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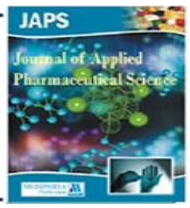

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Preparation and *In-vitro* characterization of ethylcellulose coated pectin alginate microspheres of 5-fluorouracil for colon targeting

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ABSTRACT

The main objective of the present study was to prepare and evaluate the colon-specific pectin alginate microspheres of 5-fluorouracil (5-FU) for the treatment of colon cancer. Calcium alginate beads were prepared by extruding 5-FU loaded alginate solution to calcium chloride solution and gelled spheres were formed instantaneously by ionotropic gelation reaction using different ratios of 5- FU and alginate, alginate and calcium chloride, stirring speeds (500-1500 rpm) and reaction time. The core beads were coated with ethyl cellulose to prevent drug release in the stomach and provide controlled dissolution of enteric coat in the small intestine and maximum drug release in the colon. Morphology and surface characteristics of the formulation were determined by scanning electron microscopy. *In vitro* drug release studies were performed in conditions simulating stomach to colon transit in the presence and absence of pectinase enzyme. No significant release was observed at acidic pH, however, when it reached the intestinal pH where ethyl cellulose starts to dissolve, drug release was observed. Also, release of drug was found to be higher in presence of pectinase enzyme. The DSC and FT-IR studies were also indicates there were no interactions between the drug and the polymers used.

Key words: 5-FU, Pectin alginate beads, Ethyl cellulose, Scanning Electron Microscopy and Pectinase enzyme.

INTRODUCTION

Colon targeted drug delivery has the potential to deliver bioactive agents for the treatment of a variety of colonic diseases including inflammatory bowel disease (IBD) can be effectively treated by the local delivery of drugs to the large intestine. The treatment of colonic disease such as amoebas, Crohn's diseases, ulcerative colitis, and colorectal cancer is particularly improved by their local delivery to the bowel. By this technique, absorption of the drug from the stomach and small intestine can be minimized until the drug reaches the large intestine. Various drug delivery systems have been designed that delivers the drugs quantitatively to the large bowel and subsequently to trigger the release of active drug. The treatment of large intestine disorders, such as Crohn's disease, irritable bowel syndrome, colitis, colorectal cancer, and local infectious disease, where high concentration of drug is needed (Riley, 1990) that can be improved by colon specific drug delivery systems employing various mechanism of release (Gazzaniga, 1995). However, for successful colonic drug delivery, many physiological barriers must be overcome, the major one being absorption or degradation of the active drug in the upper part of the gastrointestinal tract (GI) tract (Ashford, 1993). Among the different approaches to achieve targeted drug release to the colon, the use of polymers especially biodegradable by colonic

bacteria holds great promise (Marvola, 1999). Polysaccharides are bacterial enzymes that are available in sufficient quantity to be exploited in colon targeting of drugs. Based on this approach, various polysaccharides have been investigated for colon specific drug release. These polysaccharides include pectin, alginate, guar gum, amylase, inulin, dextran and chitosan (Krishnaiah, 2001). Based on this we planned to prepare and evaluate the colon-specific ethyl cellulose coated pectin alginate beads of 5-fluorouracil (5-FU) for the treatment of colon cancer. The rationale behind for this is ethyl cellulose prevents the drug release in the upper part of the GIT.

MATERIALS AND METHODS

Materials

Materials used included 5-fluorouracil was kindly provided as a gift sample by Merck (Bombay India) Limited. Pectin and Sodium alginate was purchased from Loba chemicals, Mumbai. Calcium chloride, hydrochloric acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, n-hexane, ethyl acetate was purchased from S.D. Chemicals, Boiser. Ethyl cellulose was provided as a gift sample from Alembic Limited, Vadodara and Peptinaze enzyme was purchased from Novozyme, U.S.A.

METHODS

Drug –polymer interaction study

FT-IR Studies

The infrared (IR) spectra were recorded using an FTIR spectrophotometer (Perkin Elmer Spectrum GX) by the KBr pellet method in the wavelength region between 4000 and 400 cm^{-1}. The spectra obtained for 5-Fluorouracil and physical mixtures of 5-Fluorouracil with polymers were compared to check compatibility of drug with polymers.

DSC Studies

Thermograms of the samples were obtained by a Perkin-Elmer differential scanning calorimeter (Pyris 6 DSC, software Pyris manager, Perkin-Elmer Schweiz AG, Hunenberg, Switzerland). Samples of 3 mg were accurately weighed into aluminum pans and then hermetically sealed with aluminum lids. The thermograms of samples were obtained at a scanning rate of 10°C/min over a temperature range of 50 to 350°C. All tests were performed twice.

Table: 1 Formulation of Pectin microspheres.

Formulation code	Amount of 5-fluoro-uracil (% w/v)	Concentraton of Pectin (% w/v)	Concentration of sodium alginate (% w/v)	Concen-tration of calcium chloride (% w/v)	Cross-linking time (min)
PMS-F1	1	1.0	2.0	6.0	10
PMS-F2	1	2.0	2.0	6.0	15
PMS-F3	1	3.0	2.0	6.0	20
PMS-F4	1	4.0	2.0	6.0	10
PMS-F5	1	5.0	2.0	6.0	15
PMS-F6	1	6.0	2.0	6.0	20

Preparation of core calcium alginate beads

The pectin microspheres were prepared by ionotropic-external gelation technique. Different formulations of calcium alginate beads were prepared as shown in table 1, the two different solutions were prepared separately. First, required amount of pectin was dispersed in a specified volume of cold water containing the drug and allowed to swell for 2 hours. In another beaker suitable amount of sodium alginate was taken and mixed well with 10 ml of water. The pectin solution containing the drug was added to sodium alginate solution with stirring at 400 rpm for 15-20 minutes to produce a viscous form. Then polymer drug solution was added drop wise by using syringe of 22 G having needle of 0.45 mm inner diameter from a height of about 5 cm into a beaker containing 6% w/v solution of calcium chloride with continuous stirring by magnetic stirrer as shown in figure 1. So the gelatinous precipitate is formed by chemical reaction between sodium alginate and calcium chloride. The prepared beads were left under stirring in the medium at 1000 rpm for 20 min and then removed by filtration and washed with distilled water and vacuum dried and stored in well closed container for further use (Krishnaiah, 2003 and Atyab, 2005).

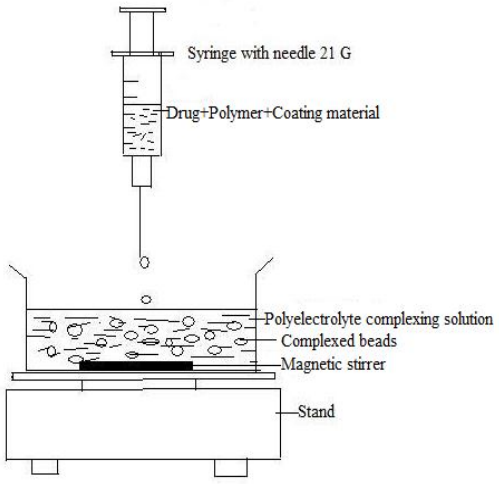

Fig 1: Schematic diagram of the preparation of hydrogel beads by ionotropic-gelation method.

Coating of pectin calcium alginate microspheres with ethyl cellulose

Drug loaded pectin microspheres were used as a core material for the preparation of double-coated system. A coacervation phase separation method was applied for this step. A known amount of the microspheres having particle size of 100-250 µm was dispersed in an in ethyl acetate (25ml) solution containing ethyl cellulose and containing 0.02% W/V span 80. This mixture was agitated for 5 min at 400 rpm. Subsequently 50 ml n-hexane (as the non-solvent) was poured into the polymeric solution containing the core material with the rate of 1 mL/min and core: coat ratio is 1:3. The medium was stirred for 60 min to complete the process of microparticles coating. Coated microspheres were then washed with an excess of n-hexane, filtered and dried at room temperature (Fatemeh Atyabi., 2004 and Paharia, 2007).

Drug Entrapment Efficiency

Amount of drug-loaded alginate beads were dispersed in phosphate buffer pH 6.8 at 37°C ± 0.5°C and kept for digestion with continuous stirring up to 24 h. Then the sample was centrifuged at 1000 rpm for 10 min to remove any insoluble solids, the supernatant layer was removed and filtered. The drug content was determined using UV - visible spectrophotometric method (Ghosh, 2007). Incorporation efficiency was calculated using the following formula:

$$\text{Drug Entrapment Efficiency} = \frac{\text{Actual drug content X 100}}{\text{Theoretical drug content}}$$

Surface morphology

The shape and surface characteristics of Pectin-alginate microspheres and ethyl cellulose coated pectin microspheres were observed by scanning electron microscopy (SEM) (FEI Quanta-200 MK2, Netherlands). The samples were imaged using a 15-kV electron beam (Atyab, 2005).

Swellability

A known weight (100 mg) of various 5- FU-loaded pectin microspheres and Ethyl cellulose coated pectin microspheres were placed in enzyme-free simulated intestinal fluid (SIF, KH_2PO_4/NaOH buffer, pH 7.4) and allowed to swell for the required period of time at 37ºC ± 0.5ºC in the dissolution apparatus (United States Pharmacopeia [USP] XXIII, model DT-06, Erweka, 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:

$$SR = Wg - W_0 / W_0 \quad (1)$$

Where, SR indicates swelling ratio; W_0, initial weight of microspheres; and Wg, final weight of microspheres.

In vitro drug release study from pectin- alginate microspheres

Uncoated pectin- alginate microspheres were evaluated for the *in vitro* drug release in pH progression medium at 37°C ± 0.5°C microspheres were weighed accurately and in 900 ml of dissolution medium. The content was rotated at 100 rpm at 37°C ±0.5°C. Perfect sink conditions prevailed during the drug dissolution study period. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 h using Simulated Gastric fluid (SGF). Then in Simulated Intestinal fluid (SIF) (KH_2PO_4(1.7g) and $Na_2HPO_4.2H_2O$ (2.25g) were added to the dissolution medium, adjusting the pH to 4. 5 with 1.0 N NaOH), and the release rate study was continued for an additional 2 h. Then, it was transferred in to the dissolution medium of PBS 7.4 (Phosphate Buffer Solutions) and maintained up to 12 hrs (Vandelli MA., 1996). The samples were withdrawn from the dissolution medium at various time intervals. The rate of 5-FU release was analyzed using UV - visible spectrophotometric method at λ_{max} of 268.0 nm.

In vitro drug release study from coated pectin alginate microspheres

Coated pectin alginate microspheres were evaluated for *in vitro* drug release study by the method similar to that of core beads; it was performed in pH progression medium at 37°C ± 0.5°C. All dissolution studies were performed in triplicate.

Stability Studies

To assess long-term stability, the core-coated microsphere formulations (E3) was put in hard gelatin capsules and sealed in aluminum packaging coated inside with polyethylene. The studies were performed at 40°C/75% relative humidity (RH) in the stability chamber (Testing Instruments, Kolkata) for 3 months. At the end of the storage period, the formulation was observed for physical appearance, size, shape, surface morphology, drug content, in vitro drug release, and Differential Scanning Calorimetry (DSC) studies.

RESULTS AND DISCUSSION

The FT-IR spectrum of 5-fluoro uracil in formulations was shown in figures 2 and 3. The spectra revealed the presence of peaks at 3124$cm^{-1}$for NH strtching,1716cm^{-1} and1657$cm^{-1}$for C=O stretching respectively, indicating that there was no interaction between the drug and excipients used in the study.

The DSC thermograms were obtained for 5-fluorouracil and the formulations. The DSC analysis of 5-fluorouracil showed a single endothermic peak at 294 ± 2^0c, due to the melting of the drug. In the DSC curve of microspheres, the characteristic peak of the drug was almost unchanged indicating the absence of strong interactions between the components and suggesting drug-excipient compatibility in all the formulations examined. The DSC thermograms were shown in the figures 4and 5.

The effect of speed of stirring, concentration of polymer and reaction time were optimized on the basis of quality of beads and entrapment of the beads. It was evaluated by determining quality of beads and entrapment efficiency at different concentrations of polymer and varying reaction time, it observes that at 1000 rpm for 20 min, proper shape and uniformity with optimized entrapment efficiency was formed.

Entrapment efficiency

Various batches were tried with different drug to polymer ratio and different cross linking time. Prepared batches were optimized using spectroscopic method for the estimation of drug (5-fluorouracil). As drug to polymer ratio and cross linking time were increased, entrapment efficiency was also increased (63.88 to 69.84%). Optimized drug to polymer ratio and cross linking time was 1: 6 and 20 minutes, the entrapment efficiency was 69.84%.

Surface morphology

By SEM study, the size of optimized alginate beads was 500 - 700 μm. This shows that the ethyl cellulose coated beads have smooth surface compared to core beads as shown in Figure 6 and 7.

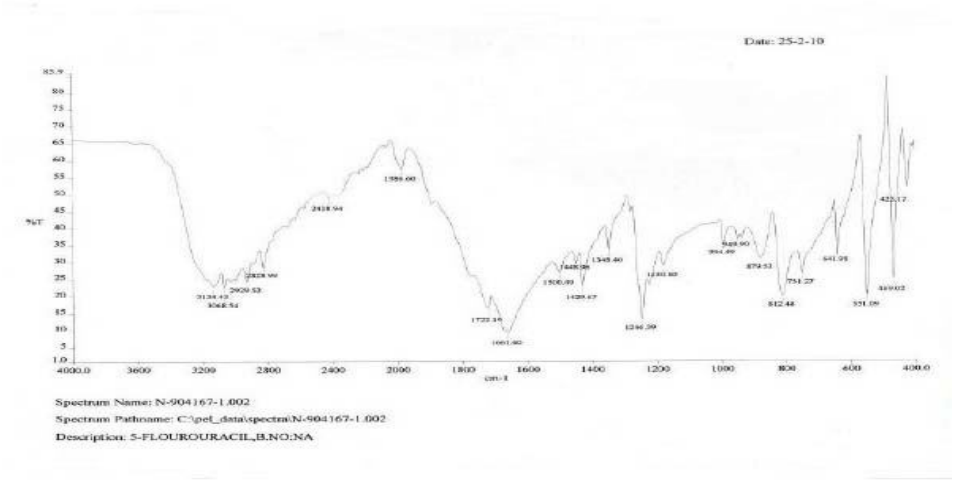

Fig: 2. FT-IR spectrum of pure 5-fluorouracil.

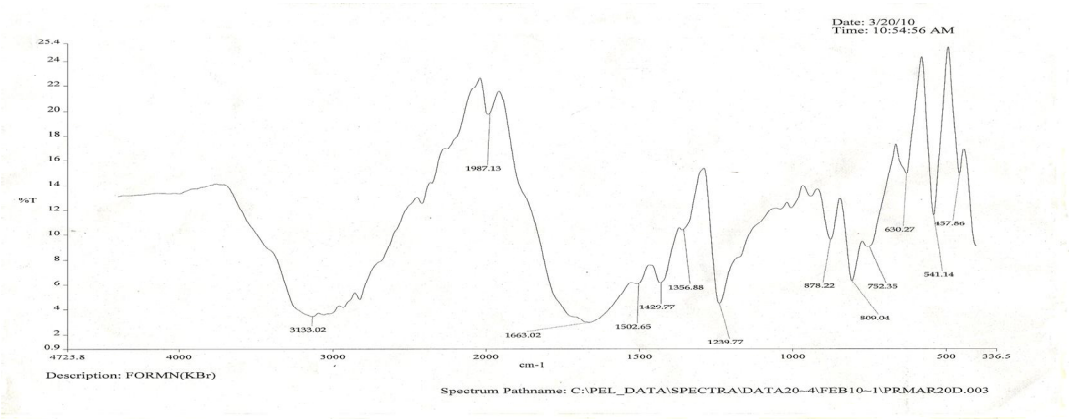

Fig: 3. FT-IR spectrum of 5-fluorouracil containing pectin alginate microspheres

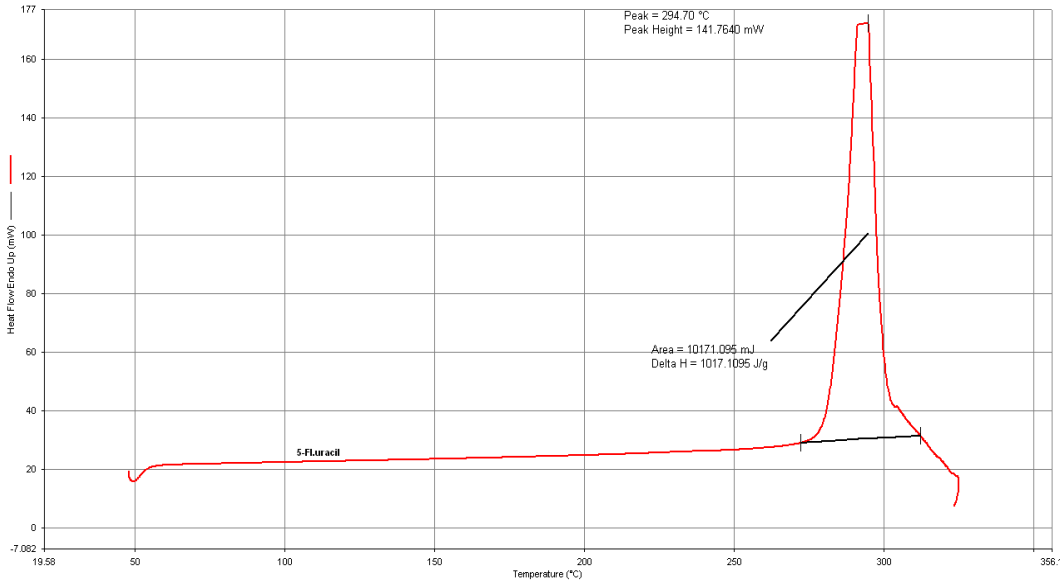

Fig: 4. DSC Thermogram of 5-Fluorouracil**.**

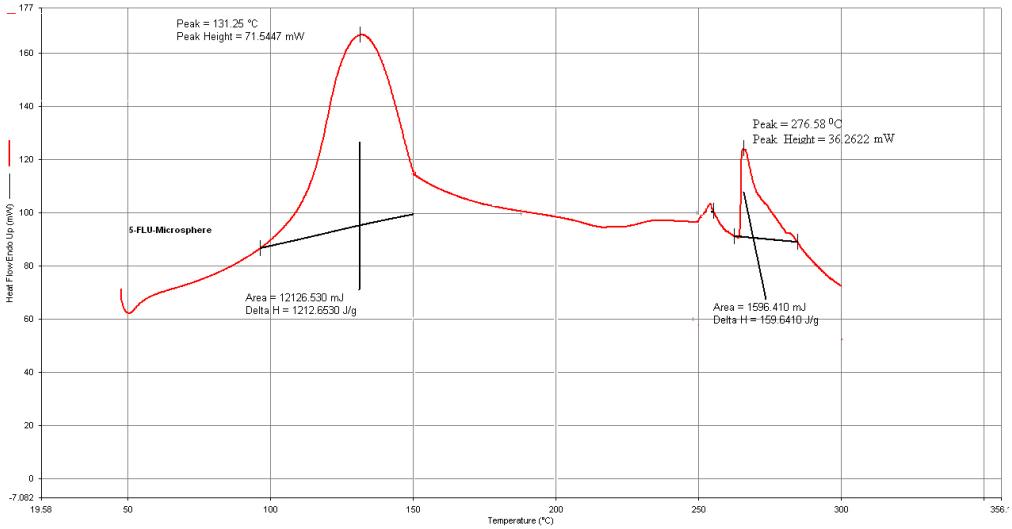

Fig: 5. DSC Thermogram of the Formulation PMS-F6.

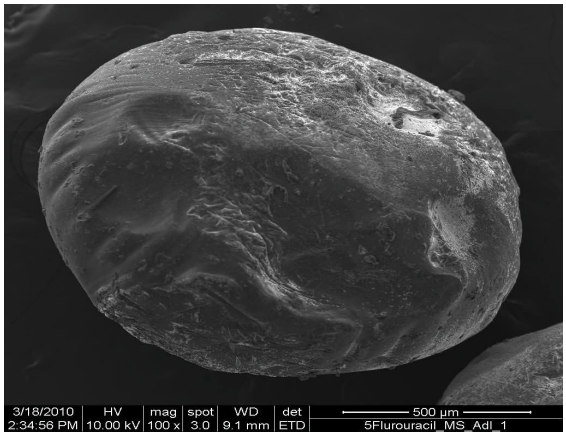

Fig: 6. SEM of 5-Fluorouracil Microspheres of PMS-F6 before Dissolution.

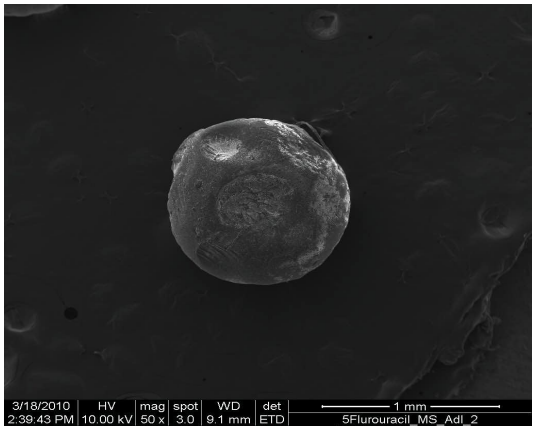

Fig: 7. SEM of Ethyl Cellulose Coated 5-Fluorouracil Microspheres of EPMS-F6.

Swellability

Swellability of different microspheres was determined. No significant swelling was observed with ethyl cellulose-coated pectin microspheres as compared with pectin microspheres as shown in table 2. Thus ensuring better resistance of ethyl cellulose - coated microspheres in the upper GI tract to swelling and preventing subsequent drug release at the non target site.

In vitro drug release

In vitro drug release of coated and uncoated beads was performed in pH progression medium at 37˚C ± 0.5˚C, in SGF, SIF, and in presence and in absence of Pectinase enzyme. As compared to core alginate beads, coated beads shows about 7.83 ± 0.96 % drug release after 4 h and rest of the drug releases up to 12 hrs as shown in Figure 8 and Figure 9. So it protects the release of drug from the upper part of GI tract and minimizes the side effects. Above pH 7.0, Ethyl cellulose coating started to dissolve and exposed the alginate beads for drug release.

The *in vitro* release of FU from pectin microspheres and Ethyl cellulose-coated microspheres in presence of pectinase enzyme in simulated colonic fluid showed faster drug release at different time periods when compared with release study without pectinase, as shown in figure 10. This finding could be attributed to the various anaerobic bacteria present in colon and responsible for digestion/degradation of pectin in order to release drug from microspheres. Therefore, we can conclude that if the Ethyl cellulose coated beads protect the drug from stomach and small intestine and start drug release upon arrival to colon and gives local action. It may provide site-specific release and reduce systemic side effects. It was further evaluated for drug release kinetics; data

Table 2: Degree of swelling of various pectin microspheres and ethyl cellulose coated pectin microspheres

Serial Number	Pectin microspheres		Ethyl cellulose-coated microspheres	
	Formulation code (Drug: Polymer)	Degree of Swelling	Formulation code (Drug: Polymer)	Degree of Swelling
1	PMS-F1 (1:1)	0.88 ± 0.20	EPMS-F1 (1:1)	0.42 ± 0.01
2	PMS-F2 (1:2)	0.98 ± 0.25	EPMS-F2 (1:2)	0.11 ± 0.02
3	PMS-F3 (1:3)	1.11 ± 0.29	EPMS-F3 (1:3)	0.14 ± 0.04
4	PMS-F4 (1:4)	1.19 ± 0.33	EPMS-F4 (1:4)	0.16 ± 0.03
5	PMS-F5 (1:5)	1.24 ± 0.40	EPMS-F5 (1:5)	0.18 ± 0.02
6	PMS-F6 (1:6)	1.31 ± 0.61	EPMS-F6 (1:6)	0.20 ± 0.04

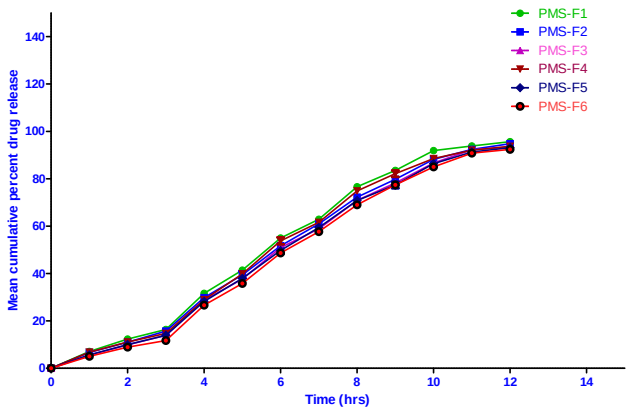

Fig 8: Percentage cumulative *in vitro* 5-FU release from different pectin microspheres in simulated gastrointestinal fluids of different pH. PMS indicates pectin microspheres.

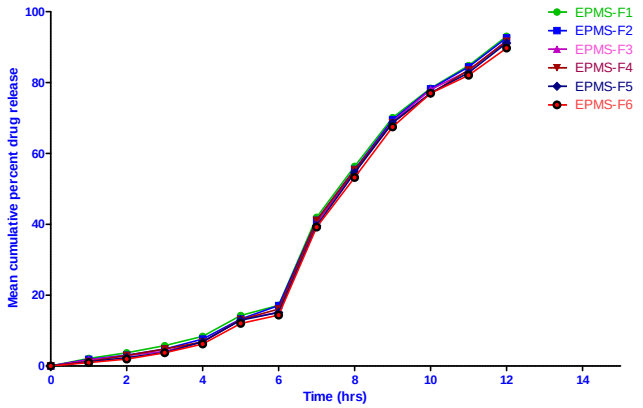

Fig 9: Percentage cumulative *in vitro* 5-FU release from different Ethyl cellulose coated microspheres in simulated gastrointestinal fluids of different pH. EPMS indicates Ethyl cellulose coated microspheres.

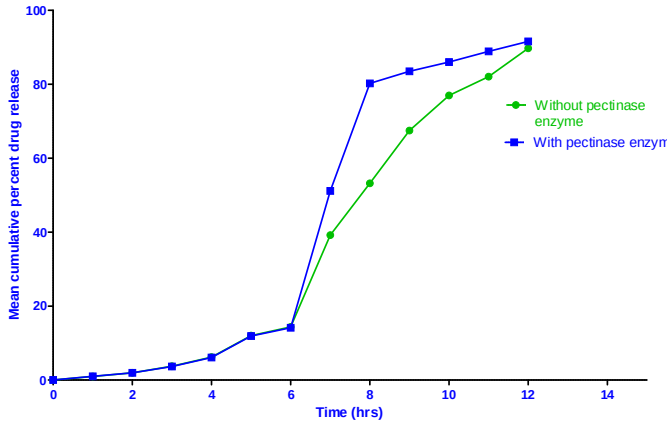

Fig 10: Effect of Pectinase enzyme on percentase 5-FU from Ethyl cellulose-coated microspheres (EPMS-F6) in simulated colonic fluid (pH 7.4) with and without pectinase enzyme.

obtained from *in vitro* drug release studies were fitted into various kinetic models such as, First Order, Higuchi law, Koresmeyer's-Peppas equation. All having R^2 values greater than 0.9. In Koresmeyer's-Peppas equation, the exponent n = 0.5 and 0.8, indicating that the release mechanism is anomalous diffusion; values were depicted in table 3.

Table 3: I*n-vitro* release kinetic parameters for 5-fluorouracil ethyl cellulose coated pectin microspheres.

Formulation code	Zero Order Model		First-Order Model		Higuchi Model		Koresmeyer's-Peppas Model	
	k0 (µg/mL/hr)	r2	k1 (hr-1)	r2	kh (mg/hr1/2)	r2	n	r2
EPMS-F1	10.6	0.89	0.244	0.975	47.44	0.975	0.695	0.977
EPMS-F2	10.57	0.888	0.234	0.962	47.3	0.964	0.578	0.961
EPMS-F3	10.52	0.886	0.227	0.983	47.08	0.971	0.543	0.985
EPMS-F4	10.51	0.893	0.237	0.988	47.04	0.987	0.674	0.974
EPMS-F5	10.46	0.885	0.232	0.986	47.03	0.987	0.499	0.973
EPMS-F6	10.51	0.892	0.221	0.992	46.79	0.999	0.724	0.981

Stability study

Stability study was performed at different temperature and also at different conditions. But it showed that there is no significant reduction in the percentage drug retained in the formulation and also there was no significant difference in drug release profile for the sample storage at 40°C/75% and at ambient temperature.

CONCLUSIONS

The designed site-specific delivery of 5-FU from the system may reduce 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. The experimental results demonstrated that Ethyl cellulose-coated pectin microspheres have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

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