IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2017 Vol.:10, Issue:3

Formulation and Evaluation of Locust Bean Gum Microspheres of Cromolyn Sodium for Treatment of Ulcerative Colitis

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

V.N.L.SIRISHA*[1], M. CHINNA ESWARIAH[2], A. SAMBASIVA RAO[3]

[1] *Research Scholar, JNTUH, Hyderabad*

[2] *Principal, Anurag Pharmacy College, Kodad, Nalgonda Dt, Telangana*

[3] *Principal, Sri Indu College of Pharmacy, Sheriguda, Ibrahimpatnam, R.R Dt, Telangana*

Submission: 19 September 2017
Accepted: 29 September 2017
Published: 30 October 2017

Keywords: Locust Bean Gum, Cromolyn Sodium, Natural Polysaccharide, Microspheres.

ABSTRACT

A multi-particulate system of Cromolyn Sodium was developed for colonic drug delivery by using natural polysaccharide Locust bean gum. Locust bean gum microspheres were formulated by ionic gelation method by using calcium chloride as gelling agent and glutaraldehyde as cross-linking agent. Optimization of microspheres was carried out by using 3^2 full factorial design. The formulated formulations were evaluated for particle size, surface morphology, percent entrapment efficiency, *in-vitro* drug release (with & without rat caecal contents) and stability studies (1 month, at 40±2ºC/75±5%RH).The SEM images revealed the rough and smooth surfaces of un-coated and coated microspheres. The *in-vitro* drug release of optimized formulation (IL4) core microspheres (for 7 hr), coated microspheres (without rat caecal contents, 24hr), coated in with rat caecal contents (24hr) were 97.36±2.45, 89.47±3.66, 98.24±2.25 respectively. The drug release kinetics of all formulations followed Higuchi diffusion; however the coated microsphere formulation followed Korsmeyer-Peppas with Fickian mechanism of release. The stability studies indicated there was no significant change in entrapment efficiency and *in-vitro* drug release hence indicating good stability of formulation.

INTRODUCTION

Ulcerative colitis is an inflammatory bowel disease (IBD) that causes long-lasting inflammation and ulcers (sores) in digestive tract. It affects the innermost lining of large intestine (colon) and rectum. Oral route of administration is the most suitable method with patient compliance but the oral route has disadvantage of systemic side effects; produced when administered through oral route. Rectal administration offers the shortest route for targeting drugs to the colon. However, reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also be uncomfortable for patients and compliance may be less than optimal [(1)]. To overcome the side effects and to improve effectiveness; targeted drug delivery is only possible way to ensure patient compliance. Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs [(2, 3)].

In the stomach, pH ranges between 1 and 2 during fasting but increases after eating[(4)].The pH is about 6.5 in the proximal small intestine and about 7.5 in the distal small intestine[(5)]. From the ileum to colon, pH declines significantly. It is about 6.4 in the cecum. However, pH values as low as 5.7 have been measured in the ascending colon, transverse colon is 6.6 and 7.0 in the descending colon in healthy volunteers [(6)]. The colon specific drug delivery system (CDDS) should be capable of protecting the drug en route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon [(7)].

The human colon has over 400 distinct species of bacteria as resident flora, a possible population of up to 1010 bacteria per gram of colonic contents. Among the reactions carried out by these gut flora are azo-reduction and enzymatic cleavage i.e. glycosides [(8)].The natural polysaccharides are the suitable polymers for targeting the drugs to colon. They can be easily modified chemically, biochemically, and are highly stable, safe, nontoxic, hydrophilic and gel forming and in addition, are biodegradable. The polysaccharides can be broken down by the colonic micro-flora to simple saccharides [(9)]. Therefore, they fall into the category of "generally recognized as safe" (GRAS)

[10].Locust bean gum, also called carob bean gum, is extracted from the outer coating of the carob seed. It is a polysaccharide comprised of galactose and mannose units.

The best Candidates for CDDS are drugs which show poor absorption from the stomach or intestine including peptides. The drugs used in the treatment of IBD, ulcerative colitis, diarrhea and colon cancer are ideal candidates for local colon delivery [11]. Cromolyn Sodium (Disodium Cromoglycate) stabilizes mast cell membranes thus preventing mast cell damage and de-granulation with subsequent release of pharmacologic mediators such as histamine, SRS-A, serotonin and bradykinin which follow certain antigen-antibody reactions. Absorption through mucosal surfaces is reported to vary from less than 2% to 8% of the administered dose, and it is rapidly excreted unchanged in the urine and bile. Since Cromolyn remains unchanged during its transit through the gastrointestinal tract and has low levels of absorption, it might well be effective in treating lesions of the gastrointestinal tract [12].

MATERIALS AND METHODS

MATERIALS

Cromolyn Sodium was obtained as a gift sample from TherDosePharmaPvt Ltd, Hyderabad. Locust Bean Gum was obtained from Nutriroma Pvt. Ltd, Hyderabad. Sodium alginate, calcium chloride and glutaraldehyde were purchased from Sigma-Aldrich. Eudragit S 100 was obtained as gift sample by Evonik, Germany. All reagents used were of analytical grade.

METHOD

Preparation of Microspheres by Ionic Gelation Method

Locust bean gum microspheres containing cromolyn sodium were prepared by dispersing the drug in a solution of locust bean gum in WFI. The microspheres were formed by dropping the above dispersion through a disposable syringe (24 gauge nozzle) into calcium chloride solution (4% w/v) and allowed for curing (1hr).Later separated, washed and dried in an oven at 50ºC for 24hrs and stored plastic bags for further use [13].

Preparation of Eudragit-Coated cross-linked locust bean Microspheres

Eudragit coating of GA cross-linked drug loaded microspheres were prepared by oil-in-oil solvent evaporation method.Eudragit-S-100 was dissolved in 10ml organic solvent (ethanol: acetone) to which 100mg of drug loaded microspheres were added and then poured into 100ml of liquid paraffin containing 3% of w/v Span-80.The above system was agitated at 1000rpm at 40ºC for 3hrs using a mechanical stirrer (Remi, Mumbai, India).The Eudragit coated microspheres were filtered and washed with n-hexane to remove the traces of oily phase on the microspheres and dried overnight in desiccators and packed in plastic bags until further [14].

Optimization and Characterization of Microspheres

A three factor two level full factorial design was used for complete study of combination of drug and polymer.

The main effects ($X_1$and X_2) represent the average result of changing one factor from its low to high values. The interaction term (X_1, X_2) shows how the response values change when two factors are simultaneously changed (Table 1).

Table.1: 3^2—full factorial design: factors, factor levels and responses

Factors –Independant Variables		
Level	Locust bean gum in %(X_1)	Cross-linking agent in ml(X_2)
Low level -1	1	1.5
High level +1	3	4.5
Responses- Dependant Variables		
Y_1	Particle size in mm	
Y_2	Entrapment Efficiency in %	
Y_3	*In-vitro* drug release in %	

Determination of Particle Size

Particle size was measured by Optical microscopy (INKO, Ambala, India) using a compound microscope (min of 500 particles) using ocular micrometer. Each measurement was made in triplicate. The mean particle size was calculated using the formula

$$ADM = \frac{(n1d1+n2d2+\ldots\ldots..nmdm)}{(n1+n2+\ldots\ldots..+nm)}$$

Shape and Surface Morphology

The shape and surface morphology of microspheres were investigated using Scanning electron microscopy (SEM)(LEO-430, Cambridge, U.K). The microspheres were fixed with carbon-glue on the supports and gold coated in a high vacuum evaporator using a gold sputter module. Samples were observed with SEM at 15Kv [15].

Encapsulation Efficiency

About 50mg of microspheres were digested in 10ml Phosphate buffer saline (PBS, pH-7.4) and extracted completely during 24 h. The solution was centrifuged at 6000rpm.The supernatant filtered through 0.22µm membrane filter (Millipore) and the amount of mesalamine was measured spectrometrically (Shimadzu, Double-Beam Spectrophotometer, 150-03, Japan) at 212nm.Each determination was made in triplicate [16, 17].

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount drug content in microspheres}}{\text{Amount of drug added}} \times 100$$

Fourier-transform infrared (FT-IR) spectroscopy

Fourier-transform infrared spectrum (FTIR) were recorded for Cromolyn sodium, locust bean gum, drug- loaded locust bean gum microspheres using spectrum BX (Perkin Elmer) infrared spectrophotometer.

Differential Scanning Calorimetry (DSC)

The thermal behavior of Cromolyn sodium, locust bean gum, drug- loaded Locust bean gum microspheres observed using a differential scanning calorimetry (DSC) Q 10V 8.1 Build 261(Universal V3.9 A TA Instruments) thermal analyzer.

***In-vitro* drug release studies**

The *in-vitro* drug release studies were performed using USP dissolution rate test (paddle apparatus, 100 rpm, 37±0.1℃). 500 mg of microspheres were suspended in 900ml of dissolution media mimicking GI tract environment(2hrs-pH 1.2,3hrs-pH 7.4 and 19hrs-pH-6.8). Samples withdrawn were quantified using UV-Visible Spectrophotometer (Shimadzu, Kyoto, Japan) at 235nm [(18)].

Preparation of rat caecal medium

Albino rats were weighed and killed by spinal traction. The contents weighed and suspended in dissolution medium to give final caecal dilution of 2% w/v. To maintain anaerobic environment CO_2 gas was bubbled into the medium [(19)].

***In-vitro* release in presence of rat caecal contents**

The release of the final optimized formulation was carried out with addition of rat caecal contents (2%w/v) to observe the effect of the caecal enzymes on release rate of drug. Samples obtained at regular intervals were filtered through 0.22µm membrane filter (Millipore, India) and analyzed.

Stability studies[(20)]

According to ICH Guidelines, an accelerated stability study have carried out on the optimized formulation at 40±2℃/75±5% RH for over a period of 30 days.

Release Kinetic Study [20]

All the release data were fitted into various kinetic models like zero order, first order Korsmeyer-Peppas, Higuchi to find out the mechanism of drug release from the polymeric matrix of microspheres.

RESULTS AND DISCUSSION

Evaluation of optimized formulation of formulation variables

To study the effect of variables on characterization of microspheres, different batches were prepared by applying 3^2 full factorial designs. Amount of polymer(X_1) and cross-linking agent(X_2) were varied three levels, low level (-1), medium (0), and high level (+1).The amount of drug and sodium alginate was kept constant. Particle size (Y_1), % entrapment efficiency (Y_2), % *in-vitro* drug release (Y_3) were selected dependent variables Fig-1.

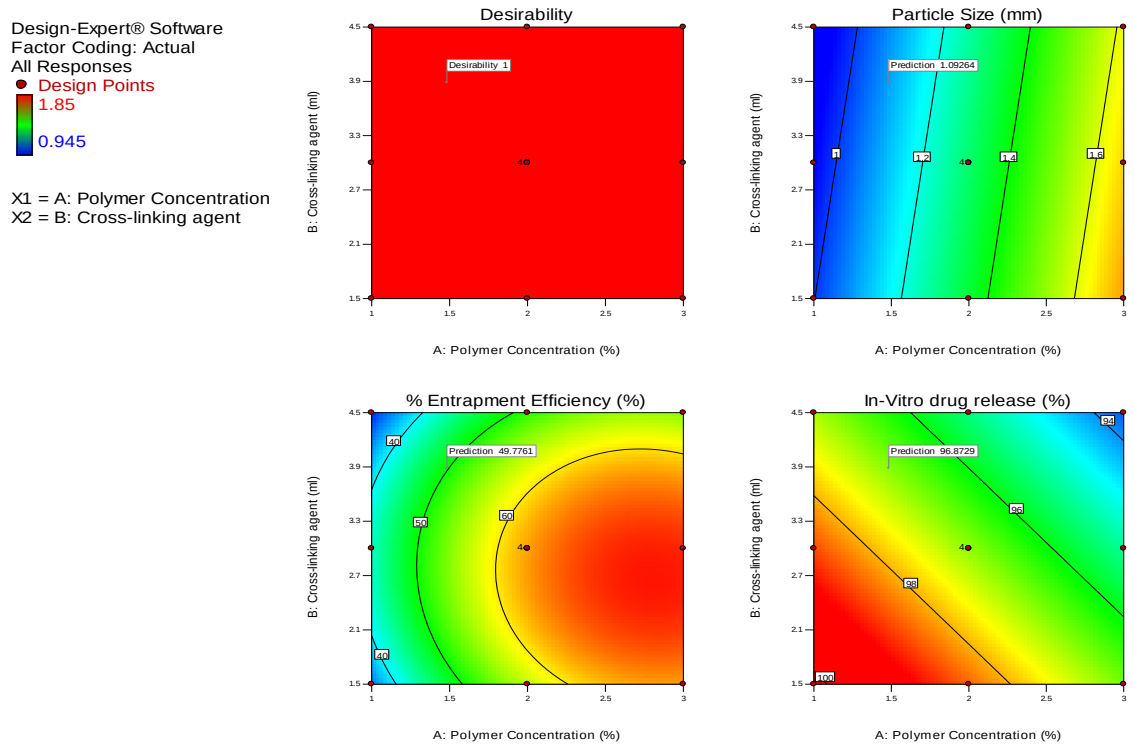


Fig-1: Surface response graphs for particle size, % entrapment efficiency, % *in-vitro* drug release.

The particle size of the microspheres increased with increase in the polymer concentration .i.e., the sphere size got increased on increase in polymer concentration. The size of the sphere decreased with increase in cross-linking agent concentration because of hardening of the polymer matrix and shrinking of size. The particle size increased with coating of the microsphere. The entrapment efficiency increased with the increase of polymer concentration and amount of cross-linking agent. Results are given in Table-2.

Table 2: Characterization of Locust bean microspheres

Batch code	Locust bean gum (X_1) (%)	Glutaraldehyde (X_2) (ml)	Particle size (mm)	Entrapment efficiency (%)	*In-vitro* drug release (%)
IL1	3	1.5	1.85±2.75	68.58±2.57	97.16±0.95
IL2	1	1.5	1.05±1.33	37.42±3.98	98.53±1.32
IL3	3	4.5	1.53±1.77	56.89±2.08	93.19±3.76
IL4	2	3	1.32±2.65	64.84±3.57	97.36±2.45
IL5	1	3	0.976±3.57	42.15±0.65	99.04±3.92
IL6	1	4.5	0.945±1.65	30.44±1.28	97.12±2.28
IL7	2	1.5	1.09±2.76	49.25±2.58	99.06±1.96
IL8	2	4.5	1.22±2.66	50.09±2.56	95.18±2.26
IL9	3	3	1.74±2.74	58.49±1.69	94.21±2.88

*****All readings are expressed as Mean± Standard deviation (n=3)

Scanning electron microscopy confirmed the spherical shape of microsphere. The surface of the un-coated formulation of microsphere was rough and the coated form of the same formulation was smooth in surface (Fig 2). To investigate the interaction between drug and polymer; FTIR studies were carried out. The spectrum of pure drug was overlapped and with the drug loaded microsphere formulation and was observed that no new bond was formed and there was no interaction with polymer indicating good compatibility between the drug and the polymer (Fig 3). The DSC studies carried out to observe the thermal behavior of drug loaded microspheres whether the drug was encapsulated in them or not. This peak in DSC curve explains the molecular encapsulation of cromolyn sodium in the matrix of the polymer (Fig 4).

a)

b)

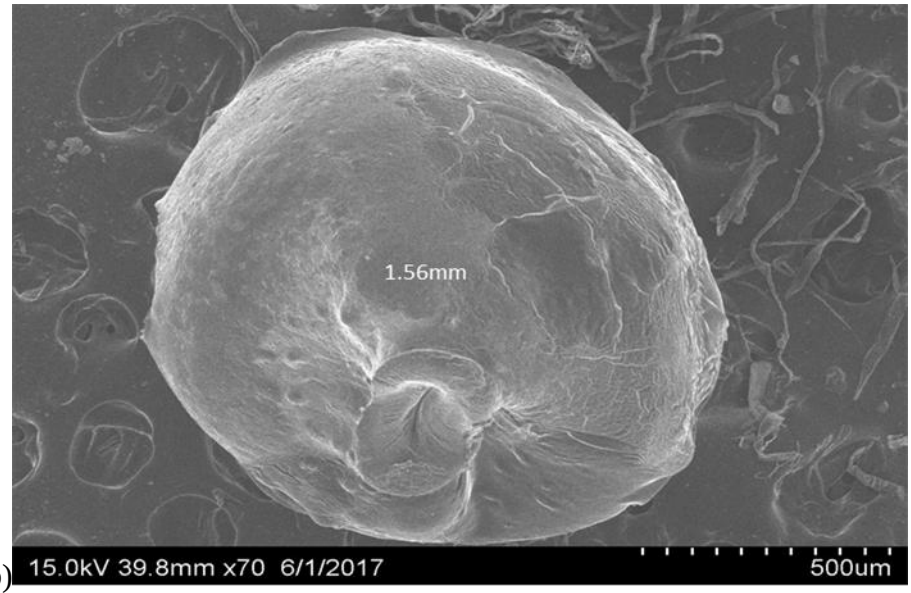


Fig 2 Scanning electronic microscopy of a) surface morphology of un-coated drug loaded microspheres, b) coated drug loaded microsphere.

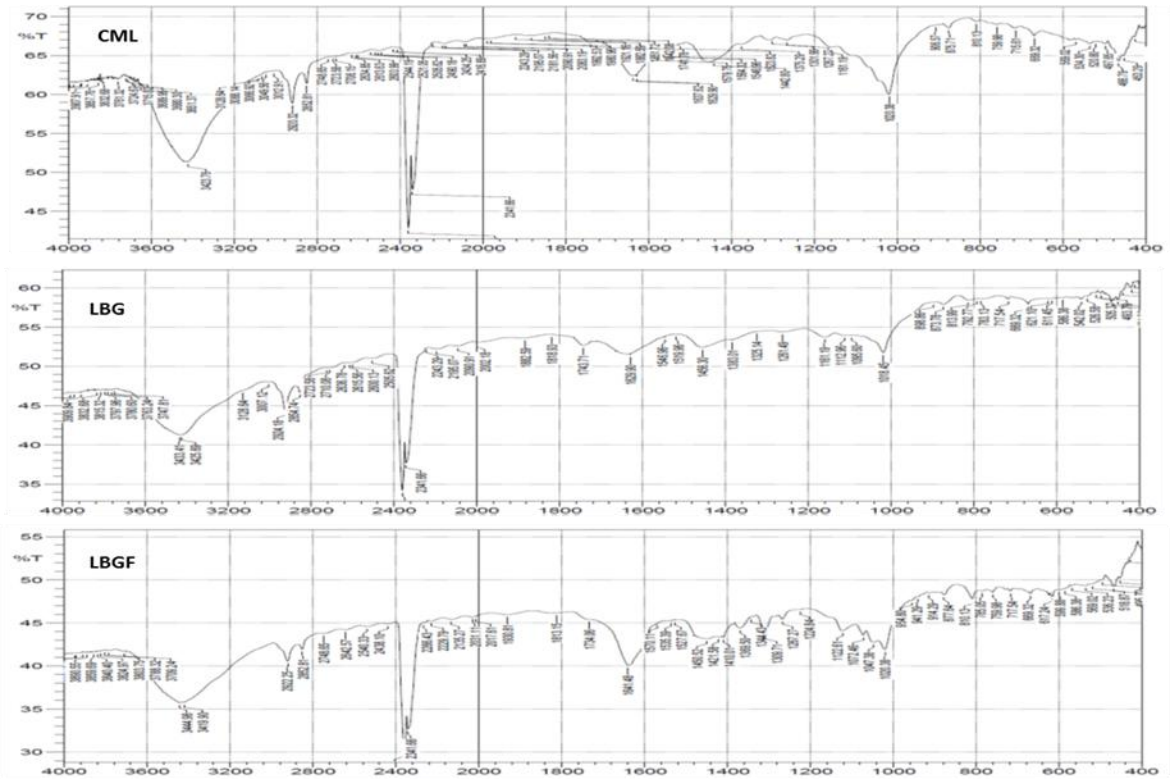


Fig 3 FTIR results of Pure drug, Locust bean gum, CML-Loaded Locust bean gum microspheres

***CML- Cromolyn sodium, LBG-Locust bean gum, LBGF-Locust bean gum formulation**

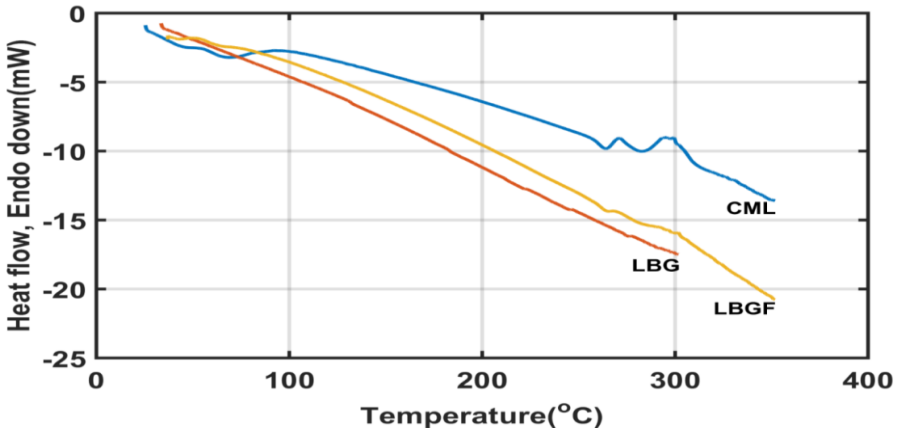
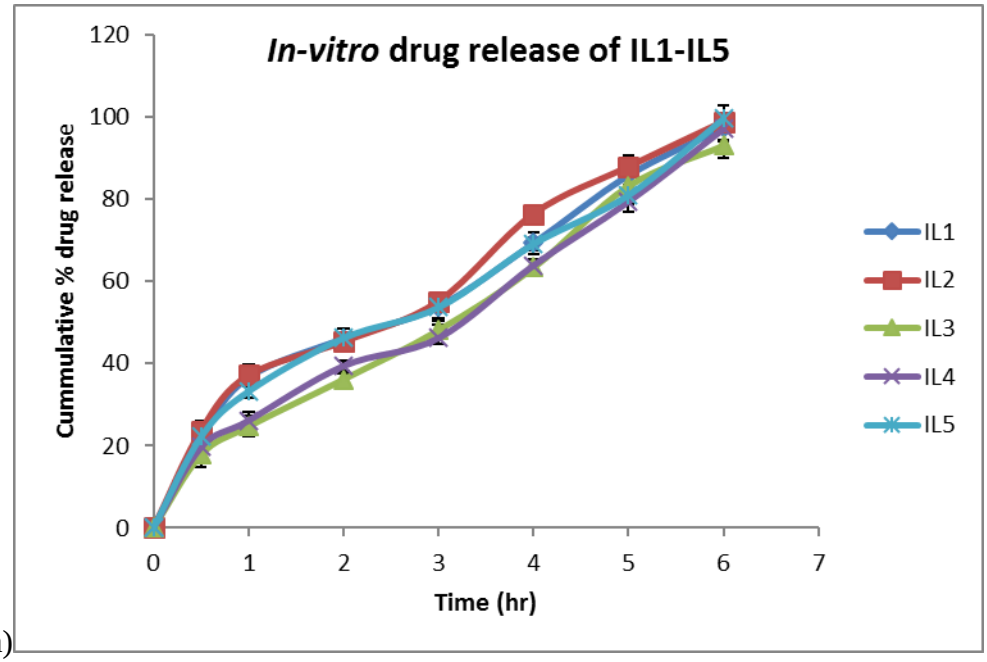


Fig 4 DSC curves of Pure drug, Locust bean gum, CML-Loaded Locust bean gum microspheres.

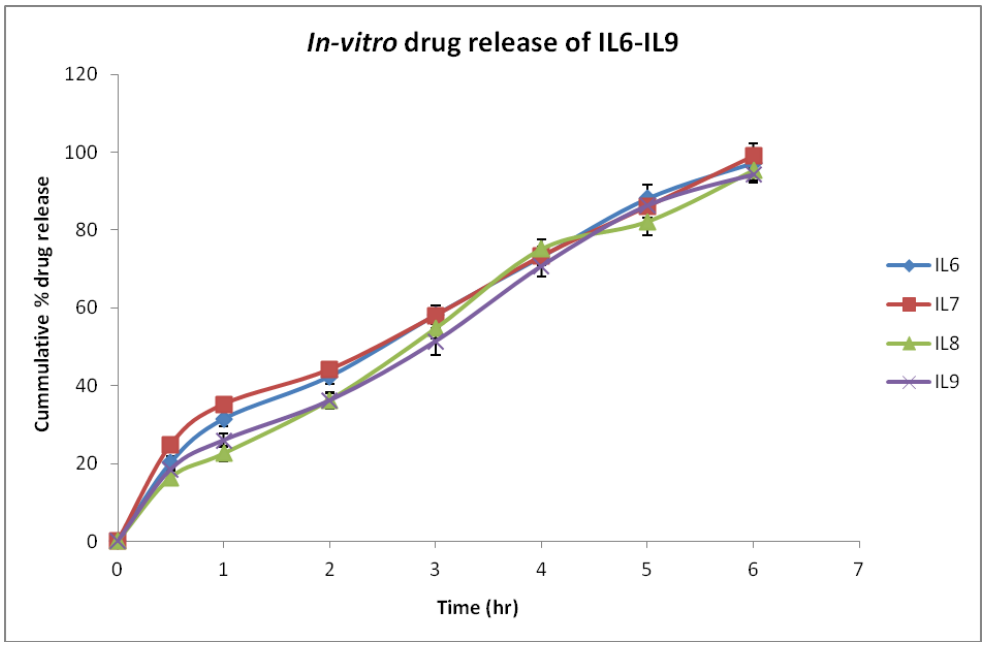
***CML-Cromolyn sodium, LBG-Locust bean gum, LBGF-Locust bean gum formulation**

The amount of drug release for the optimized formulation in first 5h studies showed that the polymer matrix remained intact in stomach and small intestine environment and the gelling property of the polymer retards the drug release from the matrix. There was an initial release of drug in the first 2h of the studies indicating the un-entrapped drug on the surface of the matrix of the microsphere but later due to the formation of the viscous gel layer around the sphere, the drug release was retarded. The polymer matrix could retard the drug release up to around 7hr.To retard the drug release up to 24hrs the optimized formulation was coated with Eudragit S-100 in three different concentrations(4 % w/v,8%w/v,15%w/v) and the drug release studies were performed for the optimized formulation.Of the three concentrations of coating solutions formulation coated with 8%w/v showed good retardation and optimized release of drug for 24hr, while the 4%w/v formulation had low retardation which had quick release & 15%w/v formulation had high retardation which had very low release even after 24hrs. The *in-vitro* drug release studies were performed with and without rat caecal contents for the final optimized formulation and the release were found to be higher in the presence of rat caecal contents (98.24±2.25) % due to the degradation of the polymer matrix by colonic enzymes released by colonic bacteria than without rat caecal contents (89.47±3.66) in the SIF medium (Fig 5). The entrapment efficiency and *in-vitro* drug release have not significantly decreased when compared with the formulation before stability studies. The *in-vitro* release from the core microsphere was found to be following Higuchi diffusion since the plots provide the highest linearity. For all LBG-microspheres, the *n* value as per Korsmeyer-Peppas model was found to be between 0.45 and 0.89, indicating anomalous release behaviour of the drug, (*i.e.*, both diffusion and dissolution of the hydrated polymer matrix).Coated microspheres followed Fickian kinetics with the value $n < 0.45$ as per the Korsmeyer-Peppas model which might be due to relaxation of the polymer matrix, followed by the diffusion matrix (Table 3).

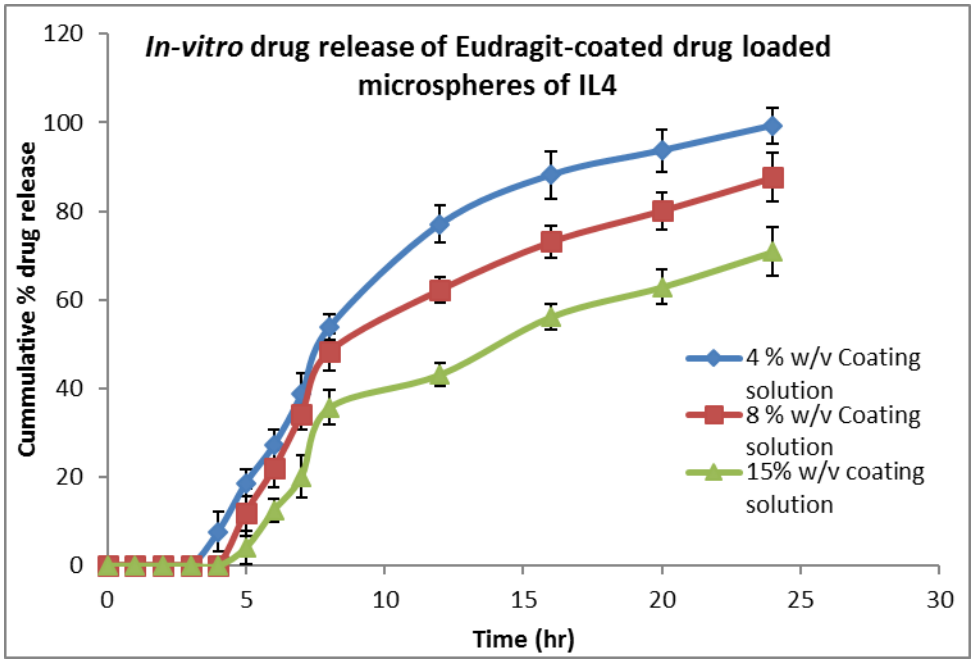
a)


In-vitro drug release of IL1-IL5
Cummulative % drug release
Time (hr)
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7
IL1
IL2
IL3
IL4
IL5

b)


In-vitro drug release of IL6-IL9
Cummulative % drug release
Time (hr)
0
20
40
60
80
100
120
0
1
2
3
4
5
6
7
IL6
IL7
IL8
IL9

c)



d)

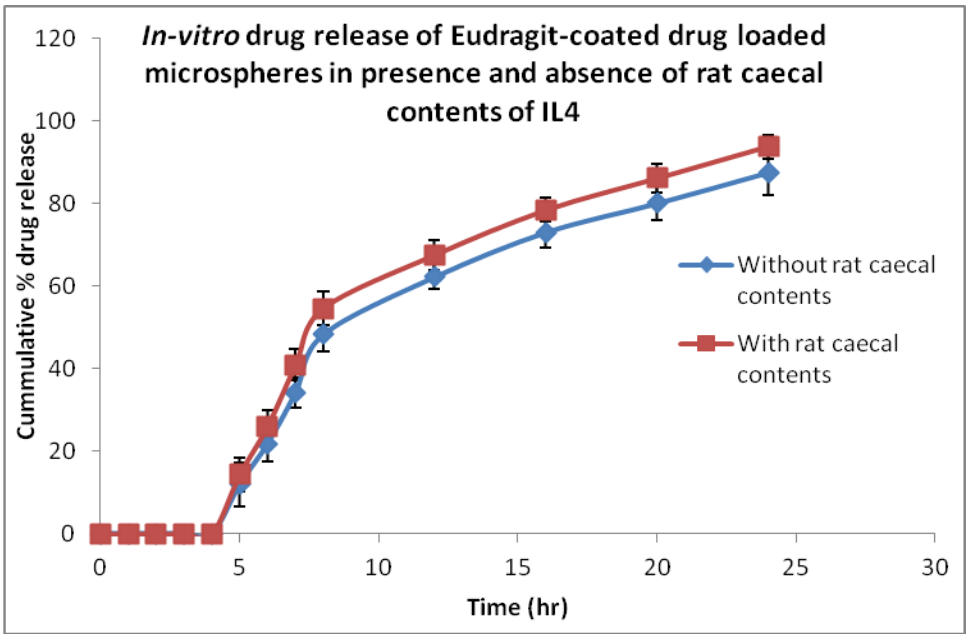


Fig 5: *In-vitro* drug release curves of a)IL1-IL5,b)IL6-IL9,c) Eudragit-coated drug loaded microspheres of IL4,d)Eudragit-coated(8%w/v) drug loaded microspheres in presence& absence of rat caecal contents of IL4.Error bars represent standard deviation(n=3).

Table 3: Comparison of different dissolution kinetic models

Formulation	Zero order	First order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
ILBG1	0.892	0.986	0.990	0.979	0.5
ILBG2	0.896	0.986	0.990	0.976	0.71
ILBG3	0.950	0.884	0.988	0.978	0.56
ILBG4	0.916	0.976	0.993	0.970	0.67
ILBG5	0.951	0.956	0.987	0.984	0.54
ILBG6	0.919	0.977	0.980	0.975	0.89
ILBG7	0.934	0.957	0.959	0.951	0.92
ILBG8	0.949	0.930	0.949	0.958	0.36
ILBG9	0.954	0.955	0.956	0.953	0.39

CONCLUSION

The ionic gelation method was proposed for the preparation of Locust bean gum microspheres and was capable of targeting the release of Cromolyn sodium(mast cell stabilizer) in colon for the management of colitis. It was concluded from the study that locust bean gum can be successfully used for colon targeted drug delivery on a daily dosage form.

ACKNOWLEDGEMENT

The authors would like to thank authority TherDosePharmaPvt Ltd for the gift sample of drug and facility.

REFERENCES

1. Watts P, Illum L. Colonic drug delivery. Drug DevInd Pharm 1997; 23:893-913.
2. Philip AK, Dabas S, Pathak K. Optimized prodrug approach: a means for achieving enhanced anti-inflammatory potential in experimentally induced colitis. J Drug Target 2009 Apr; 17(3):235-241.
3. Oluwatoyin AO, John TF. In vitro evaluation of khaya and albizia gums as compression coating for drug targeting to the colon. J Pharm Pharmacol 2005; 57:163-168.
4. Rubinstein A. Approaches and opportunities in colon-specific drug delivery.Crit Rev Ther Drug Carrier Syst 1995; 12(2-3):101-149.
5. Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 1988; 29(8):1035-1041.
6. Bussemer T, Otto I, Bodmeier R. Pulsatile drug-delivery systems. Crit Rev Ther Drug Carrier Syst 2001; 18(5):433-458.

7. Akala EO, Elekwachi O, Chase V, Johnson H, Lazarre M, Scott K. Organic redox-initiated polymerization process for the fabrication of hydrogels for colon-specific drug delivery. Drug DevInd Pharm 2003 Apr; 29(4):375-386.
8.Chien YW. Oral drug delivery and delivery systems. In: Novel drug delivery systems. New York: Marcel Dekker Inc; 1992; 139-196.
9. Ashord M, Fell JT, Attwood D, Sharma H, Woodhead P. An evaluation of pectin as a carrier for drug targeting to the colon. J Control Release 1993; 26: 213-220.
10. Philip AK, Betty Philip., Colon Targeted Drug Delivery Systems: A Review on Primary and Novel Approaches, Oman Medical Journal, Volume 25, Issue 2, 70-78, April 2010; doi:10.5001/omj.2010.24
11. Vyas SP, Khar RK. Gastroretentive systems. In: Vyas SP, Khar RK, editors.Controlled drug delivery: concepts and advances. New Delhi: Vallabh Prakashan, 2005; 218-253.
12. Yvette Piovanetti., Effects of Cromolyn on inflammatory bowel disease of childhood-Clinical and histological criteria, Yale University; spring 5-31-1978.
13.KuldeepHemrajRamteke*, VarshaBalajiJadhav, NileshShrikant Kulkarni, AmolRameshraoKharat, SonaliBhimaDiwate. Preparation, Evaluation and Optimization of Multiparticulate System of Mebendazole for Colon Targeted Drug Delivery by Using Natural Polysaccharides, Adv Pharm Bull, 2015, 5(3), 361-371 doi: 10.15171/apb.2015.050.
14.ThiruganeshRamasamy, HimaBinduRuttala, Suresh Shanmugam, and SubbiahKandasamyUmadevi. Eudragit-coated aceclofenac-loaded pectin microspheres in chronopharmacological treatment of rheumatoid arthritis, Drug Deliv, 2013; 20(2): 65–77, DOI: 10.3109/10717544.2012.762434.
15. Umadevi SK, Thiruganesh R, Suresh S, Reddy KB. Formulation and evaluation of chitosan microspheres of aceclofenac for colon-targeted drug delivery. Biopharm Drug Dispos 2010; 31(7):407-27. doi: 10.1002/bdd.722
16. Gawade P, Agrawal S. Design and characterization of eudragit coated Chitosan microspheres of deflazacort for colon targeting. J Pharm Res 2012; 5(9):4867-70.
17. Chawla A, Sharma P, Pawar P. Eudragit S-100 coated sodium alginate microspheres of naproxen sodium: formulation, optimization and in vitro evaluation. Acta Pharm 2012; 62(4):529-45. doi: 10.2478/v10007-012-0034-x
18. Kaushik D, Sardana S, Mishra D. In-vitro characterization and cytotoxicity analysis of 5- fluorouracil loaded chitosan microspheres for targeting of colon cancer. Ind J Pharm Educ Res 2010;44(3):274-82.
19. Bashardoust N, Jenita JL, Zakeri-Milani P. Preparation and In vitro Investigation of Chitosan Compressed Tablets for Colon Targeting. Adv Pharm bull 2011; 1(2):87-92. doi: 10.5681/apb.2011.013
20. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci 2001;13(2):123-33. doi: 10.1016/s0928- 0987(01)00095-1.