Non-steroidal Anti-inflammatory Drugs (NSAIDs) agents, or peptide drugs that are degraded by gastric juice and the enzymes prevalent in the upper GI tract. The colon drug delivery has a number of important implications in the field of pharmacotherapy of various diseases including inflammatory bowel disease that can be effectively treated by the local delivery of drugs to the large intestine. This technique can minimize the absorption of the drugs from the upper part of GI tract until it reaches the large intestine [1].

Since last decade a novel oral CDDS has emerged as one of the site-specific drug delivery systems. This delivery system releases the drug in the colon following oral administration without being releasing drug in the upper part of the GI tract by means of combination of one or more controlled release mechanisms. This region of GI tract is mainly targeted for delivering drugs like proteins and peptides which degrade in upper part of GI tract or for the treatment of colonic diseases like colon cancer and colititis or for the treatment of diseases sensitive to circadian rhythms such as Asthma, Angina and Rheumatoid arthritis and for the delivery of steroids, which are absorbed well in the colon. The era of slow release technologies have increased the chances for a drug to be released in the colon and thus this organ play an important role in drug absorption from oral sustained release formulations [2].

Various approaches have been utilized for targeting colon, namely pH and time-dependent systems, pressure-controlled release systems, osmotic systems, prodrugs and polysaccharide-based delivery systems. The lack of site-specificity with pH approach is being due to inter/intra subject variation and the similarity of the pH between the small intestine and the colon. The success of timed-release systems largely depends upon the consistency of the small intestinal transit times; however, the high variability in gastric emptying times makes it difficult to predict the accurate location of drug release. Inherent bacterial flora present in the colon carries out enzymatic degradation of prodrugs and polysaccharide-based delivery systems thereby releasing the drug. Site-specificity of these systems ensured due to enzyme trigger mechanism. Detailed toxicological study needs to be performed on prodrugs, as they are considered as new chemical entities from a regulatory perspective [3].

Binders are required to get pharmaceutical tablet formulation as they provide adequate mechanical properties by promoting the bonding between the different components of a powder mix in a formulation. Various natural, semi-synthetic and/or synthetic substances like starches, cellulose and gums have been employed in pharmaceutical tablet formulation as binders.

Gums are an example of hydrophilic substances employed in pharmaceutical solid dosage formulation mainly as binders. In this work *Delonix regia* seed gum (DRSG) obtained from *D. regia*, Family: Fabiaceae, has been evaluated for its activity as a sustained release excipient in the development of colon specific dosage form [4,5].

Diclofenac Sodium (DS) was selected as it has analgesic, antipyretic and anti-inflammatory activities. It is non-selective cyclooxygenase inhibitor and its potency is greater than that of indomethacin, naproxen or several other agents. In addition, DS reduces intracellular concentration of free arachidonate in leucocytes, perhaps by altering the release or uptake of the fatty acid. DS exhibit plasma protein binding of more than 99% after oral administration over 80% of the dose is absorbed from the GI tract (mainly the intestine) with Plasma half-life is 2-4 h [6,7].

MATERIALS AND METHODS

Materials

Diclofenac sodium BP was a gift sample procured from Navketan Research & Laboratories Pvt Ltd. Aurangabad India. Other chemical used were of analytical grade and were purchased from Loba Chemie Pvt Ltd, Mumbai.

Full factorial design

A 3^2 full factorial design was used for optimization of the formulations. The independent variables were the amount of polymer (X_1) and core to coating ratio (X_2). The lag time (5 h) (Y1) and drug release after lag time (Y2) were selected as dependent variables. Regression analysis was performed to identify the best formulation.

Methods

Isolation of polymer

The seeds of the chosen plant *D. regia* were collected and subjected to boiling for 5-6 h. seed coat were removed and the mesosperm was separated which was then transferred into beaker containing distilled water and boiled again to get the viscous mass. This viscous polymeric mass was then kept in an oven for complete drying. The dried polymeric material was then subjected to size reduction in multimill and sieved through sieve shaker using 60# sieve to get the desired particle size [4,5].

Drug-excipient compatibility study

Evaluation of drug-excipient interaction was carried out using Fourier Transform Infrared (FTIR) spectroscopy. KBr pellet method was used for obtaining FTIR spectra's of DRSG, DS and physical mixture of drug and polymer (1:1 ratio). The drug polymer interactions were evaluated by comparing the IR spectra of combination with that of the drug and polymer alone [8].

Development of tablets

50 mg DS containing tablets weighing around 100 mg were prepared by wet granulation techniques using other excipients. Before tableting all excipients except glidants and lubricant were passed through sieve No. 80. After that wet mass was prepared by adding binder solution i.e., starch paste (10% w/w). The dough mass was then screened through sieve No. #16 to get uniform granules. The wet granules so obtained were dried at 60°C in oven for 30 min. The dried granules were then lubricated with magnesium stearate and talc (1:1) and compressed using 10 mm round flat punches on an 8 station single hopper tablet compression machine (Karnavati Rimek, Mini press-II, Mumbai). Press coating of the core tablets with Eudragit S100 as coating material was done using 12 mm round flat punches.

Physico-chemical evaluations

Initially granules were evaluated for various pre-compression parameters like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose. The tablets were then compressed and all batches of the tablets were evaluated for weight variation, hardness, friability and drug content uniformity [7].

In vitro drug release study

Drug release study from the developed tablet formulation was carried out using USP apparatus II (paddle). The dissolution medium (900 ml) was simulated gastric fluid for first 2 h, simulated intestinal fluid for 3 h and simulated colonic fluid until complete release [9,10]. The speed of paddle was 50 rpm and temperature of dissolution medium was 37 ± 0.5°C. 5 ml aliquots were withdraw at predetermined intervals and replacement was made each time with 5 ml of fresh dissolution medium to maintain sink condition. The withdrawn sample was filtered through Whatman filter paper no. 41 and diluted up to 10 ml with respective dissolution medium and analyzed for drug content at 275.5 nm using UV-Visible Spectrophotometer, (Jasco V-630, Japan).

Drug release kinetics

Dissolution data needs to be treated as per various drug release kinetics in order to understand the mechanism of the release of the drug from the developed formulations. *In vitro* drug release data was put up in PCP Disso Software that suggested the release kinetic model that is being followed by the formulation.

Stability study

Stability study of the optimized formulation was carried out according to International Conference on Harmonisation (ICH) guidelines. The formulations were stored at accelerated (40 ± 2°C/75 ± 5% RH) test conditions in stability chamber (Remi CHM-6S) for 3 mon. At the end of every month, tablets were tested for two important parameters like drug content and *in vitro* drug release profile [11].

RESULTS AND DISCUSSION

There is obvious need to find new material for the growing research efforts in the drug delivery as continued improvement and accelerating research and development in polymeric materials have played a vital role in the progress of controlled release technologies. DRSG is one of such novel excipients which we have tried in the development of site specific drug delivery system.

Drug excipient compatibility study

The DS shows characteristic peak of N-H stretching at 3387 cm^{-1}, C=C stretching at 1573 cm^{-1} (C=C Aromatic C$_6$H$_5$), C-O bending at 1166 cm^{-1} (C-O). Polymer *D. regia* exhibited peaks for O-H stretching at 3363 cm^{-1} (O-H) and C-H stretching at 2924 cm^{-1}. The mixture of drug and polymer show N-H stretching at 3387 cm^{-1} (N-H), C=C stretching at 1573 cm^{-1} (C=C Aromatic C$_6$H$_5$) C-O bending at 1166 cm^{-1} (C-O) and polymer show O-H stretching at 3363 cm^{-1} peak. Thus it is clear from the peaks obtained for the combination of the drug and polymer (1:1) that the major reactive moieties of the drug and polymer have remain unaffected in the combination indicating compatibility between drug and novel polymer.

Development of preliminary trial batches

Prior to development of preliminary trials of core tablets, initially 9 trials (A1-A5 and C1-C4) were carried out to study effect of novel polymer concentration on drug release. A1-A5 was prepared along with Microcrystalline Cellulose (MCC) as diluents but the problem is that the MCC is superdisintigrant that's why tablets were disintegrated within an hrs. So instead of MCC, Dibasic calcium phosphate (DCP) was used as diluents and the trial batches were prepared. The tablets show Hardness in the range of 3.66-4.5 kg/cm^2 and friability in the range of 0.05-0.1%. The drug release from C1-C4 batches was 98.72, 99.94.98.34 and 90.03% respectively.

Factorial design

A 3^2 randomized full factorial design was used in this study. In this design two factors, each at 3 levels, were evaluated. The amount of novel polymer i.e., DRGS and core to coating ratio were selected as independent variables and the lag time (5 h) and drug release after 5 h were selected as dependent variables. The dependent and independent variables and used levels are summarized in Table 1.

Table 1: Experimental design

Factor	Level			Responses
	(-1)	(0)	(+1)	
Amount of polymer (%) (X1)	25	30	35	Drug release after lag time (Y1)
Core to coating ratio (X2)	1:1	1:2	1:3	Lag time of 5 h (Y2)

The nine batches (G1-G9) were prepared in 3^2 full factorial designs as per given in Table 2.

Table 2: Formulation of tablets

Name of ingredient	Formulation code								
	G1	G2	G3	G4	G5	G6	G7	G8	G9
Diclofenac sodium	50	50	50	50	50	50	50	50	50
Polymer	25	25	25	30	30	30	35	35	35
DCP	23	23	23	18	18	18	13	13	13
Magnesium stearate	1	1	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1	1	1
Coating ratio	01:01	01:02	01:03	01:01	01:02	01:03	01:01	01:02	01:03
Total	200	300	400	200	300	400	200	300	400

DS tablet characterization

Wet granulation was the method used to get the DS granules which were evaluated for pre-compression parameters like bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose to understand the suitability of DS granules for compression into tablets. The bulk density ranges from 0.62-0.66 g/cm^3, tapped density was found to be between 0.71-0.78 g/cm^3. Carr's indices were between 9.21-18.2 and Hausner's ratio was observed to be 1.08-1.23. The values for angle of repose were 12.58-18.59° indicating granules have excellent flow properties (Table 3).

Table 3: Evaluation of granules

Test code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index	Hausner's ratio	Angle of repose
G1	0.653 ± 0.011	0.746 ± 0.011	12.46 ± 2.86	1.14 ± 0.03	14.42 ± 1.28
G2	0.64 ± 0.017	0.78 ± 0.025	18.2 ± 4.62	1.22 ± 0.065	17.90 ± 1.30
G3	0.65 ± 0.030	0.71 ± 0.03	9.21 ± 5.20	1.08 ± 0.04	12.58 ± 1.14
G4	0.66 ± 0.011	0.78 ± 0.034	14.43 ± 3.38	1.16 ± 0.04	18.59 ± 1.56
G5	0.62 ± 0.011	0.77 ± 0.023	18.94 ± 0.91	1.23 ± 0.017	18.25 ± 1.30
G6	0.65 ± 0.011	0.78 ± 0.023	17.76 ± 2.12	1.18 ± 0.065	17.48 ± 4.68
G7	0.65 ± 0.030	0.71 ± 0.03	9.21 ± 5.20	1.08 ± 0.04	14.73 ± 1.73
G8	0.66 ± 0.02	0.75 ± 0.011	12.34 ± 3.90	1.13 ± 0.05	16.84 ± 3.00
G9	0.65 ± 0.02	0.74 ± 0.011	14.26 ± 3.00	1.16 ± 0.03	17.02 ± 2.36

On confirmation that granules had excellent and favorable properties for compression, tablets were compressed using 8station tablet machine. The Hardness of the compressed tablet of DS was found to be in the range of 3.3-4.6 kg/cm^2. The DS tablets were also found to comply with the friability test since the weight loss was found to be less than 1%. The mean drug content of the DS tablet was found to be 97.12-100.79% which complies with the range recommended in IP. The uniformity in the developed DS tablets suggests that the method used in the preparation of granules and subsequent compression was reproducible (Table 4).

Table 4: Evaluation of tablet

Test code	Hardness (kg/cm^2)	Friability (%)	Drug content (%)
G1	3.38 ± 0.288	0.066 ± 0.057	99.88
G2	4.66 ± 0.288	0.129 ± 0.056	97.12
G3	4.16 ± 0.288	0.22 ± 0.11	99.28
G4	3.83 ± 0.288	0.129 ± 0.056	98.98
G5	4.16 ± 0.288	0.194 ± 0.097	99.78
G6	4.15 ± 0.283	0.128 ± 0.055	100.79
G7	4.66 ± 0.288	0.178 ± 0.062	99.58
G8	4.33 ± 0.288	0.133 ± 0.057	99.98
G9	4.33 ± 0.288	0.191 ± 0.082	98.12

The expected release of the active ingredient in the colonic region was achieved with the help of Eudragit S100 coating. Eudragit S100 was tried in different concentration (25%, 30% and 35%) with varying core to coating ratio (1:1, 1:2, 1:3). Formulation G1, G2 containing 25% polymer along with 1:1 and 1:2 core to coating ratio exhibited release of the drug taking place before formulation reaches to the colon. This effect could be attributed to the small amount of coating material which failed to control the drug release in stomach and small intestine. Formulation G4 and G5 containing 30% polymer with 1:1, 1:2 core to coating ratio although exhibited 98.98% and 100.19% drug release up to 12 h, however, these formulations also released drug in upper part of GI tract. G7 and G8 released 96.28% and 92.31% drug up to 12 h though releasing small amount of the drug in the stomach as well as small intestine (Figure 1).
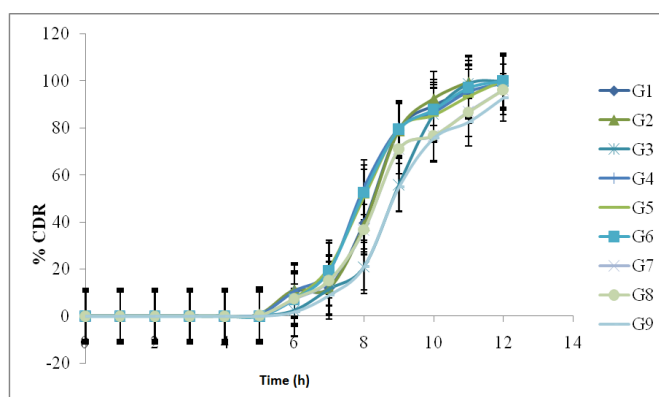
Figure 1: *In vitro* drug release study (G1-G9)

Formulation G3, G6, G9 on the other hand, did not allow the drug to be released in the upper part of the GI tract and on reaching the colonic region these formulations exhibited drug release sustained for 12 h. The *in vitro* drug release data was then put up in the Design expert software for regression analysis.

Model assessments for the dependent variables

(A) Drug release after 5 h, final equation in terms of actual factors in below:

$$\text{Drug release} = +303.33 - 5.00\ (X_1) + 25.00\ (X_2)$$

The Model F-value of 23.40 implies the model is significant. There is only a 0.15% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model (Table 5).

Table 5: Analysis of variance for Y1

Source	Sum of squares	Df	Mean square	F-value	P-value Prob > F	Significance
Model	3900.00	2	1950.00	23.40	0.0015	S
A-Polymer concentration	150.00	1	150.00	1.80	0.5583	NS
B-coating ratio	3750.00	1	3750.00	45.00	0.0005	S
Residual	500.00	6	83.33			-
Core Total	4400.0	8				-

(B) Lag time, final equation in terms of actual factors.

$$\text{Lag time} = +98.19 - 2.31(X_1) - 0.45(X_2)$$

The Model F-value of 5.62 implies the model is significant. There is only a 4.21% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model (Table 6).

Table 6: Analysis of variance for Y2

Source	Sum of squares	Df	Mean square	F-value	p-value Prob > F	Significance
Model	33.26	2	16.63	5.62	0.0421	S
A-amount of polymer	32.06	1	32.08	10.84	0.0166	S
B-coating ratio	1.2	1	1.20	0.40	0.5481	NS
Residual	17.74	6	2.96	-	-	-
Core total	51.00	8	-	-	-	-

Optimization

After analysis of both independent variables and dependent variables Design expert software gives solutions (Table 7).

Table 7: Solutions for optimized batch

S. No.	Amount of polymer	Coating ratio	Drug release (%)	lag time (min)	Desirability
1	25%	1:3	99.76	330	0.993

According to the solution provided by optimization studies formulation containing 25% polymer and 1:3 cores to coating ratio was prepared by wet granulation technique and evaluated. These DS tablet show hardness of around 4.33 ± 0.288 kg/cm^2. The DS tablets were also found to comply with the friability test since the weight loss was found to be less than 1%. The mean drug content of the DS tablet was found to be 99.25% and the formulation was found to reach colon whereupon it sustained the release of the drug for about 12 h. The drug release profile clearly reveals that there was no release of the DS that took place in stomach and intestine which proves the efficacy of the formulation for targeting the colonic region. Drug release from the developed formulation found to follow zero order kinetics.

Stability study

Optimized formulation was kept for stability studies at accelerated stability conditions to evaluate the robustness of the formulation. Samples were withdrawn according to the sampling protocol at day 0, 30, 60 and 90 and were analyzed for *in vitro* dissolution profiles (Figure 2) and drug content (Table 8).
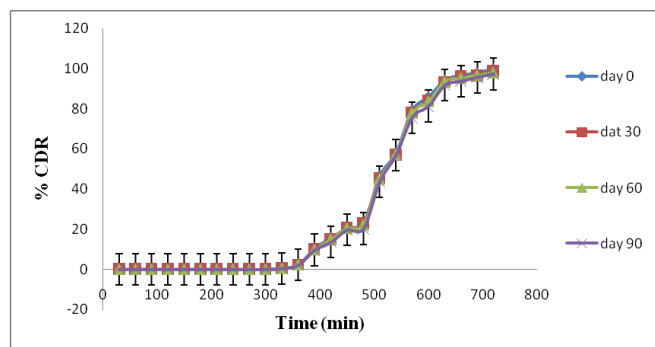

Figure 2: *In vitro* drug release study (Stability study)

Table 8: Drug content for stability study

S. No.	Day 0	Day 30	Day 60	Day 90
Drug content	99.14	98.87	100.09	99.89
Drug release	99.76 ± 1.19	98.96 ± 1.40	98.02 ± 1.65	97.25 ± 1.87

Drug release from the optimized formulation after storage as per ICH conditions for 3 mon follows zero order and thus revealing that the formulations were stable and robust. Drug content did not vary to very great extent after 3 mon storage. Thus it is evident from the stability studies that the tablets remained stable after 3 months accelerated stability.

CONCLUSION

This study showed that it is possible to control the release rate of DS over a wide time scale using *D. regia* as sustained release polymer. It was also observed that the release of DS is slower in pH 1.2 and pH 6.8 and much higher in pH 7.4. It can be thus concluded from the present investigation that the chosen novel natural polymer can be exploited for the development of colon specific drug delivery system. The results need to be substantiated by appropriate *in vivo* study.

ACKNOWLEDGMENTS

REFERENCES

[1] J. Ali, R.K. Khar, A. Ahuja, Birla Publications, **2008**.

[2] P.V. Reddy, P.R. Manthri, *Acta Pharmaceutical Sciencia*., **2010**, 52, 281-294.

[3] G. Tiwari, *Int. J. Drug Deliv.*, **2010**, 01-11.

[4] E. Gbadegesin, Adetogun, *Acta Poloniae Pharmaceutica.*, **2009**, 66, 433-438.

[5] A.R. Tekade, S.G. Gattani, *Int. J. PharmTech. Res*., **2010**, 2, 106-112.

[6] P.B. Aswer, S.S. Khadabadi, B.S. Kuchekar, T.P. Wane, N. Matake, *Arch Pharm. Sci. Res*., **2009**, 1, 48-53

[7] Indian Pharmacopeia, Ministry of Health and Family Welfare, Government of India, Ghaziabad, **2007**.

[8] G.R. Chatwal, S.K. Anand, Himalaya Publishing, **2007**, 243-245.

[9] V.K. Gupta, T.E. Beckert, J.C. Price, *Int. J. Pharm*., **2001**, 213, 83-91.

[10] R. Kaza, R. Nagarju, Y. Swapna, B.R. Hari, *J. Pharm. Sci. Technol*., **2010**, 2, 103-110.

[11] J.M. Patel, M.R. Brahmabhatt, V.B. Patel, S.V. Muley, P.G. Yeole, *Int. J. Pharm. Res.*, **2010**, 2, 28-35.