Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 3, 2015

Original Article

MULTIPARTICULATE DRUG DELIVERY SYSTEM FOR COLON TARGETING

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Received: 24 Jun 2014 Revised and Accepted: 20 Sep 2014

ABSTRACT

Objective: The objective of the present investigation was to design a multi particulate delivery system for site-specific delivery of 5-aminosalicylic acid (ASA) using natural polysaccharides (pectin) and pH-sensitive polymer (Eudragit S100) for the treatment of ulcerative colitis. This system is anticipated to protect the drug loss in the upper GI tract, which results from the inherent property of Eudragit S100 (ES), and deliver ASA in the colon only.

Methods: The use of enteric polymers (ES) as the protective coating on the microspheres makes them able to release the drug at the particular pH of colonic fluid. A combined mechanism of release is used, which combines specific biodegradability of polymer and pH-dependent drug release from the coated microspheres. The effects of polymer concentration, stirring rate, and concentration of emulsifier on particle size and drug loading were studied. Pectin microspheres were prepared by emulsion dehydration method using different ratios of drug and polymer (1:2 to 1:4), stirring speeds (1000-3000 rpm) and emulsifier concentrations (1%-3% wt/vol). Eudragit -coating of pectin microspheres was prepared by oil-in-oil solvent evaporation method. Both the pectin microspheres and Eudragit-coated pectin microspheres were evaluated for surface morphology, particle size and size distribution, percentage drug entrapment, swell ability and *In vitro* drug release in pH progression media.

Result: The release profile of 5-ASA from Eudragit-coated pectin microspheres was pH dependent. Hence, the drug released quickly at pH 7.5 but the release rate was much slower in acidic medium.

Conclusion: The designed drug delivery system can be used as a tool for colon targeting of drugs.

Keywords: 5-Aminosalicylic acid, Colon targeting, Microspheres, Pectin.

INTRODUCTION

Biodegradable pectin microspheres offer a novel approach for developing sustained release drug delivery systems that have potential for colonic drug delivery. In the controlled release area, biodegradable microspheres are one of the most useful devices to deliver materials in an effective, prolonged and safe manner. Pectin, a hetero saccharide derived from the cell wall of plants used as a gelling agent for scanning purpose. The degradation of pectin occurs mainly in the colon by pectinolytic enzymes secreted by microorganisms. As a result pectin has increasingly gained acceptance as the carrier polymer for sustained release and site specific delivery dosage forms, such as beads, pellets, tablets, and films. [1,2]. For colonic delivery of drugs, different approaches include the use of prodrugs, [3,4] pH-sensitive polymer coating, [5,6]and time-dependent formulations [7,8]. In addition, the use of biodegradable polymers such as azopolymer and polysaccharide (e. g., Pectin and Dextran) for colon targeting are also reported in the literature.[9,10]. Among the different approaches to achieve colon-target drug delivery, the use of polymers, specifically biodegraded by colonic bacteria, proves better results. The pH-dependent systems exploit the generally accepted view that pH of the human GI tract increases progressively from the stomach (pH 2-3) to the small intestine (pH 6.5-7.0) to the colon (7.0-8.0),[11]. Most commonly used pH-dependent coating polymers are meth acrylic acid copolymer (i. e., Eudragit L100-55, Eudragit L100, and Eudragit S100), which dissolve at pH 5.5, 6.0, and 7.0, respectively.

Pectin is a predominately linear polymer of mainly α-(1-4)-linked D-galacturonic acid residues interrupted by 1, 2-linkedL-rhamnase residues. Pectin has a few hundred to about 1000building blocks per molecule. As pectin is soluble in water, it is not able to shield its drug load effectively during its passage through the stomach and small intestine. Hydrophilic polymer matrix systems are widely used in oral controlled drug delivery because of their flexibility to obtain a desirable drug release profile, cost-effectiveness, and broad regulatory acceptance,[12,13]. The ability of the hydrophilic polymer matrices to release an entrapped drug in aqueous medium and to regulate the release of such drug by control of swelling and cross-linking makes them particularly suitable for controlled-release applications,[14]. These matrices can be applied for the release of both hydrophilic and hydrophobic drugs and charged solutes.

5-ASA is a drug used for treating ulcerative colitis. The exact mechanism of 5-ASA is not known but is believed to reduce inflammation in the colon. Ulcerative colitis and other inflammatory diseases cause excessive production of chemicals, for example, prostaglandins, that produce inflammation in the colon. Prostaglandins are produced by the enzymes, cyclooxygenase and lipoxygenase. These enzymes are over-active in individuals with ulcerative colitis. 5-ASA may work by blocking the activity of cyclooxygenase and lipoxygenase, thereby, reducing the production of prostaglandins. Reduced production of prostaglandins decreases inflammation in the colon and the symptoms associated with ulcerative colitis. Site-specific delivery of 5-ASA may reduce the systemic side effects and provide effective and safe therapy that may reduce the dose and duration of therapy when compared with the conventional treatment.

Microspheres of Eudragit S100 were developed for delivery of 5-Aminosalicylic acid specifically into the colon. This type of advanced pharmaceutical dosage forms (Multi particulate systems) is of great practical importance for instance, for the treatment of ulcerative colitis. The present investigation reveals that Eudragit-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of 5-ASA.

MATERIALS AND METHODS

The 5-ASA was a gift from S. A. Pharma (Saga r, India), pectin (Sigma-Aldrich) and Eudragit S-100 (Rohm, GmbH, Germany) were obtained from Alembic Ltd (Gujarat, India). Glutaraldehyde, castor oil and Magnesium Chloride were procured from Hi- media, India. Hydrochloric Acid procured from Qualigens Fine chemicals, India. Isopropyl alcohol, HPLC grade methanol and water was obtained

from Hi-Media Chemical (Bombay), India. All other materials used in dissolution studies were of analytical reagent grade and were used as received.

Preparation of Eudragit-coated pectin microspheres

Pectin microspheres were prepared by using a combined method i. e. water-in-oil (w/o) emulsification and cross linking method.15, whereas Eudragit coating of pectin microspheres was performed using oil-in-oil (o/o) solvent evaporation method, 16. For preparation of pectin microspheres, Pectin (3 g) was dissolved in 30 ml of de ionized water and to it 5 ml of MgCl2 (5% w/v) solution was added. In a 1 liter beaker at 50±1°C, 5-ASA (1 g) was added to the pectin solution through syringe into a continuous oil phase (which consists of 300 ml of castor oil, 100 ml of isopropanol and 2% (w/v) span 80). The dispersion was stirred using a stainless steel stirrer at 2000 rpm for 10 min and thereafter with continuous stirring,15 ml of glutaraldehyde was added to the beaker. The cross linking reaction was allowed to proceed for 3 hrs. Hardened microspheres were filtered, washed repeatedly with isopropanol and water, so as to remove castor oil and un reacted glutaraldehyde. The microspheres were dried under vacuum at 40°C overnight and kept in a desiccators.

Similarly pectin microspheres were prepared by taking polymer: drug in a ratio of 1:2, 1:3 and 1:4, stirring rate 1000 rpm, 2000 rpm and 3000 rpm and emulsifier (span 80) concentration 1%, 2% and 3%. Eudragit coating of pectin microspheres was performed using oil-in-oil (o/o) solvent evaporation method. 250 mg of Eudragit S-100 was dissolved in 10 ml of organic solvent (2:1, ethanol: acetone). 50 mg of pectin microspheres were added to the above solution. This organic phase was then poured into 100 ml of light liquid paraffin containing 2% w/v span 80. To facilitate for the evaporation of solvent, the system was maintained under agitation speed of 1000 rpm at 40°C for 3 hrs. Finally the coated microspheres were washed with n-hexane and dried overnight in the vacuum desiccators.

Surface morphology

The shape and surface morphology of pectin microspheres and Eudragit coated pectin microspheres were investigated using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope.

Particle size and particle size distribution

The particle size and particle size distribution was measured in particle size analyzer (Malvern, USA). Microspheres were suspended in distilled water and the particle size and size distribution was determined using the software provided by the manufacturer.

Swell ability

A known weight (100 mg) of various ASA-loaded pectin microspheres and Eudragit-coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (SIF, KH2PO4/ Na OH buffer, pH 7.4) and allowed to swell for the required period of time at 37°C ± 0.5°C in the dissolution apparatus (United States Pharmacopoeia [USP] XXIII, model DT-06, Eureka, Germany). The microspheres were periodically removed and blotted with filter paper; then their change in weight (after correcting for drug loss) was measured until attainment of equilibrium. The swelling ratio (SR) was then calculated using the following formula

$$SR = \frac{wg - wo}{wo}$$

Where SR indicates swelling ratio, w_o is an initial weight of microspheres and w_g is final weight of microspheres.

Percentage drug entrapment

The percentage of drug entrapped in the microspheres was determined by digesting the microspheres (50 mg) in sufficient saline phosphate buffer pH 7.4 for 48 hrs. It was centrifuged at 3000 rpm for 30 min and the supernatant were analyzed. Spectro photo metrically at 267.7 nm. The percentage drug entrapment of coated pectin microspheres was determined in the same manner.

$$Percent\ drug\ entrapment = \frac{\{\%drugloading\ \}}{\{\%theoreticalloading\ \}} X\ 100$$

$$Percent\ drug\ loading = \frac{\{weight\ of\ drug\ in\ microspheres\}}{\{weight\ of\ microspheres\}} X\ 100$$

***In vitro* drug release studies in pH progression media**

Eudragit-coated pectin microspheres and uncoated pectin microspheres were evaluated for the *in vitro* drug release in pH progression media. An accurately weighed amount of microspheres, equivalent to 100 mg of 5-ASA, was added to 900 ml of dissolution medium and the release of 5-ASA from microspheres was investigated using rotating paddle dissolution test apparatus (Electro lab, India) at 100 rpm and 37±0.5°C. The simulation of gastrointestinal transit conditions was achieved by altering the pH of the dissolution medium. Initially it was kept at pH 1.2 for 2 hrs with 0.1N H Cl. Then KH_2PO_4 (1.7 g) and $Na_2HPO_4.2H_2O$ (2.225 g) were added to the dissolution medium adjusting the pH 4.5 for 3rdand 4thhr and adjusted with Na OH to 6.8 for 5thhr. After 5thhr, the pH of the dissolution medium was adjusted to 7.5 and maintained up to 8hr. The final volume in all case was kept 900 ml. The samples were withdrawn from dissolution medium at various time intervals using a pipette fitted with micro-filter at its tips and analyzed Spectro photometrically at 267.7 nm.

Statistical analysis

The mean percentage of ASA released in pH progression media from both pectin microspheres and Eudragit-coated pectin microspheres was prepared by using various drug: polymer ratios and compared. The Student t test was used to find the statistical significance. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preparation of Eudragit-coated pectin microspheres

Pectin microspheres of ASA were successfully prepared by emulsion dehydration technique. The pectin microspheres were coated with Eudragit S100 by oil-in-oil solvent evaporation method, using coat: core ratio 5:1. Both the coated microspheres and uncoated microspheres were found to be of spherical shape (SEM). The method was optimized using different stirring rate and emulsifier concentration to produce microspheres of small size and narrow size distribution, high drug loading efficiency, and controlled drug release at pH progression media.

Table 1: Formulations prepared by different approaches

S. No.	Formulation code	Variables	Values
1	MA1	Drug: Polymer Ratio	1:2
2	MA2		1:3
3	MA3		1:4
4	MB1	Emulsifier (Span80) concentration	1% w/v
5	MB2		2% w/v
6	MB3		3% w/v
7	MC1	Stirring speed	1000 rpm
8	MC2		2000 rpm
9	MC3		3000 rpm

Table 2: Particle size of microspheres prepared by different formulation approaches

S. No.	Formulation code	Mean diameter of microspheres (in µm)
1	MA1	7.19 ±0.32
2	MA2	15.06 ±0.24
3	MA3	20.96 ±0.71
4	MB1	22.40 ±0.53
5	MB2	14.22 ±0.90
6	MB3	6.59 ±0.35
7	MC1	23.32 ±0.64
8	MC2	17.81 ±0.85
9	MC3	8.42 ±0.26

Particle size and particle size distribution

The particle size distributions of the microspheres of different formulation approaches are given in the table-2.

As the drug: polymer ratio was increased from 1:2 to 1:4, the particle size of the microspheres increased from 7.19±0.32 µ m to 20.96±0.71 µ m. The increase in size of the microspheres is due to an increase in viscosity of polymer solution with increasing concentration, which resulted in the formation of larger emulsion droplets and finally greater size of microspheres. As the concentration of the emulsifying agent (Span 80) was increased from 1% to 3% w/v, the particle size of the microspheres was decreased from 22.40±0.53 µ m to 6.59±0.35 µ m. This may be due to the decrease of interfacial energy between the two droplets and the presence of emulsifying agent in the cross linking medium, allowing the stabilization of the preformed microspheres to maintain their size until completion of the cross linking reaction. As the stirring rate was increased from 1000 rpm to 3000 rpm, the particle size of the microspheres was decreased from 23.32±0.64 µ m to 8.42±0.26 µ m. This may be due to formation of small size droplets on higher stirring rate.

Swell ability

The swell ability of different formulations performed in pH 1.2 and pH 7.5at 37±0.5°C are given in the Table3. The result indicates that swelling ratio was increased with an increase in drug: polymer ratio (from 1:2 to 1:4). It may be due to the denser crosslink between the pectin molecules, producing more packed structures in the formulations having more concentration of polymer (drug: polymer ratio less).

Table 3: Swelling ratio of microspheres prepared by different formulation approaches

S. No.	Formulation code	% Swelling ratio	
		In pH 1.2	In pH 7.5
1	MA1	2.63±0.11	1.83±0.05
2	MA2	3.70±0.06	2.36±0.11
3	MA3	4.59±0.08	3.27±0.02
4	MB1	3.11±0.15	2.89±0.07
5	MB2	3.29±0.12	3.26±0.06
6	MB3	3.66±0.08	3.35±0.08
7	MC1	2.96±0.06	2.23±0.06
8	MC2	3.51±0.04	3.11±0.05
9	MC3	4.12±0.06	3.90±0.10

Table 4: Percentage drug entrapment of microspheres prepared by different formulation approaches

S. No.	Formulation code	%drug entrapment
1	MA1	73.56±1.47
2	MA2	79.92±1.19
3	MA3	85.14±2.68
4	MB1	72.15±0.79
5	MB2	74.52±2.31
6	MB3	75.16±1.20
7	MC1	85.23±0.75
8	MC2	81.01±0.21
9	MC3	76.15±1.05

The result indicates that on increasing drug: polymer ratio from 1:2 to 1:4, the entrapment efficiency was increased from 73.56±1.47 % to 85.18±2.68 %. However, the change in the concentration of the emulsifying agent (span 80) had no significant effect in entrapment efficiency of the microspheres. But, As the stirring rate was increased from 1000 rpm to 3000 rpm, the entrapment efficiency was decreased from 85.23±0.75 % to 76.15±1.05 %, which may be due to formation of small size microspheres with increased surface area. Higher stirring rate enhanced the diffusion of drug from such microspheres, resulting in the loss of drug from microspheres with a consequent lowering in the entrapment efficiency.

In-vitro drug release

In-vitro drug release was carried out for uncoated and Eudragit coated microspheres in pH progression medium. The in-vitro release from uncoated microspheres in pH progression media is represented in fig. 1. The result indicates that, when drug: polymer ratio was increased in the preparation of cross linked pectin microspheres, the in-vitro drug release from microspheres was decreased which may be due to increased path length for diffusion of drug molecule from microspheres. Drug release after 8 hrs was found to be 96.25±1.75% in case of microspheres prepared using 1:2 drug: polymer ratio, while it was 88.65±1.26% for microspheres prepared with 1:4 drug: polymer ratio.

Microspheres which were prepared using 1% w/v of emulsifying concentration, released 94.53±1.11% of drug after 8 hrs while those prepared using 2% and 3% w/v of emulsifying agent released 95.23±2.01% and 96.23±1.71% of drug after the same period. The

result revealed that the concentration of emulsifying agent had no significant effect on drug release of the microspheres.

Microspheres which were prepared at stirring speed of 3000 rpm, released 96.23±2.15% of drug after 8 hrs, while those prepared at 2000 rpm released 92.43±1.18% of drug after 8 hrs. The size of the microspheres prepared at 1000 rpm was large and hence effective surface area was less in comparison to those prepared at 2000 rpm and 3000 rpm, which could probably be the reason for the lesser amount of drug release (89.15±2.56% after 8 hr) from microspheres prepared at 1000 rpm.

Drug release from Uncoated and Eudragit coated microspheres in pH progression media

The cumulative percentage drug release from Eudragit-coated pectin microspheres showed the desired rate, as there was no measurable drug release observed up to 2 hours in SGF (pH 1.2), while at pH 4.5, the drug release was quite insignificant (<2%) up to 4 hours. Drug release from Eudragit-coated pectin microspheres in pH progression media is represented in fig. 2. However in contrast, the in-vitro drug release study of the uncoated microspheres (fig 1) shows that more than 30% of drug release in GI Fluid, so less drug remains for colonic drug delivery. Hence Eudragit coated microspheres were considered to be more promising for colonic drug delivery.

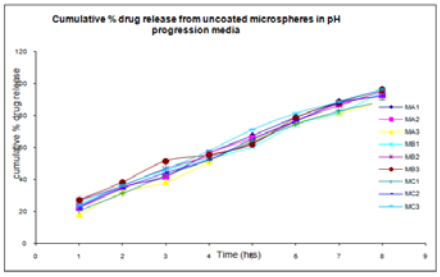

Fig. 1: Drug release from uncoated microspheres in pH progression media

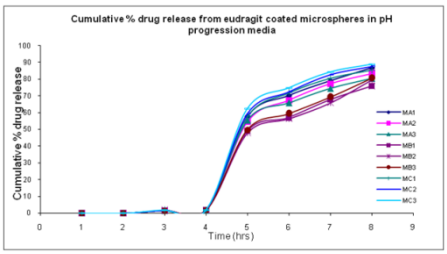

Fig. 2: Drug release from Eudragit coated microspheres in pH progression media

DISCUSSION

Eudragit coated pectin microspheres were found more suitable for colonic drug delivery in comparison to uncoated pectin microsphere. Different formulation approaches (i. e. drug: polymer ratio, emulsifier conc. and stirring speed) were carried out for preparing uncoated and coated microspheres. Among all the formulation approaches, in case of microspheres prepared using 1:2 drug: polymer ratio MA1 is considered to be the best, (as the %drug release is more i. e. 96.25%±1.75%).

However, when stirring speed is considered, microspheres which were prepared at stirring speed of 3000 rpm, MC3 is found to be the best (96.23±2.15% drug release after 8 hrs).

CONCLUSION

The experimental results demonstrated that Eudragit-coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system. However it reduces the side effects of the drug caused by its absorption from the upper part of the GI tract when given in conventional dosage forms such as tablets and capsules.

CONFLICT OF INTERESTS

Declared None

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