Research Article

FORMULATION AND EVALUATION OF KARAYA GUM MICROSPHERES OF CROMOLYN SODIUM: TREATMENT OF ULCERATIVE COLITIS

V.N.L. Sirisha ^{1*}, M. Chinna Eswariah ², A. Sambasiva Rao ³

¹Research Scholar, JNTUH, Hyderabad, India

²Principal, Anurag Pharmacy College, Kodad, Nalgonda Dt, Telangana, India

³Principal, Sri Indu College of Pharmacy, Sheriguda, Ibrahimpatnam, R.R Dt, Telangana, India

*Corresponding Author Email: sirishaphd2011@gmail.com

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ABSTRACT

The objective of the present study was to formulate site specific drug delivery of Cromolyn sodium using karaya gum. The microspheres were prepared by both ionic gelation method and emulsion-ionic gelation. Microspheres were characterized by FT-IR spectroscopy, Differential scanning calorimetry and evaluated by scanning electron microscopy (SEM), particle size analysis, entrapment efficiency and in-vitro drug release studies in different simulated gastric fluids. Stability studies were carried out for one month at $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH. The SEM images revealed the surface morphology was rough and smooth for core and coated microspheres, respectively. The optimized batch (IK1,EK8) coated microspheres (15% w/v) for 24hr exhibited $95.24 \pm 3.48\%$ and $99.74 \pm 2.82\%$ drug release, respectively. The drug release was quick in presence of rat caecal contents because of the enzymatic effect on the polymer matrix. The drug release from all karaya gum microsphere formulations followed Higuchi kinetics. Moreover, drug release from Eudragit S-100 coated microspheres followed the Korsmeyer-Peppas equation with a Fickian kinetics mechanism. Finally stability studies suggested the change in entrapment efficiency and in-vitro drug release of microspheres was minimal, indicating good stability of the formulation.

Keywords: Karaya Gum, Cromolyn Sodium, Natural Polysaccharide, Microspheres.

INTRODUCTION

The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. This region of the colon is recognized as having a somewhat less hostile environment with less diversity and intensity of activity than the stomach and small intestine. Additionally, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Colon itself is susceptible to many disease states such as constipation, irritable bowel syndrome and more serious diseases including Crohn's disease, ulcerative colitis, carcinomas and infections. Development of new therapeutic agents for the treatment of colonic diseases has required colon-specific delivery systems to maximize the effectiveness of these drugs. In the long-term management of ulcerative colitis delivering drugs to the colon may be regarded as a better method of drug delivery with less side effects and higher efficacy. A colon-specific drug delivery system should prevent drug release in the stomach and small intestine and affect an abrupt onset of drug release upon entry into the colon¹.

Karaya gum also known as Indian tragacanth is the dried exudate of the trees *Sterculia* species². Gum Karaya is a polysaccharide of high molecular weight which on hydrolysis yields D-galactose, L-rhamnose, and D-galacturonic acid, together with minor amounts of D-glucuronic acid³.

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 delivery⁴. Cromolyn Sodium (Disodium Cromoglycate) stabilizes

mast cell membranes thus preventing mast cell damage and degranulation 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 tract⁵. 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 intestine⁶.

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

Cromolyn Sodium was obtained as a gift sample from TherDose Pharma Pvt Ltd, Hyderabad. Karaya Gum was obtained from Nutriroma Pvt. Ltd, Hyderabad. Sodium Alginate, Calcium Chloride and Glutaldehyde were purchased from Sigma-Aldrich. Eudragit S 100 was obtained as gift sample by Evonik, Germany. All reagents used were Analytical grade.

Preparation of microspheres by ionic gelation method

Karaya gum microspheres containing mesalamine were prepared by dispersing the drug in a solution of karaya 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 °C for 24hrs and stored plastic bags for further use⁷.

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 30 minutes 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 dried⁸.

Preparation of eudragit-coated cross-linked karaya gum 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 40 °C 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 further⁹.

Optimization and characterization of microspheres

A three level two factor full factorial design was used for complete study of combination of drug and polymer. The main effects (X1 and X2) represent the average result of changing one factor from its low to high values. The interaction term (X1, X2) shows how the response values change when two factors are simultaneously changed (Table 1).

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.

$$ADM = (n_1d_1 + n_2d_2 + \dots + n_md_m) / (n_1 + n_2 + \dots + n_m)$$

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 15Kv¹⁰.

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 235nm. Each determination was made in triplicate^{11,12}.

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

Fourier-transform infrared (FT-IR) spectroscopy

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

Differential Scanning Calorimetry (DSC)

The thermal behavior of for Cromolyn sodium, Karaya gum, drug- loaded Karaya 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, and 37±0.1 °C). 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 235nm¹³.

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 CO₂ gas was bubbled in to the medium. This study was approved by Institutional Animal Ethics Committee¹⁴.

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 analyzed¹⁴.

Stability studies

According to ICH Guidelines, an accelerated stability study has to be carried out on the optimized formulation at $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH for over a period of 30 days¹⁵.

Release Kinetic Study

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 microspheres¹⁵.

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(X1) and cross-linking agent(X2) 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 (Y1), % entrapment efficiency (Y2), % in-vitro drug release (Y3) were selected dependent variables figure 1.

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&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 (figure 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 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 (figure3). 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 (figure 4).

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 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 15% w/v showed good retardation and optimized release of drug for 24hr, while the 4% w/v & 8% w/v formulations had low retardation and quick release in both the methods. This might be due to the low retarding power of karaya gum. 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 karaya gum has low retarding nature and hence the release high in case of lower coating concentrations but in case of 15% w/v the higher concentration of eudragit coating took time to get dissolved and hence the release was retarded up to 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- 99.66 ± 2.75 , emulsion method- 98.96 ± 2.87 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- 95.24 ± 3.48 , emulsion method- 99.74 ± 2.83 for 24hrs) in the SIF medium (figure 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 caecal contents which show the caecal contents have 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 Karaya gum microspheres, the n value as per korsmeyer-peppas model was found to be between 0.44 and 0.94, 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).

Table 1: 3^2 -Full factorial design: factors, factor levels and responses

Factors –In-Dependent Variables		
Level	Karaya gum in % (X1)	Cross-linking agent in ml (X2)
Low level -1	1.5	1.5
High level +1	4	4.5
Responses- Dependent Variables		
Y1	Particle size in mm	
Y2	Entrapment Efficiency in %	
Y3	In-vitro drug release in %	

Table 2: Characterization of Karaya gum microspheres (Ionic gelation method)

Batch code	Karaya gum(X1) (%)	Glutaraldehyde (X2)(ml)	Particle size (mm)	Entrapment efficiency (%)	In-vitro drug release (%)
IK1	2.75	3	1.38±3.55	65.38±3.35	96.54±2.66
IK2	4	4.5	1.35±3.37	51.68±2.98	94.33±2.69
IK3	2.75	1.5	1.58±2.87	59.67±3.08	99.16±2.65
IK4	1.5	4.5	0.982±1.65	54.34±2.45	97.08±3.92
IK5	4	3	1.46±3.57	62.75±3.65	98.22±3.23
IK6	4	1.5	1.66±2.65	56.84±3.28	96.12±3.19
IK7	1.5	1.5	1.22±3.76	48.75±3.58	98.34±3.75
IK8	2.75	4.5	1.18±2.86	54.39±1.56	94.08±3.62
IK9	1.5	3	1.17±2.74	57.69±2.86	99.03±3.55

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

Table 3: Characterization of Karaya gum microspheres (Emulsion method)

Batch code	Karaya gum(X1) (%)	Glutaraldehyde (X2)(ml)	Particle size (mm)	Entrapment efficiency (%)	In-vitro drug release (%)
EK1	2.75	3	0.425±3.27	67.24±3.17	97.16±3.21
EK2	4	4.5	0.506±1.33	57.92±2.58	96.12±3.54
EK3	2.75	1.5	0.453±2.67	69.49±3.28	99.56±3.62
EK4	1.5	4.5	0.246±3.86	52.84±3.57	99.18±2.66
EK5	4	3	0.576±2.57	65.73±3.95	95.62±3.54
EK6	4	1.5	0.645±3.65	71.54±3.78	97.78±3.08
EK7	1.5	1.5	0.329±3.47	59.96±1.85	98.68±2.87
EK8	2.75	4.5	0.380±3.62	65.92±3.25	97.46±2.64
EK9	1.5	3	0.314±3.95	55.49±1.69	95.94±2.86

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

Table 4: Comparison of different dissolution kinetic models

Formulation	Zero order	First order	Higuchi	Koresmeyer -Peppas	
	R2	R2	R2	R2	n
IK1	0.792	0.886	0.990	0.979	0.45
IK2	0.796	0.786	0.980	0.966	0.62
IK3	0.850	0.784	0.978	0.968	0.68
IK4	0.716	0.876	0.983	0.975	0.77
IK5	0.851	0.856	0.967	0.974	0.44
IK6	0.719	0.777	0.970	0.975	0.79
IK7	0.834	0.857	0.969	0.961	0.94
IK8	0.849	0.830	0.959	0.948	0.46
IK9	0.854	0.755	0.966	0.933	0.59

Table 5: Comparison of different dissolution kinetic models

Formulation	Zero order	First order	Higuchi	Koresmeyer -Peppas	
	R2	R2	R2	R2	N
EK1	0.892	0.986	0.990	0.979	0.5
EK2	0.896	0.986	0.990	0.976	0.71
EK3	0.950	0.884	0.988	0.978	0.56
EK4	0.916	0.976	0.993	0.970	0.67
EK5	0.951	0.956	0.987	0.984	0.54
EK6	0.919	0.977	0.980	0.975	0.89
EK7	0.934	0.957	0.959	0.951	0.92
EK8	0.949	0.930	0.949	0.958	0.36
EK9	0.954	0.955	0.956	0.953	0.39

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

Figure 2: Scanning electronic 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

Figure 3: FTIR results of Pure drug, Karaya gum, CML-Loaded Karaya gum microspheres
*CML- Cromolyn sodium, KG-Karaya gum, KGF- Karaya gum formulation

Figure 4: DSC curves of pure drug, Karaya gum, and CML-Loaded Karaya gum microspheres
*CML-Cromolyn sodium, KG-Karaya gum, KGF-Karaya gum formulation

Figure 5: In-vitro drug release curves of a)IK1-IK9 by ionic gelation method, b)EK1-EK9 by emulsion method, c) Eudragit-coated drug loaded microspheres in presence & absence of rat caecal contents of IK1,d)Eudragit-coated drug loaded microspheres in presence & absence of rat caecal contents of EK8.Error bars represent standard deviation(n=3).

CONCLUSION

Karaya gum microspheres were capable of targeting the release of cromolyn sodium (mast cell stabilizer) in 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 karaya gum can be successfully used for colon targeted drug delivery on a daily dosage form.

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