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Formulation and evaluation of polysaccharide based colon targeted system

Chaurasia M[1,#] and Ray S[1]
Amity Institute of Pharmacy, Amity University, Lucknow, India

Contact email: mohinichaurasia@gmail.com

INTRODUCTION AND OBJECTIVES

High intracolonic drug concentration is required for the treatment of diseases associated with the colon such as colon cancer, IBD, ulcerative colitis and Crohn's disease. Several strategies can be utilized to selectively target the drug release to colon (Chourasia et al., 2003). The classic idea is to use different polysaccharides as delivery vehicle due to the fact that they are degraded by polysaccharidases present in the colon. Common examples of these polysaccharides, which have been explored for colon specific delivery, are chitosan, pectin, guar gum, chondroitin sulphate, dextran and amylase (Chourasia et al., 2006). The only problem associated with the use of these polymers is their high solubility in GI fluids that implies the need of cross-linking to assess their integrity until they reach the colonic region. In the present investigation, pectin is used for preparing colon-specific microspheres of methotrexate (MTX) as it is degraded by pectinase enzymes present exclusively in the colon. It is used as its calcium salt to overcome solubility problems in physiological environment of upper GIT.

MATERIALS AND METHODS

MTX was received as a gift sample from Unimed Technologies Ltd, Gujrat, India. Pectin (6% methoxy content) was procured from CDH, India. Pancreatin (from pig pancreas), pepsin (bovine), pectinase, iso-octane, and calcium chloride were procured from Loba Chemie, India. All other chemicals were of analytical grade.

Preparation of calcium pectinate microspheres Drug was added to the 50 g aqueous solution containing 3% w/w of pectin and dispersed in 100 ml isooctane containing Span 80 (2 g), with continuous stirring at 1500 rpm, with the help of mechanical stirrer. After 10 minutes, 5 g of Tween 80 (1% aqueous solution) was added and the dispersion was stirred for another 5 minutes followed by addition of 20 gm of 35% w/w solution of calcium-chloride dihydrate and allowed to react with the pectin-globules for 10 minutes. The microspheres formed were collected, washed with distilled water and dried at 45°C until constant weight. Triplicate batches were prepared for each formulation, mixed uniformly and randomly sampled for characterization.

Characterization Particle size of microspheres was determined using laser diffraction particle size analyzer (Cilas 1064 L, France). Shape and surface morphology was investigated using SEM. For determination of drug content, an accurately weighed (100 mg) amount of microspheres was digested in minimum quantity of pectinase solution (120 FDU/ml) for 24 hours. The digested homogenate was centrifuged at 3000 rpm for 5 minutes and supernatant was assayed for MTX content using HPLC method. The in vitro release studies were carried out using USP paddle apparatus (type II) at rotational speed of 100 rpm at 37±0.1°C. The drug release studies were conducted in simulated gastric fluid (SGF) for first 2 hours and in simulated intestinal fluid (SIF) for 3 hours. The dissolution medium was then replaced with simulated colonic fluid (SCF) and release rate studies were continued for 24 hours. *In vitro* drug release was carried out in presence of rat caecal contents to assess the biodegradability of polysaccharide by colonic bacteria (Rama Prasad et al., 1998).

RESULTS AND DISCUSSION

Ca-pectinate microspheres were prepared by the modified emulsification technique. Drug and polymer solution in PBS (pH 7.4) was finely dispersed in isooctane as discrete droplets, thereby forming a w/o type emulsion. The calcium chloride was then added as cross-linking agent. Ca^{++} binds preferentially to the polyglucuronic acid of pectin in a planner two dimensional manner causing gelation, producing the so called egg-box structure and thus results in hardening of the microsphere sheath. This cross-linking reduces solubility of polymer in water, forming gel like film at the surface of droplets. The microspheres prepared discrete upon dispersion in an aqueous medium and having uniform size ranges from 20.8±1.34 to 32.2±1.59 µm (Table 1). Particle size was observed to be increased with increasing pectin concentration. Mean particles size was found to be 22.5±1.12 µm in case of microspheres having 2% pectin while it was significantly increased to 32.2±1.59 µm ($p<0.005$) with 3.5% pectin concentration. *In vitro* release rate studies were carried out in SGF for 2 hours and SIF for 3 hours to ensure the efficacy of the formulation to withstand the physiological environment of stomach and small intestine. After 5 hours release rate study only 8.15±0.49% drug was released which showed that formulation maintained the integrity in the stomach and small intestine. Release of drug was significantly increased ($p<0.05$) when medium was replaced with SCF containing pectinase. *In vitro* release in presence of pectinase after 24 hours was found to be 96.34±4.75% whereas in control study it was only 20.89±1.12%. This increased released of drug in SCF showed that pectinase is respon-

sible for the degradation of polymer matrix and the release of drug.

Table 1. Composition and characteristics of different calcium pectinate microspheres formulations

Formulation code	Variables	Average particle size (µm)	Encapsulation efficiency (%)
MD_0	Methotrexate (0%, 10%, 20%, 30% and 40%w/w)	20.8±1.34	-
MD_1		21.4±1.23	65.82±4.18
MD_2		22.9±1.56	66.91±2.29
MD_3		23.0±1.25	68.93±1.70
MD_4^*		23.3±1.38	52.28±0.32
MP_1	Pectin (2%, 2.5%, 3% and 3.5% w/w)	22.5±1.12	70.13±0.14
MP_2		26.2±1.85	70.30±5.20
MP_3		28.7±1.46	70.33±5.60
MP_4^*		32.2±1.59	74.01±3.32
MC_1	Calcium chloride (20 ml) (20%, 25%, 30% and 35% w/w)	25.0±1.37	66.73±3.40
MC_2		23.8±1.24	69.63±4.81
MC_3^*		22.2±0.84	69.91±4.52
MC_4		21.8±1.05	68.32±2.14

To investigate the biodegradability of pectin, *in vitro* release rate studies were also carried out in presence of rat caecal contents. Release of the drug after 24 hours was significantly increased ($p<0.05$) from dissolution media having rat caecal contents in comparison to the dissolution media devoid of rat caecal contents. Amount of the drug released from the microspheres after 24 hours in presence of 1, 2 and 3% rat caecal matter was found to be 43.24±2.14, 54.86±2.63 and 69.94±3.46%, respectively whereas in control experiment it was only 20.89±1.12 % (Fig. 1).

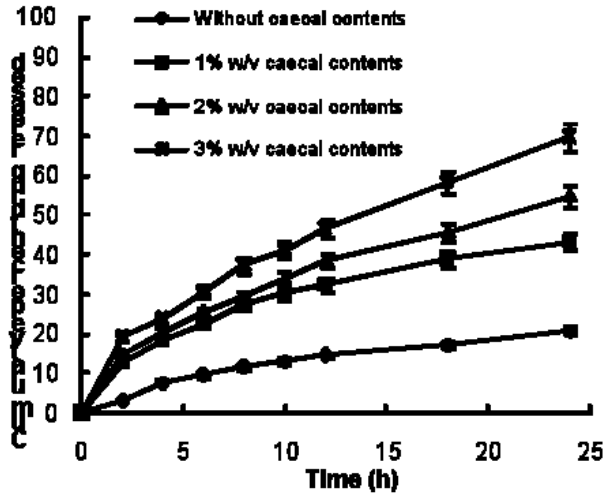

Figure 1 : *In vitro* drug release in SCF pH 7.0 with and without rat caecal contents

Presence of rat caecal contents in the dissolution media exerted a degradative effect on polymer matrix and higher amount of drug was released. Despite release of high high amount of drug in presence of 3% rat caecal contents compared to 1 or 2% level, there was considerable amount of drug to be released form formulation and hence induction of enzymes was carried out and release rate studies were repeated with 1, 2 and 3% rat caecal contents. To induce the enzymes different sets of animals were administered with 1 ml of 1% aqueous pectin solution for 7 days. Induction of enzymes for 7 days resulted in improved activity of colonic enzymes that is reflected from the release of higher amount of drug in comparison to those involved rat caecal contents without induction. The percentage of drug released after 24 hours was found to be 59.55±2.98 and 78.23±3.26% with 1 and 2% rat caecal contents, respectively whereas with 3% rat caecal contents it was further increased to 94.43±4.48% (Fig. 2).

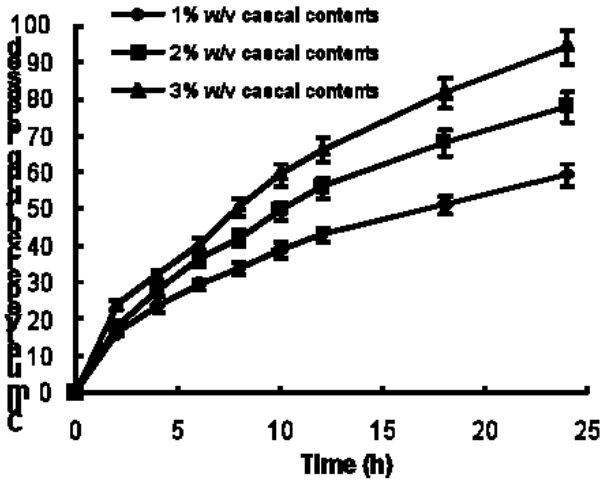

Figure 2 : *In vitro* drug release in presence of rat caecal contents after 7 days of enzyme induction

CONCLUSIONS

In conclusion, the capability of pectin in form of Ca-pectinate microspheres has been investigated to achieve colon specific release. The microspheres of pectin were prepared by emulsification method, which showed optimum performance in terms of size, drug content and encapsulation efficiency. Results of in vitro release rate studies revealed that Ca-pectinate microspheres offered a high degree of protection from premature drug release in simulated upper gastrointestinal conditions and delivers its most of the drug load in colonic environemnt.

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