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KONDAGOGU MICROSPHERES FOR COLON-SPECIFIC DRUG DELIVERY: AN IN-VITRO EVALUATION

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ABSTRACT

A high dosage of Cromolyn sodium (CML) is required for treating ulcerative colitis because of its low solubility. The present work is aiming at target the drug directly into the colon for local activity and reducing the dose and systemic side effects. In this research work, cromolyn microspheres are prepared by ionic gelation and emulsion-ionic gelation methods using kondagogu gum as the natural polysaccharide gum and coated with Eudragit to retard the drug for a longer period of time. The formulated microspheres were evaluated for particle size, surface morphology, entrapment efficiency, FTIR, DSC, in vitro % drug release, kinetic study. The microspheres formed using natural polysaccharide kondagogu gum are capable of colon targeting the mast cell stabilizer drug, mesalamine for the treatment of ulcerative colitis.

Keywords: Kondagogu gum, Cromolyn Sodium, Natural Polysaccharide, Microspheres.

INTRODUCTION

Colonic drug delivery has gained increased attention not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides. In colonic delivery, a drug needs to be protected from absorption and/or the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon-targeted delivery of drugs.[1]

Colon is referred to as the optimal absorption site for protein and polypeptide after oral administration, because of the existence relatively low proteolytic enzyme activities and quite long transit time in the colon.[2] Colon targeting is naturally of value for the topical treatment of diseases of the colon such as Chron's diseases, ulcerative colitis, colorectal cancer and amebiasis.[3] Natural polysaccharides have been used extensively in designing colon-targeted tablet dosage forms because they are biocompatible and biodegradable, highly stable, safe, nontoxic, and hydrophilic and fall under the category "generally regarded as safe" (GRAS).[4] Natural gums are obtained as exudates from different tree species, which exhibit unique and diverse physicochemical properties and have a wide variety of applications.[5] Gum kondagogu (Cochlospermum Gossypium) belongs to Cochlospermum spp, family Bixaceae.[6]

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.[7] 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.[8] Eudragit belongs to another class of biocompatible polymers. Eudragit S-100 is a pH-sensitive anionic copolymer consisting of methacrylic acid and methacrylate in the ratio 1:2. It does not degrade below pH 7. Eudragit S-100 has been used to prevent drug release from microspheres in the small intestine.[9]

The objective of present study was to develop a colon targeted microspheres of cromolyn sodium with a view of retarding the drug release in the physiological environment of stomach and small intestine and to ensure maximum drug release in the physiological environment of colon with an improved patient

compliance, lesser side effects, and most aspects of an ideal drug delivery system.

MATERIALS AND METHODS

Materials

Cromolyn Sodium was obtained as a gift sample from TherDose Pharma Pvt Ltd, Hyderabad. Kondagogu 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 analytical grade.

Method

Preparation of microspheres by ionic gelation method

Kondagogu gum microspheres containing cromolyn sodium were prepared by dispersing the drug in a solution of kondagogu 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 50^0C for 24hrs and stored plastic bags for further use.[10]

Preparation of microspheres by emulsion-ionic gelation method

Cromolyn sodium microspheres were prepared by using different ratios of drug: natural gum. The pure drug is dispersed in the solution of sodium alginate and water and to this, the gum was added and stirred to get a viscous aqueous dispersion which was then extruded through a syringe needle 24# into light liquid paraffin containing 1.5% span-80 and 0.2% glacial acetic acid being kept under magnetic stirring (Remi MS-301) at 500 rpm to undergo emulsification which then leads to form spheres dispersed. Needed amount of 4% w/v calcium chloride solution is poured by continuing stirring, by which the formed spheres are exposed towards the calcium chloride and allowed 30 minutes for curing. The formed spheres were allowed to keep as such for 30minutes to finish curing process. The microspheres were decanted and washed with petroleum ether to remove liquid paraffin and water. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil and dried.[11]

Preparation of eudragit-coated cross-linked kondagogu gum microspheres

Eudragit coating of GA cross-linked drug-loaded microspheres were prepared by an oil-in-oil solvent evaporation method.Eudragit-S-100 was dissolved in a 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 40^0C 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.[12]

Optimization and characterization of microspheres

A three-factor two-level full factorial design was used for the complete study of the 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 –In-Dependant Variables		
Level	**Kondagogu gum in %(X_1)**	**Cross-linking agent in ml(X_2)**
Low level -1	2	1.5
High level +1	4	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 an ocular micrometer. Each measurement was made in triplicate. The mean particle size was calculated using the formula

$$ADM = \frac{n1d1 + n2d2 + \cdots \ldots \ldots \ldots + nmdm}{n1 + n2 + \cdots \ldots \ldots \ldots + nm}$$

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.[13]

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 a 0.22µm membrane filter (Millipore) and the amount of mesalamine was measured spectrometrically (Shimadzu, Double-Beam Spectrophotometer, 150-03, Japan) at 235nm.Each determination was made in triplicate.[14, 15]

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

Fourier-transform infrared (FT-IR) spectroscopy

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

Differential scanning calorimetry (DSC)

The thermal behavior of for Cromolyn sodium, Kondagogu gum, drug-loaded Kondagogu 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 ± 0.1^0C). 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.[16]

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. This study was approved by Institutional Animal Ethics Committee.[17]

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

The release of the final optimized formulation was carried out with the addition of rat caecal contents (2%w/v) to observe the effect of the caecal enzymes on the release rate of the drug.[16] Samples obtained at regular intervals were filtered through a 0.22µm membrane filter (Millipore, India) and analyzed.

Stability studies

According to ICH Guidelines, an accelerated stability study has to be carried out on the optimized formulation at 40 ± 2^0C/75±5% RH for over a period of 30 days.[18]

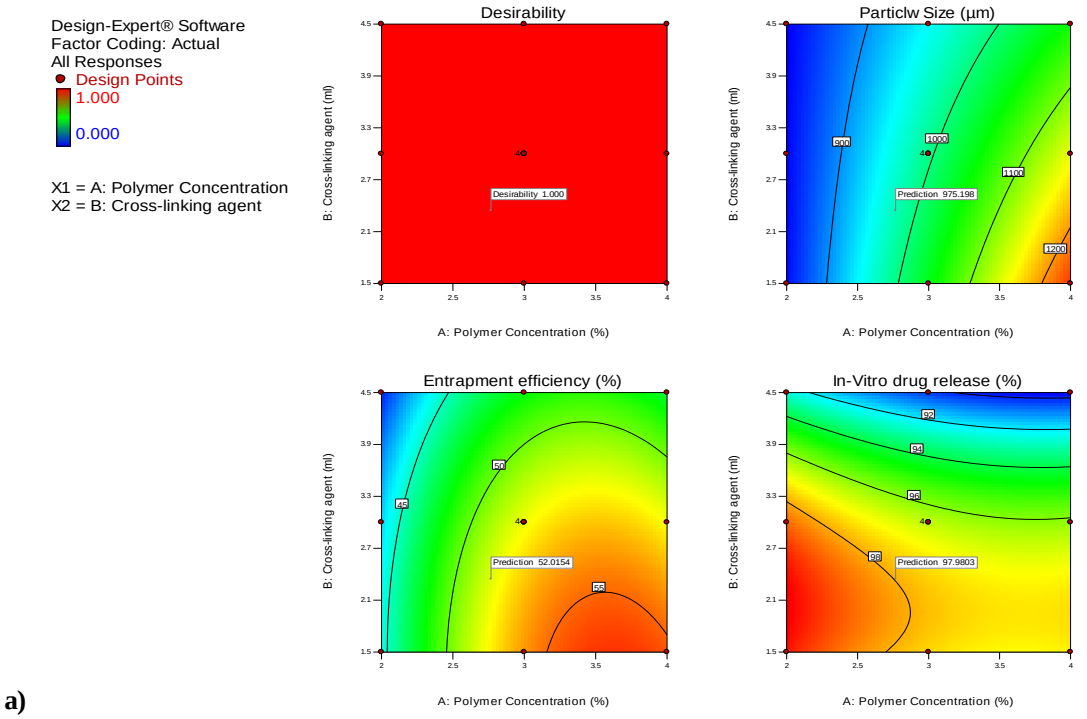
Release kinetic study

All the release data were fitted to 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.[18]

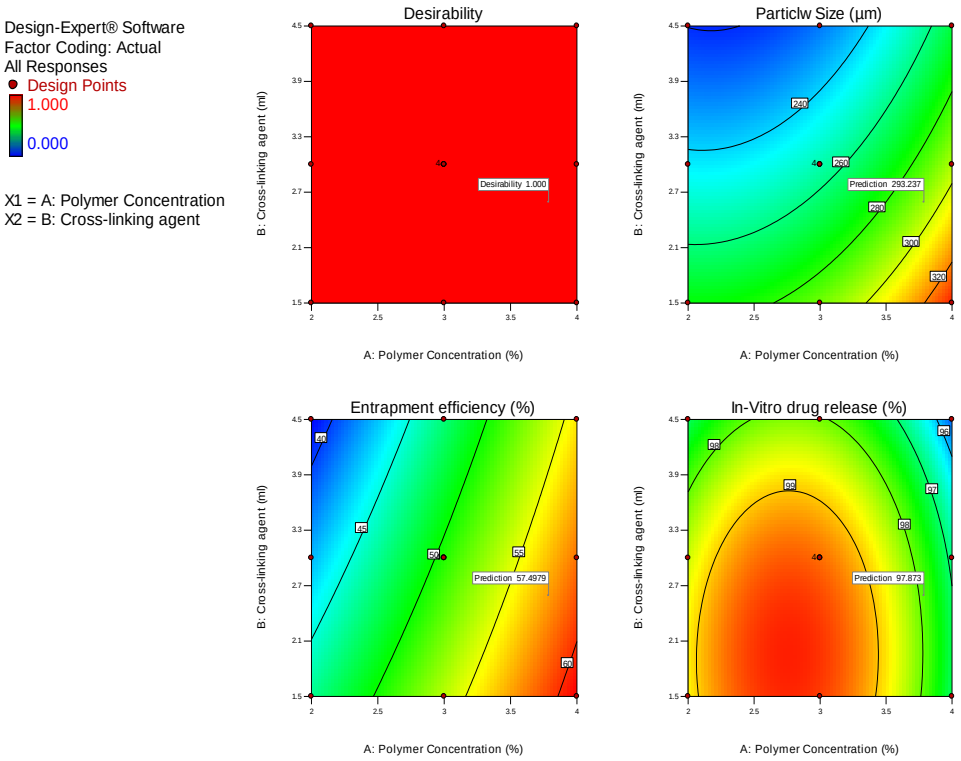
RESULTS AND DISCUSSION

Evaluation of optimized formulation of formulation variables

To study the effect of variables on the 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.



a)



b)

Fig-1: Surface response graphs for particle size, % entrapment efficiency, % *in-vitro* drug release, a) by ionic gelation method, b) by emulsion method.

The particle size of the microspheres increased with increase in the polymer concentration .i.e., the sphere size got increased with 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 a 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&3.

Table 2: Characterization of Kondagogu gum microspheres (ionic gelation method)

Batch code	Kondagogu gum(X_1) (%)	Glutaraldehyde (X_2)(ml)	Particle size (mm)	Entrapment efficiency (%)	*In-vitro* drug release (%)
IKG1	4	3	1.27±3.55	61.58±2.74	96.14±3.46
IKG2	3	4.5	0.913±3.37	57.24±2.98	89.74±3.69
IKG3	2	1.5	1.04±2.87	49.85±2.78	98.44±1.65
IKG4	3	1.5	1.27±1.65	62.32±2.45	99.11±2.92
IKG5	4	1.5	1.45±3.57	63.82±2.65	96.85±2.82
IKG6	2	4.5	0.854±2.65	38.69±2.62	92.44±2.79
IKG7	3	3	0.982±3.47	60.38±3.58	96.28±2.47
IKG8	4	4.5	1.09±2.86	56.72±3.56	90.72±2.86
IKG9	2	3	937±2.74	42.39±3.28	98.15±2.65

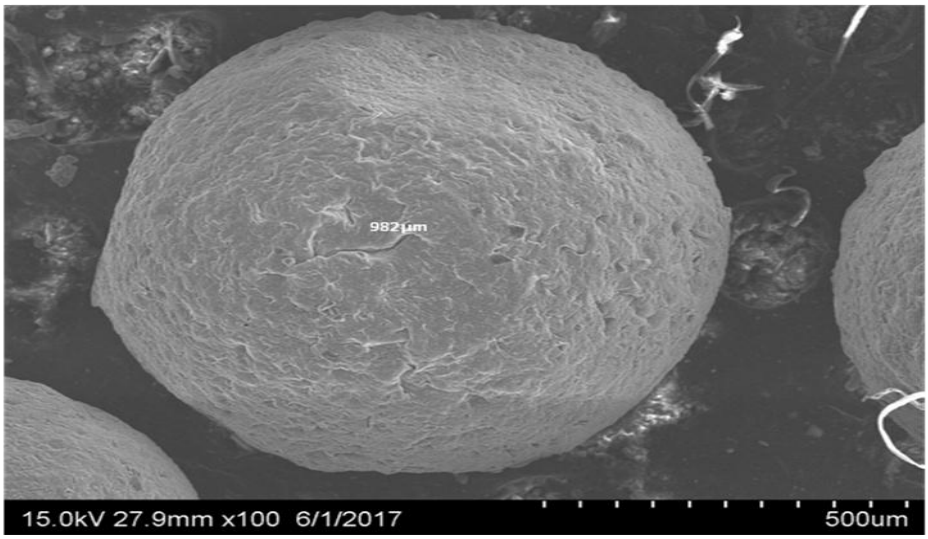
*****All readings are expressed as Mean± Standard deviation (n=3)

Table 3: Characterization of Kondagogu gum microspheres (emulsion method).

Batch code	Kondagogu Gum (X_1) (%)	Glutaraldehyde (X_2)(ml)	Particle size (µm)	Entrapment efficiency (%)	*In-vitro* drug release (%)
EKG1	2	4.5	215±3.27	38.62±2.37	96.65±2.82
EKG2	3	4.5	235±1.33	47.92±3.65	98.92±2.85
EKG3	2	1.5	274±2.67	46.34±2.82	98.62±2.62
EKG4	3	1.5	285±3.86	54.84±3.57	99.96±3.86
EKG5	4	1.5	335±2.57	60.87±2.95	96.97±3.43
EKG6	4	3	293±3.65	58.25±2.67	97.36±3.74
EKG7	3	3	254±3.47	50.29±3.48	99.14±3.28
EKG8	4	4.5	267±3.62	55.92±2.92	95.14±3.64
EKG9	2	3	248±3.95	42.74±2.76	99.19±3.68

Scanning electron microscopy confirmed the spherical shape of the microsphere. The surface of the un-coated formulation of microsphere was rough and the coated form of the same formulation was smooth on the surface (Fig 2). The microspheres formed by ionic gelation had higher particle size when compared with emulsion method. To investigate the interaction between drug and polymer FTIR studies were carried out. The spectrum of the 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 electron microscopy of a) surface morphology of drug-loaded microspheres by ionic gelation method, b) surface morphology of drug loaded microsphere by emulsion- ionic gelation method.

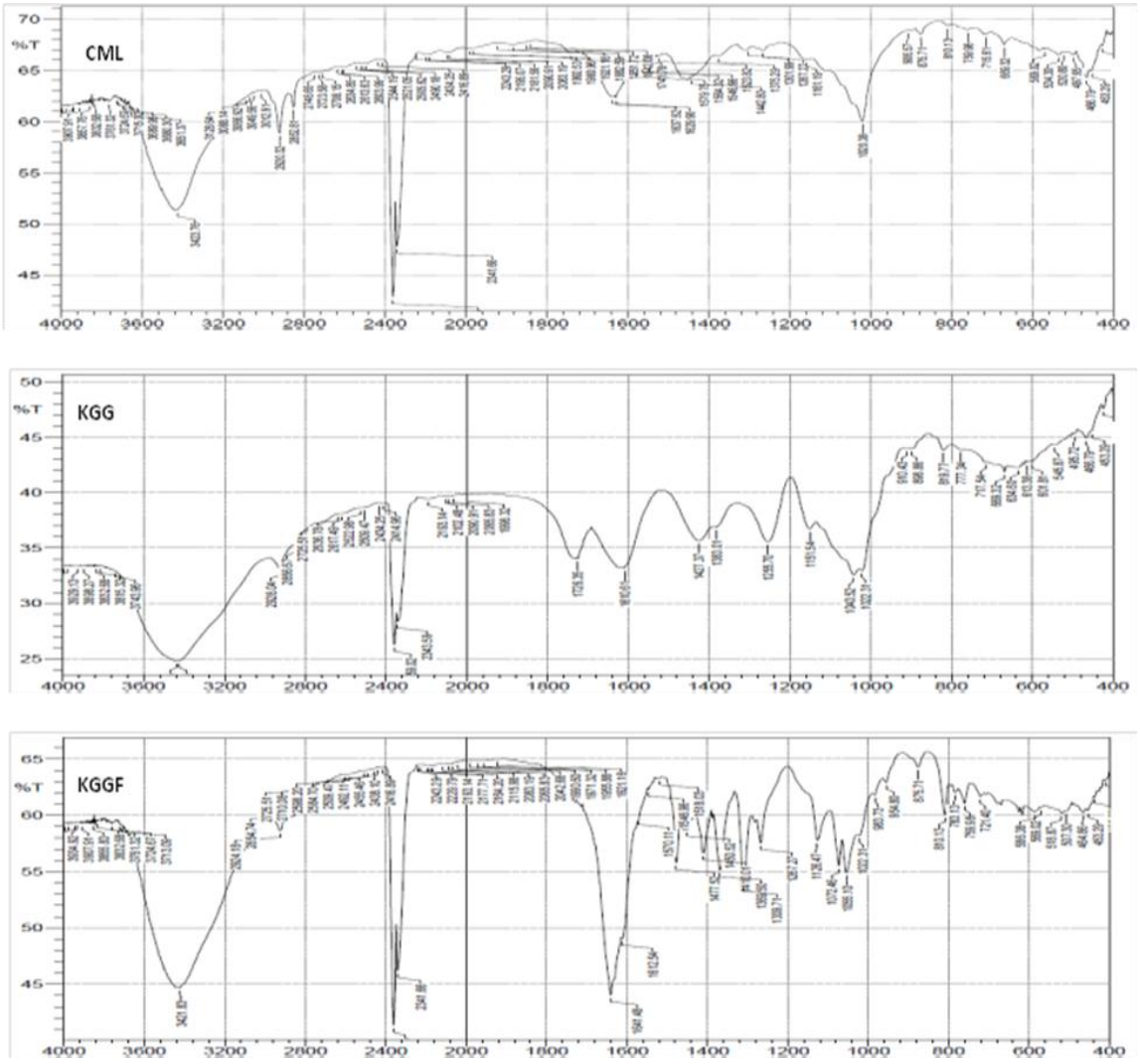


Fig 3 FTIR results of Pure drug, Kondagogu gum, CML-Loaded Kondagogu gum microspheres
***CML-Cromolyn sodium, KGG-Kondagogu gum, KGGF-Kondagogu gum formulation**

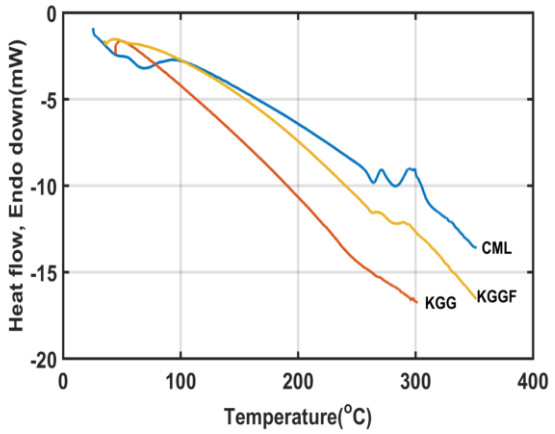
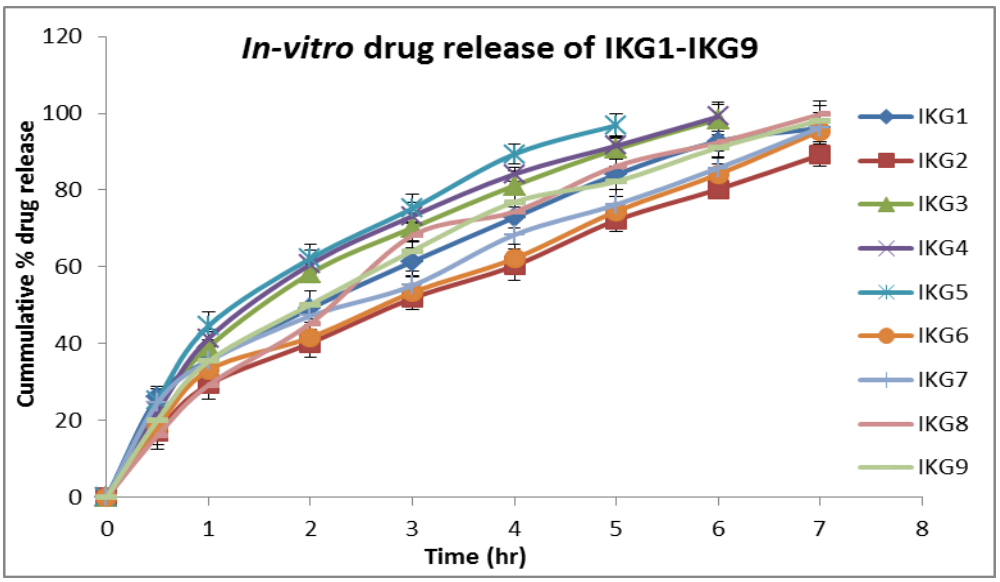


Fig 4 DSC curves of pure drug, Kondagogu gum, CML-Loaded Kondagogu gum microspheres.

***CML-Cromolyn sodium, KGG-Kondagogu gum, KGGF-Kondagogu gum formulation**

The amount of drug release for the optimized formulation in first 5h studies showed that the polymer matrix remained intact in the 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 6hr.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 4%w/v showed good retardation and optimized release of drug for 24hr, while the 8%w/v&15%w/v formulations had very high retardation and slow release in both the methods. This might be due to the dense structure by which kondagogu gum has retarding nature. Once the formulation reaches the large intestine the Eudragit coating gets easily dissolved and the natural polymer is exposed directly to the intestinal pH. The kondagogu gum has high retarding nature and hence the release was optimum in case of lower coating concentrations but in case of 8%w/v&15%w/v the higher concentration of Eudragit coating took a long time to get dissolved optimum release was reached within 24hrs. The *in-vitro* drug release was quick in case of emulsion-ionic gelation method than ionic gelation method because of the low particle size and higher surface area available for dissolution. 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 (Ionic gelation method-97.16±3.54 for 24 hrs, emulsion method-99.42±3.65 for 20hrs) % due to the degradation of the polymer matrix by colonic enzymes released by colonic bacteria than without rat caecal contents (Ionic gelation method-89.17±3.48, emulsion method-94.78±2.98for 24hrs)in the SIF medium (Fig 5). The entrapment efficiency and *in-vitro* drug release had no significant decrease when compared with the formulation before stability studies. The drug release was complete by 20hr in presence of rat cecal contents which show the cecal contents have a significant effect on drug release. The *in vitro* release from the core microsphere was found to be following Higuchi diffusion since the plots provide the highest linearity. For all KG-microspheres, the *n* value as per Korsmeyer-Peppas model was found to be between 0.43 and 0.96, indicating anomalous release behavior 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 4&5).

a)



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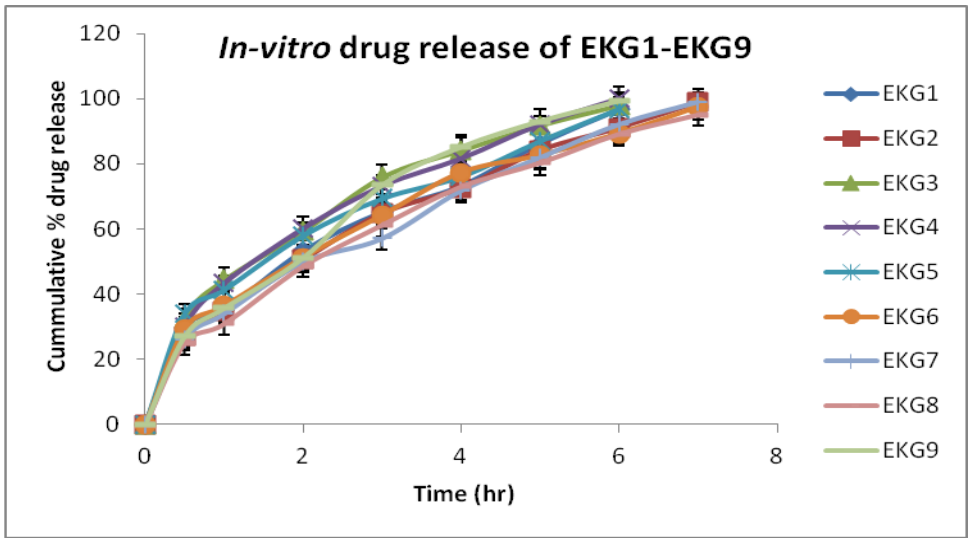

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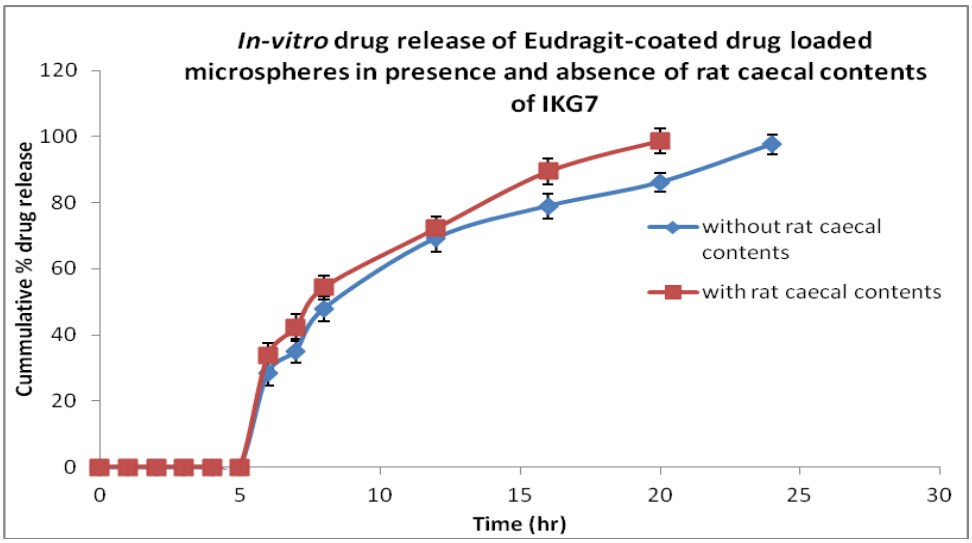

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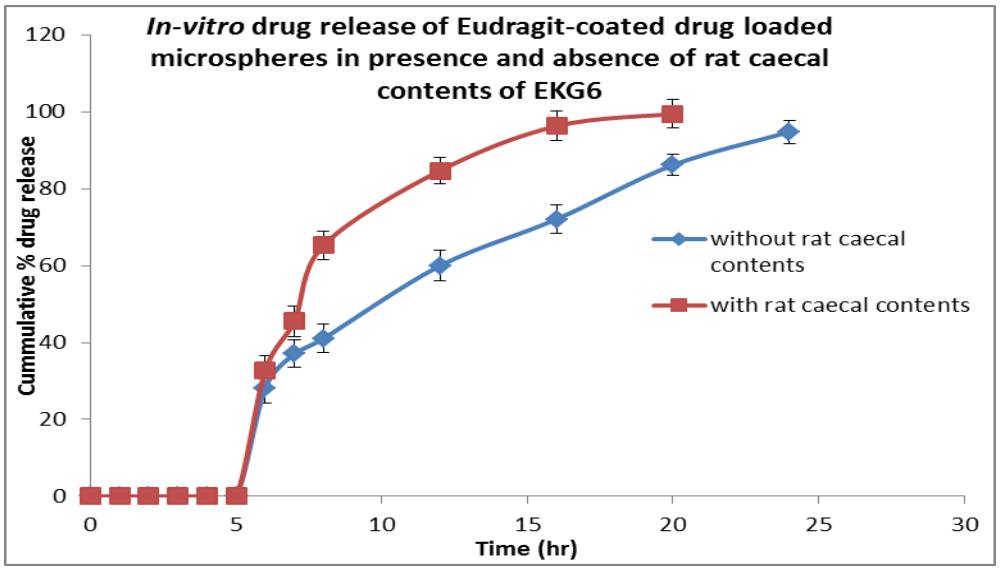

Fig 5: *In-vitro* drug release curves of a) IKG1-IKG9 by ionic gelation method, b) EKG1-EKG9 by emulsion method, c) Eudragit-coated drug-loaded microspheres in presence & absence of rat caecal contents of IKG7, d) Eudragit-coated drug-loaded microspheres in presence & absence of rat caecal contents of EKG6. Error bars represent standard deviation (n=3).

Table 4: Comparison of different dissolution kinetic models

Formulation	Zero-order	First order	Higuchi	Korsmeyer -Peppas	
	R^2	R^2	R^2	R^2	n
IKG1	0.764	0.924	0.976	0.959	0.43
IKG2	0.896	0.886	0.960	0.926	0.54
IKG3	0.874	0.894	0.958	0.898	0.56
IKG4	0.766	0.896	0.976	0.955	0.67
IKG5	0.841	0.886	0.987	0.954	0.84
IKG6	0.794	0.787	0.980	0.958	0.57
IKG7	0.854	0.877	0.975	0.891	0.96
IKG8	0.874	0.843	0.979	0.958	0.66
IKG9	0.884	0.785	0.976	0.963	0.49

Table 5: Comparison of different dissolution kinetic models.

Formulation	Zero-order	First order	Higuchi	Korsmeyer -Peppas	
	R^2	R^2	R^2	R^2	n
EKG1	0.889	0.956	0.988	0.969	0.45
EKG2	0.869	0.968	0.979	0.967	0.67
EKG3	0.935	0.848	0.986	0.987	0.64
EKG4	0.946	0.966	0.983	0.975	0.86
EKG5	0.965	0.976	0.989	0.968	0.58
EKG6	0.939	0.987	0.996	0.985	0.88
EKG7	0.983	0.977	0.969	0.981	0.52
EKG8	0.979	0.960	0.979	0.968	0.66
EKG9	0.964	0.975	0.996	0.973	0.79

CONCLUSION

Kondagogu gum microspheres were capable of targeting the release of cromolyn sodium (mast cell stabilizer) in the colon for the management of colitis in both the methods. The release was retarded until it reaches the large intestine by both the methods.It was concluded from the study that kondagogu gum can be successfully used for colon targeted drug delivery on a daily dosage form.

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