Asian Journal of Biomaterial Research 2018; 4(4):8-20

Research Article

Meropenem Loaded Pectin Microspheres for Colon Delivery

Tahseen Khan* Ankur Vaidya, Ruhee Jain,
Bhagyoday Tirth Pharmacy College, Khurai Road, Sagar (MP) 470001

Received: 23 July 2018 Revised: 29 July 2018 Accepted: 02 Aug 2018

Abstract

Objective: The objective of present study is to prepare the meropenem loaded pectin microspheres. **Material and Methods:** Pectin microspheres were prepared by the method reported method with slight modification. Meropenem loaded pectin microspheres were prepared
by water/oil emulsion method. Meropenam loaded pectin microspheres were characterized by size and size distribution, morphology, SEM, Entrapment efficiency, In-vitro drug release in simulated gastric fluid of pH 1.2, Mixture of simulated gastric and intestinal fluid of pH 4.5, Simulated intestinal fluid of pH 6.8, Simulated intestinal fluid of pH 7.5 and Simulated colonic fluids of pH 7.5. **Results:** Meropenem loaded Pectin microspheres were prepared successfully by the selected method. Coating of Eudragit S-100 on pectin microspheres were done effectively. Pectin microsphere and Eudragit coated pectin microsphere were found spherical in optical and SEM microscopy. Surface of Pectin microsphere and Eudragit coated pectin microsphere were found smooth in SEM studied. Average particle size of pectin microsphere was found 10.24 µm Entrapment efficiency of both Pectin microspheres were found 70.25±2.04%. In-vitro release of meropenem from pectin microspheres was found 97.3% whereas from Eudragit coated pectin microspheres release 83.7% at 12hrs. **Conclusion:** The experimental results demonstrated that Eudragit S-100 help the drug to release in the colon by degrading its coating at pH 7 results in exposure of the pectin microspheres which on enzymatic influence and due to pH sensitive nature degraded and release the drug to the colon. Thus the Eudragit coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

Introduction

In past era, the way of consumption of food and medicines had no perceptible difference. To date, this problem is sorted by delivering the drug through the suitable routes. The oral route is considered to be most convenient for administration of drug to patients, due to its several advantages such as less pain, greater convenience, patient compliance etc. This route is convenient especially for chronic therapies where repeated administration is required (Florence et al, 1993; Chen et al, 1998).

When conventional drug is orally administrated, it normally dissolves in the gastrointestinal fluids and is absorbed from these regions of the gastrointestinal tract, which depends upon the physicochemical property of the drug. In spite of these advantages, the oral route is not acquiescent to the administration of most protein and polypeptide drugs, because of their high susceptibility to digestive enzymes in the gastrointestinal (GI) tract, poor absorption,

***Corresponding Author**

Tahseen Khan
Assistant Professor
Bhagyoday Tirth Pharmacy College,
Khurai Road, Sagar (MP) 470001
E.mail: heenakhan.khan55@gmail.com

and their limited ability to transport across the intestinal epithelial barrier. (Sinha and Kumria, 2001) The colon is one of the attracting interests as a site for drug delivery where poorly absorbed drug molecules may have improved bioavailability and also have gained the importance for systemic delivery of proteins and peptide drugs. This is because of the acidic environment of stomach and pancreatic enzymes of small intestine leads to the inactivation of these protein and peptide drugs. (Longer *et al.*, 1992; Yang et al., 2002) Drug colon targeting is profitable in the treatment of diseases associated with the colon such as amoebiasis, ulcerative colitis, crohn's disease, or colorectal cancer. (Macfarlane et al., 1990)

There are several important bacterias present in the colon such as *Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus, Clostridium* secrete a wide range of reductive and hydrolytic enzymes which are responsible for degradation of di-, tri- and polysaccharides such enzymes are β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase. (Sinha et al., 2003; Cavalcanti et al., 2008) Furthermore, the colon appears highly responsible to agents that enhance the absorption of poorly absorbed drugs due to its longer retention time. For colon drug targeting the simplest method is to obtain slower release rates or longer release periods by the application of thicker layers of conventional enteric coating or extremely slow releasing matrices. (Vaidya et al., 2009)

Colon drug delivery has its important value where intestinal delayed drug absorption is desired from therapeutic point of view in the treatment of disease which have peak symptoms in the early morning such as nocturnal asthma, angina or arthritis whereas, this region face drawbacks such as impaction of faeces (which might act to entrap drug) and the presence of bacterial enzymes and toxins but these apprehension are easier to deal with than the exhaustive destruction drug experiences in the stomach and small intestine. (Vaidya et al., 2009)

The conventional tablet dosage form provides minimal amount of the drug in the colon with undesirable adverse effects due to variation in the transit time. The drugs are targeted directly to the site of action in the colon, would be effective. Better treatment may be possible with a minimum dose of the drug. Latest approaches have been used for colon drug delivery which base upon time dependent system, pH dependent system, microbiologically controlled system, multiparticulate system and luminal pressure controlled system.

The aim of present study is to prepare the meropenem loaded pectin microspheres. These prepared microspheres will not maintain their integrity in upper part of GIT and release the drug at desired site i.e; colon. To protect the drug release from microspheres, these multiparticulte systems were coated with enteric coating material i.e. Eudragit S-100. These meropenem loaded Eudragit coated pectin microspheres will not only target the drug to colon but also avoid diffusion of drug through formulation, lead to inhibit any absorption through small intestine. This concept will also maximize the drug utilization that will result lowering of the dose.

Materials and Methods

Material

Pectin was purchased from Central Drug House (CDH) Ltd., New Delhi, India. Mylan laboratories limited Nashik (M.H.) supplied Meropenem as gift sample. Span-80 was procured from Research Lab Fine Chem Industries Mumbai India. Isooctane was purchased from CDH, Mumbai, India. All other chemicals were of analytical reagent grade.

Preparation of Pectin Microspheres

Pectin microspheres were prepared by the method reported by Vaidya et al., (2009) with slight modification. Briefly the drug-polymer solution (0.5%, in distilled water) was dispersed in 50 ml isooctane containing span 80 (1.5% w/v) and the

dispersion was continuously stirred at varied speed to obtain stable water/oil emulsion. The dispersion was rapidly cooled to 10ºC followed by addition of 50 ml of acetone, for the dehydration of pectin droplets. For the complete solvent evaporation, the formulation was continuously stirred at 1000 rpm for 30 min at room temperature. The formulation containing Microspheres were freeze-dried and kept in airtight container for further studies. Similarly, the pectin microspheres with varying compositions and varying formulation variables were prepared and optimized.

Optimization

Various formulation variables e.g. Meropenem concentration, polymer concentration, span 80 concentration and process variables viz. stirring speed and stirring time, which could affect the preparation and properties of microspheres were identified and studied. The formulation compositions of designed pectin microspheres are given in Table 1.

Optimization of process variables

Various process variables that could affect the preparation and properties of microspheres were optimized i.e. stirring speed: 1000, 1500, and 2000 rpm stirring time 20, 30, and 40 minutes and span 80 concentrations (0.5, 0.75, 1.00 and 1.25). Effect of these variables were observed on final particle size, size distribution and shape of microspheres and meropenem loading efficiency are reported in Table 1 and shown graphically in fig 1-6. On the basis of meropenem loading efficiency the optimum condition are reported in Table 2.

Entrapment Efficiency

The drug entrapment efficiency in microspheres was determined using the method reported by Vaidya et al., 2009. The prepared microspheres were digested in 10 ml of PBS (pH 7.4) for 12 h which contain pectinase solution (4% wt/wt) which is followed by the centrifugation at 3000 rpm for 5 min, and the supernatant obtained was assayed. The digested homogenate was centrifuged at 3000 rpm for 5 min, and the supernatant was assayed spectrophotometrically at 297 nm (UV- Thermo scientific) for meropenam drug.

Coating of Pectin Microspheres

The prepared pectin microspheres were coated with Eudragit S-100 using the method reported by Vaidya et al., 2009 with slight modifications. Briefly, the prepared pectin microspheres (50 mg) were dispersed in 10 ml of organic solvents mixture (acetone/ ethanol, 2:1) containing Eudragit S-100 to provide 1:5, 1:10 or 1:15 core/coat ratio. This untreated phase was added into 70 ml of light liquid paraffin which hold 1% w/v span 80. After this the system was continuously stirred for 3 h at 1000 rpm in order to evaporate the solvent at room temperature. The Eudragit coated microspheres were centrifuged at 1000 rpm for 15 min for separation and washed with n-hexane. Finally coated microspheres were lyophilized and stored in tightly capped container.

Characterization of pectin microspheres:

Shape and Surface Morphology.

The shape and surface morphology of the prepared pectin microspheres were studied by using scanning electron microscopy, where the microsphere powder was lightly sprinkled on a double adhesive tape which was stuck on aluminium stub. By using the sputter coater the stubs were then coated with the gold to of about 300Å thick and then observed under scanning electron microscopy (Vaidya et al., 2009).

Particle Size Determination.

The size of both uncoated and Eudragit coated pectin microspheres was appraised using Laser diffraction based particle size analyzer (1064L, Cilas, Marcoussis, France).

***In- vitro* Drug Release.**

In-vitro drug release studies were performed according to Vaidya et al., 2009 extraction technique using USP dissolution test apparatus Type 2 (Paddle type). The dissolution studies were achieved in 900 ml dissolution medium, with

continuously stirring at 100 rpm at room temperature.

The scheme for using the simulated gastrointestinal fluids at different pH was as follows:

- 1st hour: Simulated gastric fluid of pH 1.2.
- 2nd and 3rd hours: Mixture of simulated gastric and intestinal fluid of pH 4.5.
- 4th and 5th hours: Simulated intestinal fluid of pH 6.8.
- 6th and 7th hours: Simulated intestinal fluid of pH 7.5.
- 8th to 24th hours: Simulated colonic fluids of pH 7.5.

Aliquots of samples were periodically withdrawn and balanced with an equal amount of fresh dissolution media. The spectrophotometric measurement at 297 nm (UV-Thermo scientific)

RESULTS AND DISCUSSION

The aim of present study is to prepare meropenem loaded pectin micropsheres coated with Eudragit S-100 for colonic drug delivery. Eudragit S-100 is an enteric coated polymer it protect the release of meropenem from the pectin microspheres in upper GI tract. Pectin is itself an enzyme degradable polymer which releases the entrapped drug (meropenem) in colonic site. So the present study utilizes the dual advantage of pectin enzymatic degradation as well as enteric coating of Eudragit S-100.

Pectin microspheres bearing meropenem were prepared by solvent evaporation method. There were several formulations and process variables viz, pectin concentration, Span 80 (emulsifier concentration), stirring time and stirring speed which were optimized to obtain spherical microspheres with optimum particle size and maximum drug entrapment efficiency.

Shape and surface morphology of pectin microspheres were observed using scanning electron microscopy (SEM). Shape of pectin was found to be spherical with smooth surface while in the case of enteric coated pectin microspheres with the rough surface due to the Eudragit S-100 coating.

The particle size and percent entrapment efficiency of pectin microspheres increases from 9.17±2.25 µm to 10.34±1.43 µm and 68.42±2.28 % to 73.50±2.18% respectively, as the polymer concentration was increases from 1% to 2%. The optimized polymer concentration was 1.5% with the particle size of 9.39±1.35 µm and entrapment efficiency 70.61±3.19% as on increasing the polymer concentration, no significant increase in the entrapment efficiency observed. The high polymer concentration is more viscous so it is difficult to formulate the uniform pectin microspheres. The obtained results good agreement of previous report (Ashford et al., 1993).

In optimization of drug concentration which is varied from 5 to 15%, as the drug conc. increased the particle size also increased from 10.19±0.59µm to 10.42±0.91µm and entrapment efficiency also increased from 62.28±30.07% to 73.89±3.22% respectively. The optimized drug concentration was 10% with particle size 10.31±0.58µm and entrapment efficiency was 66.31±2.81%. The similar results reported by other researcher (Dandagi et al., 2007).

On optimizing the Span 80 (emulsifier) concentration, it was found that on increasing the concentration from 0.5 % to 1.25%, particle size decreased from 10.86±1.80 µm to 8.68±2.61µm and the entrapment efficiency increased from 70.05±3.22% to 75.12±3.18%. The optimum span 80 conc. was 1.0% and particle size was 9.18±1.19 µm and 72.37±2.54% was optimum entrapment efficiency. The similar results reported by other researcher (Pradesh et al., 2005).

On optimizing the stirring speed, the stirring speed was increased from 1000 rpm to 2000 rpm which results the decreased particle size from 13.61±1.59 µm to 9.89±2.09 µm and first increased and then gradual decreased entrapment efficiency from 72.23±3.31% to 69.03±2.31%.The optimum stirring speed considered was 1500 rpm with 10.30±1.90 µm particle size and 74.51±2.65% entrapment efficiency. The similar

results reported by other researcher (Pradesh et al., 2005).

The particle size and the entrapment efficiency decreased from 12.30±2.44 µm to 10.30±2.15 µm and 70.21±2.87% to 63.89±2.99% with the increase in the stirring time from 20 mins. to 30 mins. The optimum speed was 30 minutes with the particle size and entrapment efficiency was 10.89±1.60 µm and 71.13±3.54%. The similar results reported by other researcher (Pradesh et al., 2005).

Further the plain pectin microspheres were coated with Eudragit S-100 and core: coat ratio was optimized to achieve the uniform coated pectin microspheres, when the core: coat ratio was increased from 1:5 to 1:15 the average diameter was increased from 13.34 ± 1.90 µm to 17.78 ± 1.76 µm. The optimized core: coat ratio was found at 1:10 with the average particle diameter was 16.32 ± 1.22 µm. The similar results reported by other researcher (Trivedi P et al., 2008).

In vitro drug release from optimized uncoated and Eudragit S-100 coated pectin microspheres were carried out in different medium (pH- 1.2, 4.5, 6.8, 7.5) similar to the simulated pH of GIT fluids, that is with and without enzymatic medium. It showed significant difference in drug release profile from uncoated pectin microspheres and enteric coated pectin microspheres. The similar results reported by other researcher (Vaidya et al., 2008).

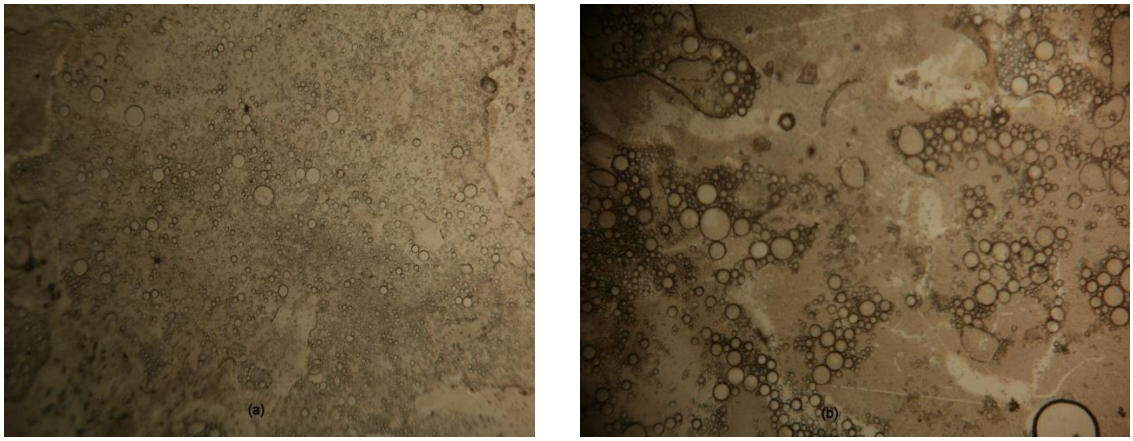
The drug release studies were carried out in SGF for 1-3 hrs, SIF for 4-7 hrs and CIF for 8-24 hrs to ensure the efficacy of the formulation to withstand the physiological environment of stomach, small intestine, and colon. There was regular difference in drug release between uncoated and coated pectin microspheres. After 1hr there was 12.4 % drug release from uncoated formulation whereas no release was observed from the enteric coated microspheres after 1h. Consequently the drug release after 8 hrs from uncoated formulation was 88.6% and from coated formulation was 48.4%. This data explained Eudragit S-100 coated formulation shown delayed released as compare to uncoated microspheres. This delayed release was due to Eudragit S-100 polymer coating contains carboxyl group which gets ionized from neutral to alkaline media. At pH 7.4 in small intestines, the coating dissolves and the pectin microspheres were released, results in polymer material swelling and erosion, thus drug was released. The results are well correlated with previously published reports (Vaidya et al., 2008).

The results clearly suggests that enteric coated pectin microsphere formulation could be utilized for sustained and colon drug delivery purpose.

CONCLUSION

The present studies divulge the positive upshot of Eudragit S-100 coated meropenem loaded pectin microspheres for the colon delivery. These microspheres maintain their integrity in upper part of GIT and reduce the side effects of the drug caused by its absorption from the upper part of GIT when the drug is given in conventional dosage forms such as tablets and capsules. The experimental results demonstrated that Eudragit S-100 help the drug to release in the colon by degrading its coating at pH 7 results in exposure of the pectin microspheres which on enzymatic influence and due to pH sensitive nature degraded and release the drug to the colon. Thus the Eudragit coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

Asian Journal of Biomaterial Research 2018; 4(4):8-20



(a) **(b)**

Fig. 1.: Photograph of (a) Uncoated Pectin Microspheres (b) Eudragit S-100 Coated Pectin Microspheres.

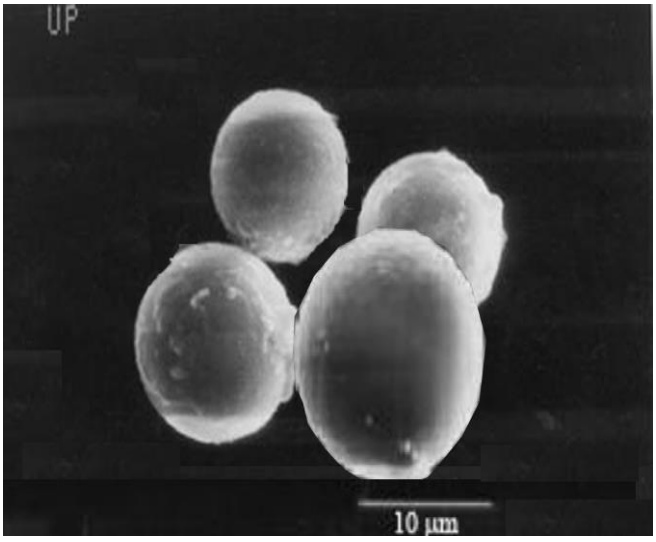


Fig. 2: SEM photograph of uncoated pectin microspheres.

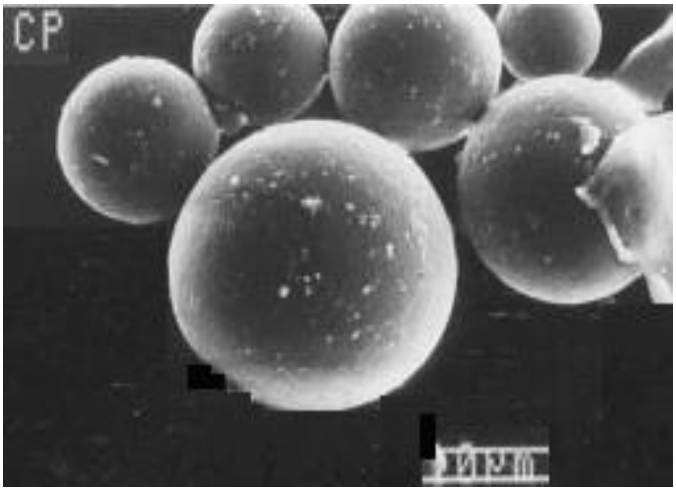


Fig 3: SEM photograph of Eudragit S - 100 coated pectin microspheres.

Asian Journal of Biomaterial Research 2018; 4(4):8-20

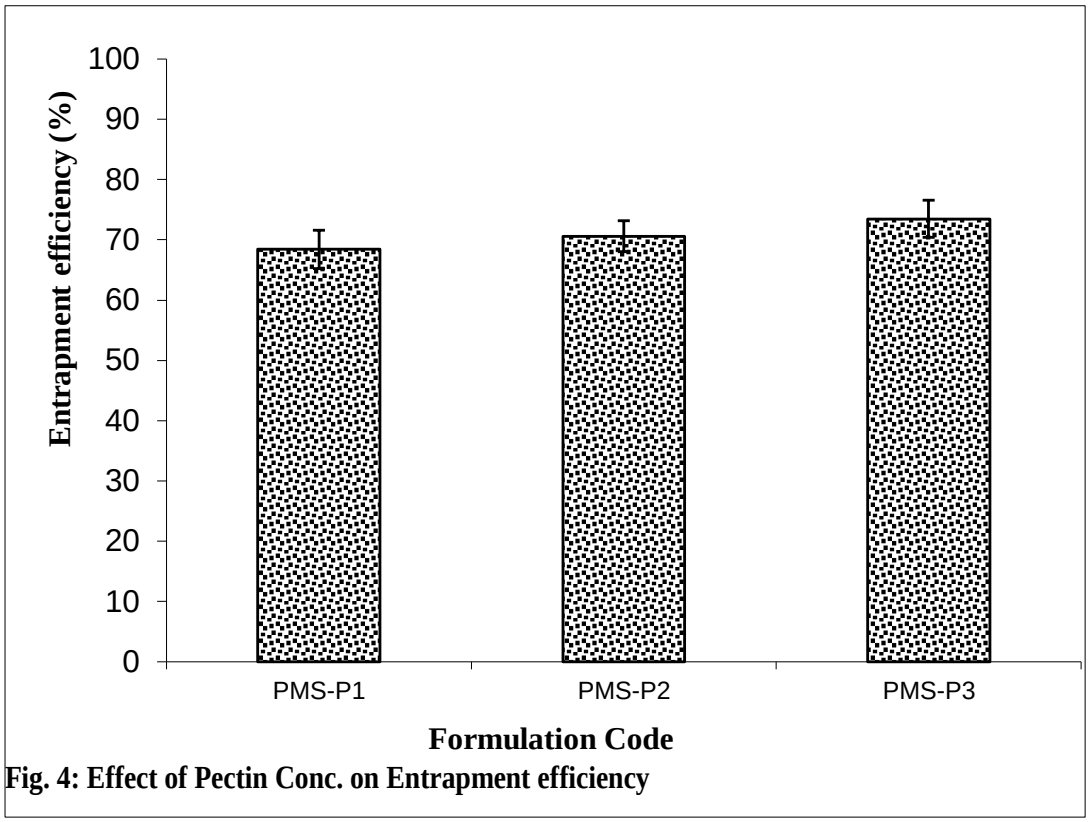


Fig. 4: Effect of Pectin Conc. on Entrapment efficiency

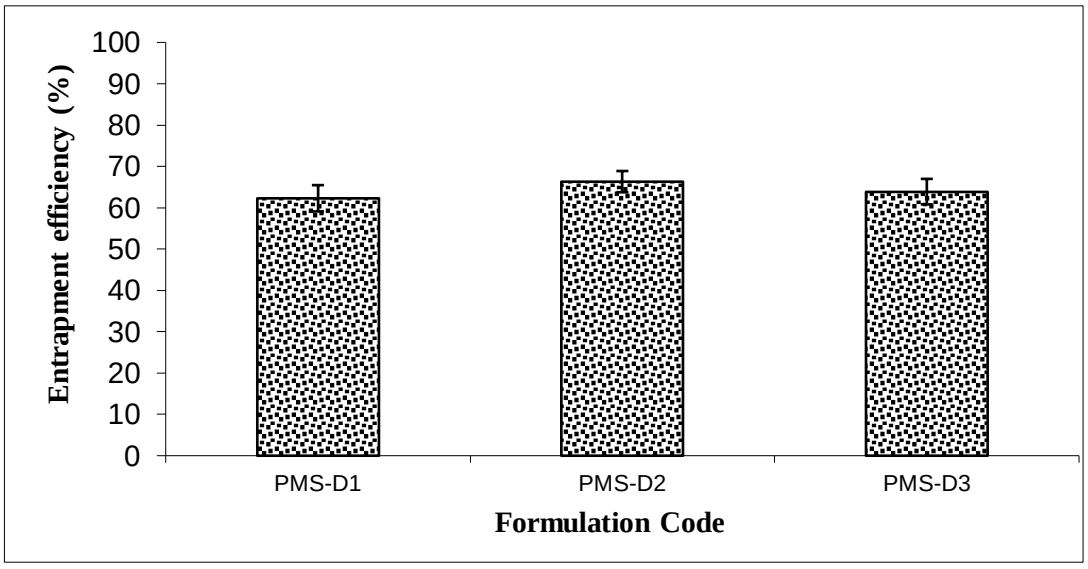


Fig. 5: Effect of Drug Conc. on Entrapment efficiency

Asian Journal of Biomaterial Research 2018; 4(4):8-20

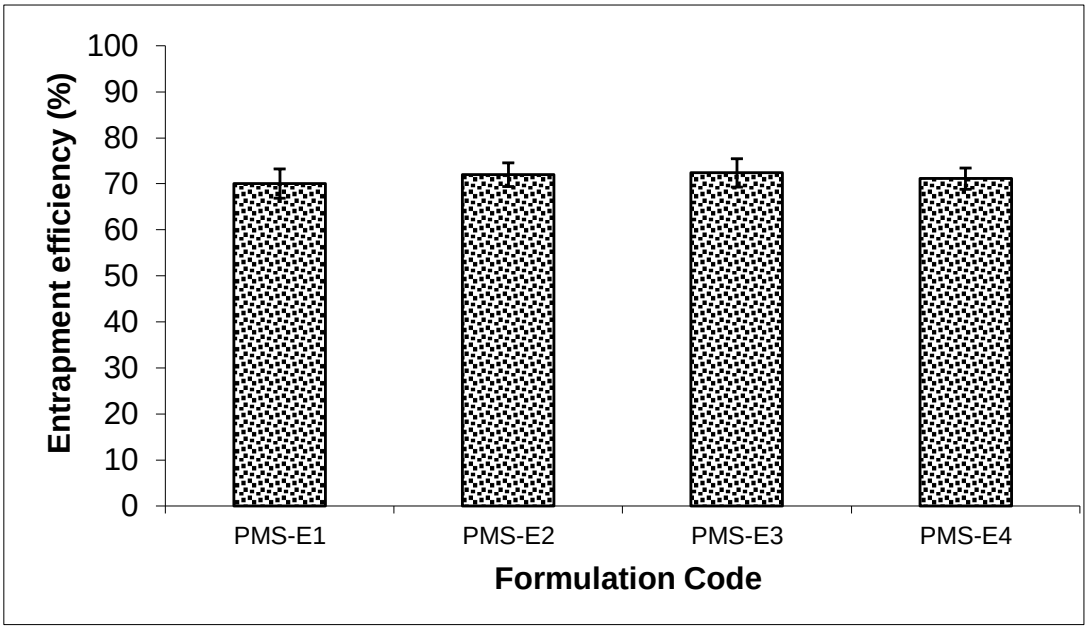


Fig. 6: Effect of Emulsifying Conc. on Entrapment efficiency

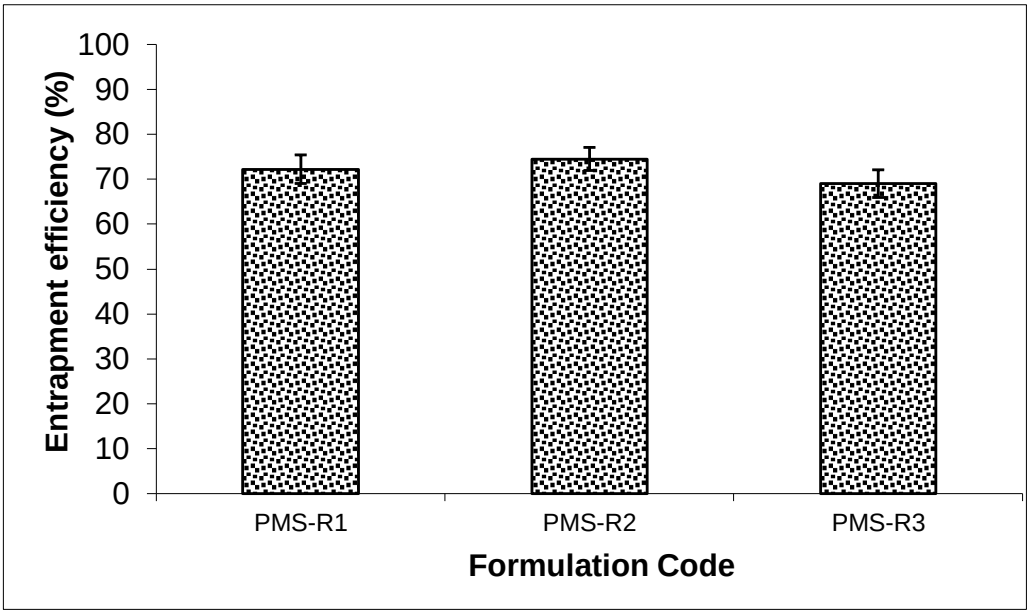


Fig. 7: Effect of Stirring speed on Entrapment efficiency

Asian Journal of Biomaterial Research 2018; 4(4):8-20

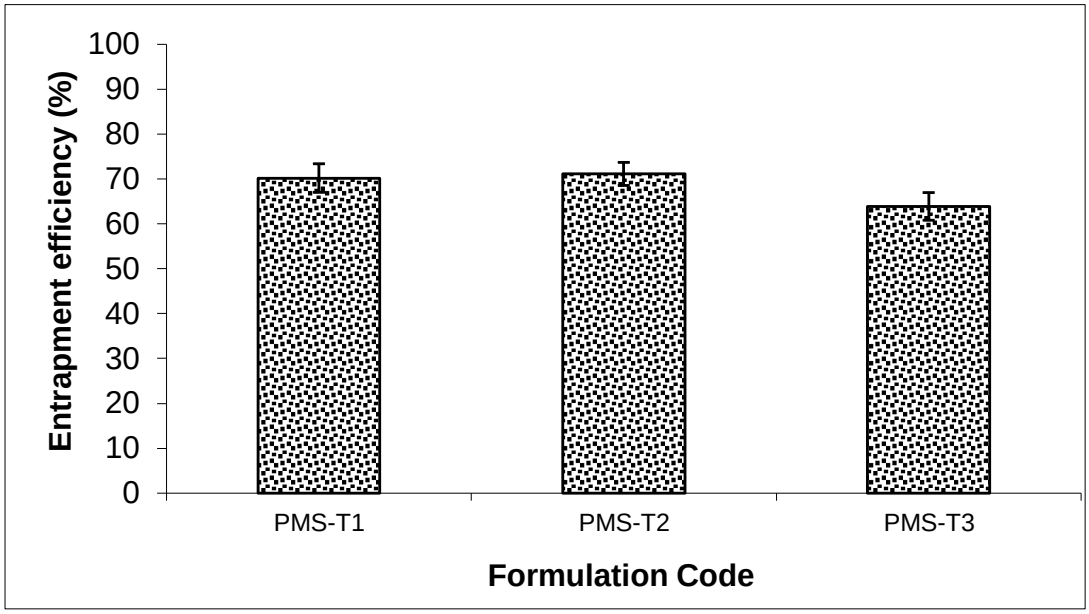
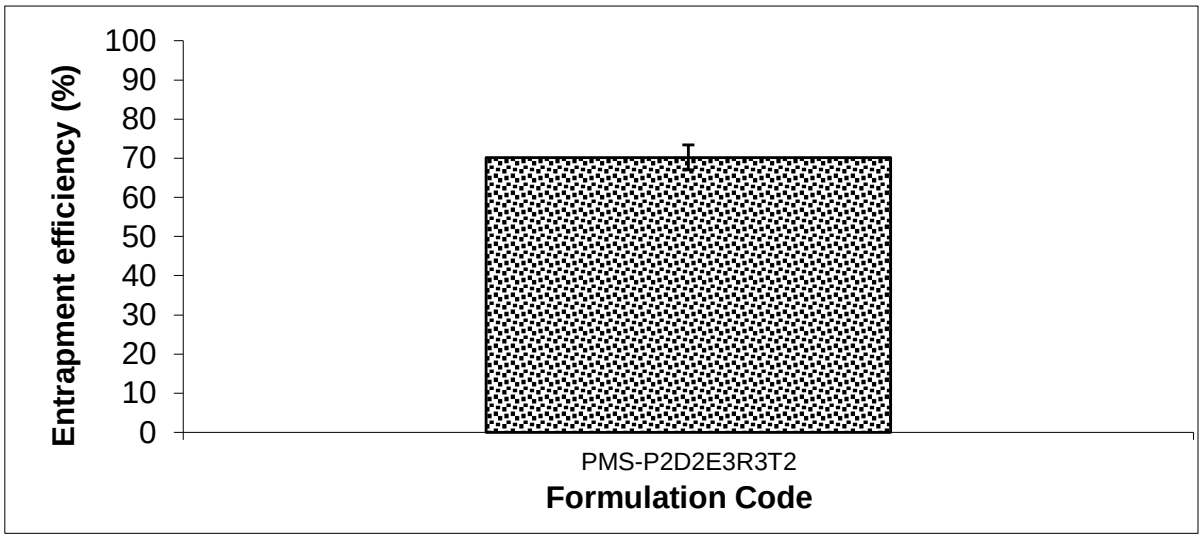


Fig. 8: Effect of Stirring Time on Entrapment efficiency



Asian Journal of Biomaterial Research 2018; 4(4):8-20

Fig. 9: Effect of optimized condition on entrapment efficiency of pectin microspheres

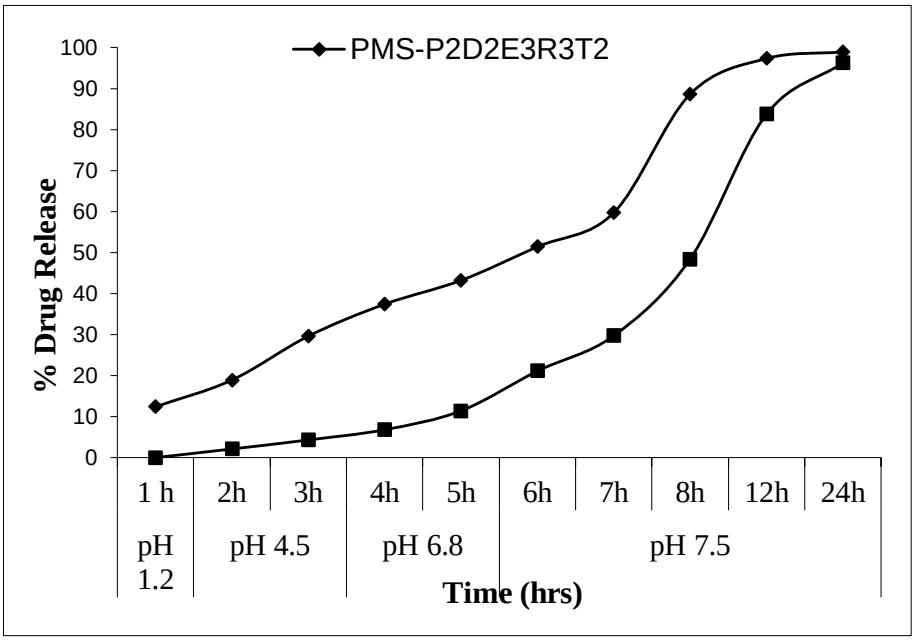


Fig 10: Percent drug release from uncoated and Eudragit S-100 coated pectin microspheres

Table 1: Average particle size, Entrapment efficiency of uncoated pectin microspheres:

Formulation code	Variables	Values	Particle size (µm)	Entrapment efficiency (%)
PMS-P1	Pectin concentration (%)	1	9.17±2.28	68.42±2.28
PMS-P2		**1.5**	**9.39±1.35**	**70.61±3.19**
PMS-P3		2	10.34±1.43	73.50±2.18
PMS-D1	Drug concentration (%)	5	10.19±0.59	62.28±3.07
PMS-D2		**10**	**10.31±0.58**	**66.31±2.81**
PMS-D3		15	10.42±0.91	63.89±3.22
PMS-E1	Emulsifier concentration [Span 80(w/v) (%)]	0.5	10.86±1.80	70.05±3.22
PMS-E2		0.75	10.06±1.15	71.98±2.65
PMS-E3		**1.0**	**9.18±1.19**	**72.37±2.54**
PMS-E4		1.25	8.68±2.61	71.12±3.18
PMS-R1	Stirring speed (rpm)	1000	13.61±1.59	72.23±3.31
PMS-R2		**1500**	**10.30±1.90**	**74.51±2.65**
PMS-R3		2000	9.89±2.09	69.01±2.31
PMS-T1	Stirring Time (min)	20	12.30±2.44	70.21±2.87
PMS-T2		**30**	**10.89±1.60**	**71.13±3.54**
PMS-T3		40	10.30±2.15	63.89±2.99

Table 2: Optimized condition for preparation of microspheres:

Formulation Code	% pectin solution	% drug concentration	% span80	Stirring speed	Stirring time
PMS-P2D2E3R3T2	1.5	10	1.0	1500	30

Table 3: Effect of optimized conditions on Pectin microspheres size, shape and entrapment efficiency:

Formulation Code	Diameter (µm)	Shape	Entrapment efficiency
PMS-P2D2E3R3T2	10.24	Spherical	70.25±2.04

Table: 4 Effect of core: coat ratio on size and shape of microspheres:

Formulation Code	Core: Coat ratio	Average Diameter (µm)	Shape
PMS-P2D2E3R3T2C1	1:5	13.34±1.90	Uncoated Particles seen, coating insufficient
PMS-P2D2E3R3T2C2	**1:10**	**16.32±1.22**	**Spherical with uniform coating**
PMS-P2D2E3R3T2C3	1:15	17.78±1.76	Coating material found in solution showing over concentration

Table 5: *In vitro* percent drug release from uncoated and Eudragit S 100 coated pectin microsphere

Formulation Code	pH 1.2	pH 4.5		pH 6.8		pH 7.5			
	1 h	2h	3h	4h	5h	6h	7h	8h	12h	24h
PMS-P2D2E3R3T2	12.4	18.9	29.6	37.4	43.2	51.4	59.7	88.6	97.3	98.9
PMS-P2D2E3R3T2C2	0	2.1	4.3	6.8	11.4	21.1	29.7	48.4	83.7	96.2

References

Ashford M, Fell J, Attwood D, Sharma H, Woodhead P. 1993. An evaluation of pectin as a carrier for drug targeting to the colon. Journal of Controlled Release, 213.

Ashford M, Fell J, Attwood D, Sharma H, Woodhead P. 1993. *In vitro* investigation into the suitability of pH dependent polymer for colonic targeting. International Journal of Pharmaceutics, 193-199.

Cavalcanti OA, Van den, Mooter G, Caramico-Soares I, Kinget R. 2002. Polysaccharides as excipients for colon-specific coatings, permeability and swelling properties of coasted films. Journal of Industrial Pharmacy, 57-64.

Chen H, Langer R. 1998. Oral particulate delivery: status and future trends. International Journal of Pharmaceutics, 339–50.

Dandagi MP, Masthiolimath SV, Gadad PA, Iliger R.S. 2007. Mucoadhesive microspheres of propanalol Hcl for nasal delivery. Indian Journal of Pharmaceutical Sciences, 402-07.

Englyst HN, Hay S, Macfarlane GT. 1987. Polysaccharide breakdown by mixed populations of human faecal bacteria. International Journal of Pharmaceutics, 163.

Fatemeh A, Rudabeh V, Rassoul D. 2004. Preparation of ethylcellulose coated gelatin microspheres as a multiparticulate colonic delivery system for 5-aminosalicilic acid. Iranian Journal of Pharmaceutical Research, 81-86

Florence AT, Jani PU. 1993. Particulate delivery the challenge of the oral route. International Journal of Pharmaceutics, 65–107.

Gordon, P H. and Nivatvongs S. 1992. Principles and Practice of surgery for the colon, rectum, and anus. St. Louis: Quality Medical Publishing Inc, 334-368.

Imming P, Sinning C, Meyer A. 2006. Drugs, their targets and the nature and number of drug targets. Nature Review Drug Discovery, 821-34.

Kanazawa K, Nouda H, Sunagawa M. 1997. Structure-activity relationships of carbapenem compounds to anti-Haemophilus influenzae activity and affinity for penicillin-binding proteins. Journal of Antibiotics, (Tokyo), 162.

Keighley, M R, Williams, N.S. 2001. Surgery of the anus, rectum and colon. W.B. Saunders, 225-245.

Khan Z, Zeljkopreeg, Kurjakoviee N**.** 1999. pH dependent colon targeted oral drug delivery system sing methacrylic acid polymers. Journal of Controlled Release, 215-222.

Lee F Siew, Abdul W Basit, and J Michael Newton. 2000. The potential of organic-based amylose-ethylcellulose film coatings as oral colon-specific drug delivery systems AAPS. PharmSciTech, 53–61.

Macfarlane GT, Hay S, Macfarlane S, Gibson GR. 1990. Effect of different carbohydrates on growth polysaccharidases and glycosidase production of bacterosides ovatus in batch and continuous culture. Journal Applied Bacteriology, 179–187.

Marta Rodriguez, Jose Vila Jato**.** 1998. Design of a new multiparticulate dosage form consisting of a hydrophobic core coated with a pH dependent polymer for colonic drug delivery. Journal of Controlled Release, 67-77.

Pradesh TS, Sunny M, Varma KH, Ramesh P. 2005. Preparation of microstructured hydroxyapatite microspheres using oil in water emulsion. The Bulletin of Materials Science, 383- 90.

Sinha VR, Kumria R. 2001. Polysaccharides in colon-specific drug delivery. International Journal of Pharmaceutics, 19–38.

Sinha VR, Rachna K. 2003. Coating polymers for colon specific drug delivery: A comparative in vitro evaluation. Acta Pharmaceutica, 41-73.

Trivedi P, Verma AML, Garud N. 2008. Preparation and characterization of acclofenac microspheres. Asian Journal of Pharmaceutics, 10-15.

Vaidya A, Jain A, Khare P, Agarwal RK, Jain SK. 2009. Metronidazole loaded pectin microspheres for colon Targeting. Journal of Pharmaceutical Sciences, 4229–4236.

Vaidya A, Jain S, Agrawal R, Jain SK. 2012. Pectin–metronidazole prodrug bearing microspheres for colon targeting. Journal of Saudi Chemical Society, 2-3.

Yang L, James S C, Joseph A. 2002. Colon-specific drug delivery. International Journal of Pharmaceutics, 1-15.