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Development of Resistant Starch-Pectin Microsphere for Improving Oral Colon-specific Drug Delivery of 5-Fluorouracil

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Abstract

Conventional chemotherapy is ineffective for colorectal cancer as the drug moiety does not reach its intended site. 5-Fluorouracil (5-FU) microspheres aimed at colon-specific drug delivery have been developed by using the solvent evaporation technique in this study. The influence of utilizing different percentages of resistant starch-pectin polymer on the drug loading, encapsulation efficiency, and release behavior of the drug was investigated. The characterization of prepared microspheres was done by shape, surface morphology, drug loading, encapsulation efficiency and *in vitro* drug release studies. Drug-polymer interactions and drug stability were investigated by Differential Scanning Calorimetry (DSC) and FTIR spectroscopy. The 5-Fluorouracil polymeric microspheres that were formulated utilizing a resistant starch (RS) and pectin polymer displayed ideal physicochemical properties and spherical particles. The targeted microspheres demonstrated a high drug loading, encapsulation efficiency, and a satisfactory drug release pattern over the time period of 8 hours. Studies on drug release conducted under conditions that simulate stomach to colon transit have demonstrated that the drug was prevented from being released in the physiological conditions of the stomach and small intestine. Creating a new multiparticulate system using RS-pectin microspheres in order to forecast the protection that the combination might provide to the dosage form that will ensure localized drug release in the colon. Finally, it was found that the 5-Fluorouracil polymeric microsphere is a suitable micro-carrier for effective colon-specific drug delivery tools with increased chemotherapeutic efficacy.

Key words: Microspheres, 5-Fluorouracil, resistant starch, solvent evaporation technique, pectin, chemotherapy.

Introduction

The clinical potential of technologies that deliver drugs specifically to the colon is being studied more and more nowadays. In the treatment of many colonic diseases, such as ulcerative colitis, Crohn's disease, luminal amebiosis, colon cancer, diarrhea, or other colon-specific pathologies, delivery of the drug moiety to the colon enhances efficacy and minimizes systemic side effects (Philip *et al*., 2009). Polysaccharide-based delivery systems which are specifically stimulated by the physiological circumstances of the colon carry significant promise among the numerous approaches created for delivering drugs to the colon because they offer better site specificity and fulfill the necessary therapeutic needs (Zhou, 1994).

5-Fluorouracil is a cytotoxic chemotherapeutic agent. For several decades, it has been the drug of choice against colorectal cancer (McQuade *et al.*, 2017). Patients prefer oral treatment to intravenous treatment, according to a survey study on cancer patients, but they were hesitant to forgo tumor

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response level for convenience of drug administration (Liu *et al*., 1997). After surgery, giving these high risk patients a course of 5-Fluorouracil based chemotherapy lowers their risk of developing cancer; recurrence rate can drop to 40%, total survival can rise up to 60%, and is currently the most recommended treatment for people with stage III colon cancer (Markowitz *et al.*, 2002).

In the past few decades, drug delivery has made significant progress and in the years to come, many more advances are anticipated. Microspheres are one of the most popular varieties and offer various advantages as compared to other controlled release drug delivery systems. Microspheres can be defined as microscopic particles that are spherical and the size ranges from 1 µm to 1,000 µm (Malik *et al.*, 2014). Small compounds, proteins, and nucleic acids can all be enclosed within microspheres to form a variety of therapeutic formulations. They are typically biocompatible, have a high bioavailability, and can release drugs for a very long time. Utilizing specialized polymers as a carrier for colon-specific drug offers a number of advantages, including as biocompatibility, affordability, non-toxicity, resistance to the digestion in the upper GI tract and targeted delivery specifically to the colon (Khan *et al*., 2020)

Due to lower enzymatic activity, lower diversity, near neutral pH and lower intensity, the colon presents less hostile conditions for drug delivery. Formulations stay in the colon for two to three days, which offers a significant timeframe for drug absorption (Watts and Illum, 1997). Colon specific delivery systems based primarily on time or pH dependency of drug release (Rubinstein, 1995) are unreliable due to variable transit time through the upper GIT (factors altering gastric emptying time) and the inherent variability of pH (pH drops to 6.4 ± 0.6 on entering the colon which is unsuitable for drugs released at pH values over 7). Microflora-activated systems (colonic microflora count: 10^{12} CFU/mL) seem more promising because the sudden rise in bacterial population and related enzymatic activity in the colon reflect a discrete event that is independent of the gastrointestinal transit time (Yang *et al.*, 2002). Polysaccharide based delivery systems which are specifically stimulated by the physiological circumstances of the colon carry significant promise among the numerous approaches created for delivering medications to the colon because they offer increased site specificity and satisfy the necessary therapeutic needs (Zhou, 1994). Utilizing naturally occurring dietary polysaccharides as a drug carrier for colonic delivery simplifies the concerns of toxicity, safety and availability. Ideal candidates are those that have low absorption from both the stomach and intestine; and degrade through anaerobic bacteria in the colon.

The term "resistant starch" refers to the portion of starch which is not digested in the small intestine, and therefore only broken down and fermented in the colon (Champ, 1992). It can be delivered to the lower gut for anaerobic fermentation by gut microbiota indicating a potential material which can be utilized in the development of colon-specific systems for drug delivery. The high resistant starch content assures the resistance of components against pancreatin's enzymatic digestion (Meneguina *et al.*, 2014). On the other hand pectin, a hydrophilic polysaccharide derived from plant cell walls, is one of the most well researched polysaccharides for its suitability for targeting drugs to the colon (Wakerly *et al*., 1996; Liu *et al*., 2003; Dev *et al*., 2011). Degree of esterification greatly influences the solubility of pectin, which controls the efficiency of colon specific pectin-based system for drug delivery (Shah *et al*., 2011).

Colon targeted administration of 5-Fluorouracil offers an efficient and secure treatment for colon cancer with a lower dose and shorter course of treatment, while also reducing systemic side effects. In light of this information, the present study was the first approach to prepare RS-pectin microspehres, where RS and pectin were utilized as carriers, and to design and develop an effective orally available 5-Fluorouracil colon-specific drug delivery system.

Materials and Methods

Materials: 5-Fluorouracil, pectin, starch, calcium chloride, paraffin oil and span 85 and all other reagents were purchased from local suppliers. The other excipients used were of analytical grade.

Preparation of 5-Fluorouracil microspheres: The 5-Fluorouracil microspheres were prepared by solvent evaporation technique (Jalil and Nixon, 1989). To prepare microspheres, 5-Fluorouracil was suspended by ultrasonication in the mixed polymeric dispersion. Amount of 5-Fluorouracil for making different percentage of drug loading was calculated according to the filmogenic content of RS-pectin dispersion. This suspension was then poured into the mineral oil containing 1.0% w/v Span 85 (sorbitan trioleate) and at high speed, stirred constantly to create stable water in oil (w/o) emulsion. Finally, along with continuous stirring, the emulsion was warmed up to a high temperature with the help of water bath when microspheres appear and was continued until the solvent was removed by evaporation. Yield microspheres were filtered (HVLP filter, Millipore, pore size 0.45 µm), washed extensively with *n*-hexane and dried at 50°C overnight. The formulation composition is provided in Table 1.

Table 1. Formulation composition of 5-Fluorouracil microspheres.

Formulations	Polymer drug ratio
Pectin and 5-FU	
F1	1:3
F2	1:1
F3	3:1
Pectin, RS and 5-FU	
F4	1:3
F5	1:1
F6	3:1

In RS-containing formulations (F4, F5 and F6), pectin and RS were used in equal amount.

Fourier-transform infra-red (FTIR) spectroscopy: FT-IR analysis was conducted with Shimadzu & portable IR Spirit FTIR spectrophotometer to thoroughly evaluate the physicochemical characteristics of microspheres and to examine any potential interaction between the components (Chaturvedi *et al*., 2012).

Differential scanning calorimetry (DSC) analysis: Utilizing a differential scanning calorimeter with a computer analyzer (Shimadzu TA–60 differential scanning calorimeter, Shimadzu Corporation, Kyoto, Japan), DSC studies of the 5-Fluorouracil, pectin, resistant starch and microspheres were performed (Arefin *et al*., 2016). On an aluminum pan, samples (3–7 mg) were heated at a rate of 10°C/min throughout a temperature range of 10–500°C while maintaining a nitrogen atmosphere.

Scanning electron microscopy: By using Scanning Electron Microscopy, the exterior morphology of microspheres was examined (Ramachandran, 2011). SEM analysis of prepared microspheres was done using the instrument model JEOL JSM 6490 LA. The prepared microspheres were attached to supports using carbon-glue and coated with gold using a high-vacuum evaporator and a gold sputter module. After that, samples were examined with the scanning electron microscope.

Determination of drug loading: Drug loading means amount of drug loaded or entrapped in the microspheres. Approximately 20 mg of prepared microspheres was taken in a mortar and crushed fully using pestle. Then they were totally dissolved in 20 mL of freshly prepared phosphate buffer solution (pH 7.4), continuously stirred for three hours and finally the solution was filtered. Then analysis of the solution was done with the help of the ultraviolet spectrophotometer at 266 nm. The drug loading was calculated using the formula (Arefin *et al*., 2016)–

$$\text{Drug loading (\%)} = \frac{\text{Amount of drug}}{\text{Weight of microspheres}} \text{ X } 100$$

Determination of drug encapsulation efficiency: Drug entrapment efficiency means the ratio of the actual drug content and theoretical drug content. Approximately 20 mg of the prepared microspheres was taken in a mortar powdered completely using pestle. Then they were totally dissolved in 20 mL of freshly prepared phosphate buffer solution (pH 7.4), continuously stirred for three hours and finally the

solution was filtered. Analysis was done with an ultraviolet spectrophotometer at 266 nm. The drug entrapment was calculated using the formula (Patel, 2007).

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \text{ X } 100$$

In vitro dissolution study of the microspheres: Dissolution study was carried out following established methods in dissolution testing machine using USP I (basket type) dissolution tester at three different pH (pH 1.2, pH 6.8 and pH 7.4) (Costa, 2001). The temperature was maintained at 37±0.5°C and the rpm was set to 50. At first 650 mL of 0.1N HCl (pH 1.2) was given in each basket and 80 mg microspheres containing drug was placed in each basket. After each time interval 5 mL of sample from basket was withdrawn and for each sampling the volume was adjusted with 5 mL of 0.1N HCl to maintain the sink condition.

After 2 hours, 250 mL of freshly prepared phosphate buffer was poured into the basket and the pH of the medium was adjusted to 6.8. The volume was adjusted after each sampling like before. Again after 2 hours, the pH was adjusted to 7.4. The sink condition was maintained with phosphate buffer. This dissolution was continued for a time period of 8 hours. The released drug was assayed by using a UV spectrophotometer at 266 nm.

Results and Discussion

Fourier transform infrared spectroscopy (FT-IR): The FTIR spectrum of 5-Fluorouracil is presented in Figure 1 (A). It showed the absorption bands at 1719 cm^{-1}, 1645 cm^{-1} and 1243 cm^{-1} which were responsible for cyclic imide (CO–NH–CO), amide I band (C=O), and amide III band (C=O), respectively. FTIR spectra of microspheres containing pectin, resistant starch & 5-Fluorouracil in Figure 1 (B) was found to have no chemical shift, proving that 5-Fluorouracil was completely entrapped in the microspheres and that there was no chemical interaction amongst the constituents of the microspheres.

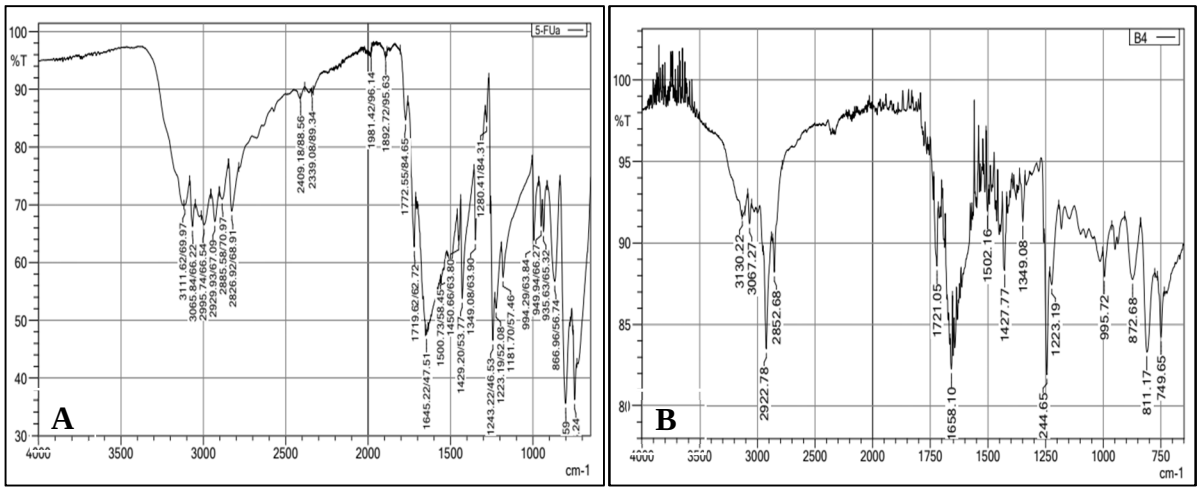

Figure 1. FTIR spectrum of (A) 5-Fluorouracil and (B) Microspheres containing 5-FU, RS and petin.

Thermal characterization of microspheres: When a drug is present in polymeric microspheres, there will not be any identifiable endotherm of the drug (Utreja *et al*, 2010). The DSC thermograms are presented in figure 2 (A-D). The melting endotherm of pure 5-Fluorouracil was determined to be 291.67°C. Endothermic peaks at 156.90 °C and 128.20°C, respectively corresponded to pectin and resistant starch melting temperatures. The melting endotherm of 5-Fluorouracil microspheres was at

135.28°C. These DSC thermograms showed no discernible 5-Fluorouracil endothermic peak in the prepared microspheres. Therefore, it might be concluded that the polymer completely encapsulated the drug.

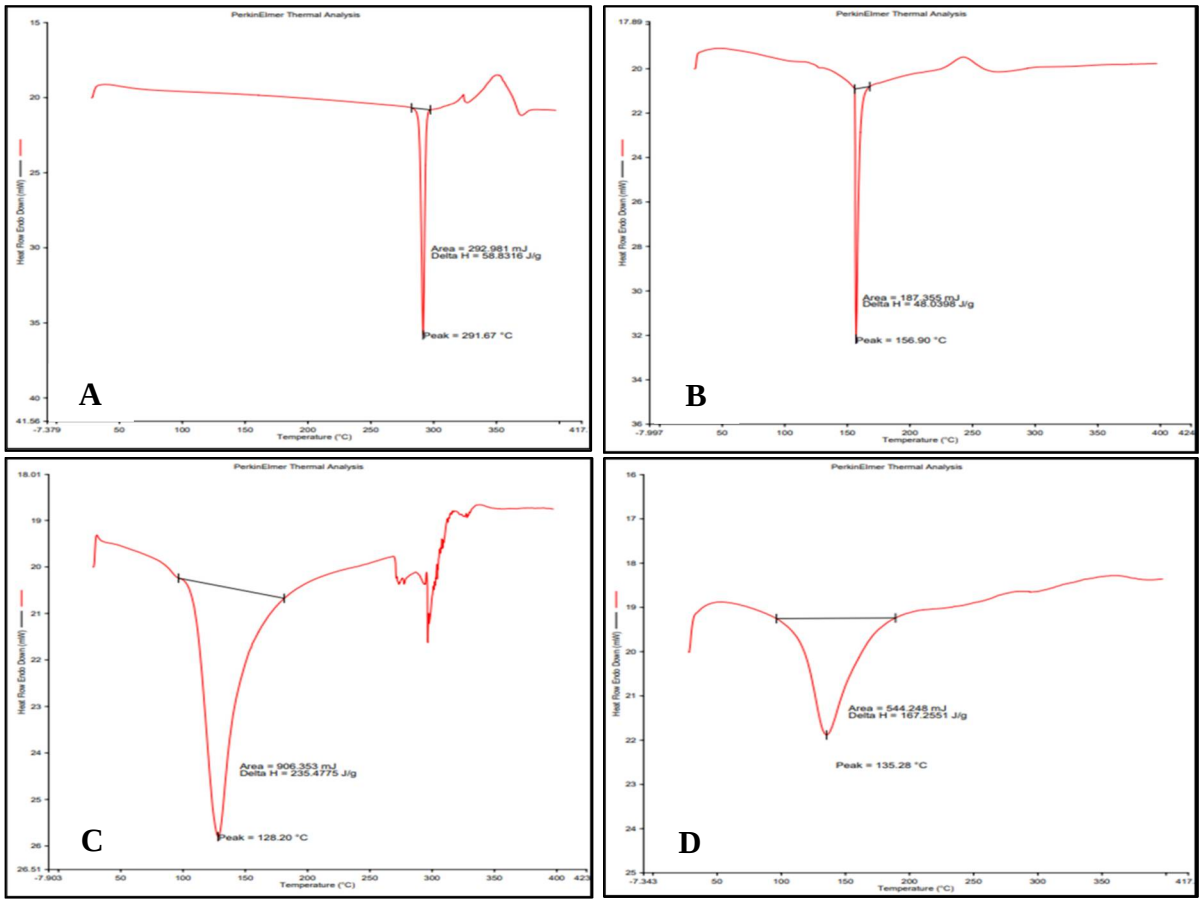

Figure 2. DSC thermogram of (A) 5- Fluorouracil, (B) pectin, (C) resistant starch and (D) 5-Fluorouracil microspheres.

Surface morphology analysis: The prepared microspheres were seen to be spherical in shape on the SEM picture. The surface tension of water on some of the microspheres during dehydration also caused some of them to be bound together. The microspheres that only contained pectin had rough outer surfaces due to the presence of pectin residues on their surface. Microspheres with an exterior surface that was slightly rough were formed when both resistant starch and pectin were used. Figure 3 (A, B) and Figure 4 (A, B) represent SEM images of prepared 5-Fluorouracil microspheres containing only pectin and RS-pectin, respectively, at different magnifications.

Drug loading: The drug loading was observed to be higher when the percentage of polymer was high. In case of microspheres with 50% drug loading, the actual drug loading was slightly lower. It might be due to inefficient drug encapsulation, which allowed drug crystals to stay on the microspheres' outer surface resulting in a low drug content. Low drug yield was observed because the drug crystals on the outer had been removed during the washing stage of the encapsulation procedure. The production yield was also dependent on the polymer adhering to the

beaker wall and stirrer blades during the formation of the microspheres. Similar instances were observed in previous studies (Jagtap *et al*., 2012). The drug loading of microspheres with different polymer concentrations is presented in table 2.

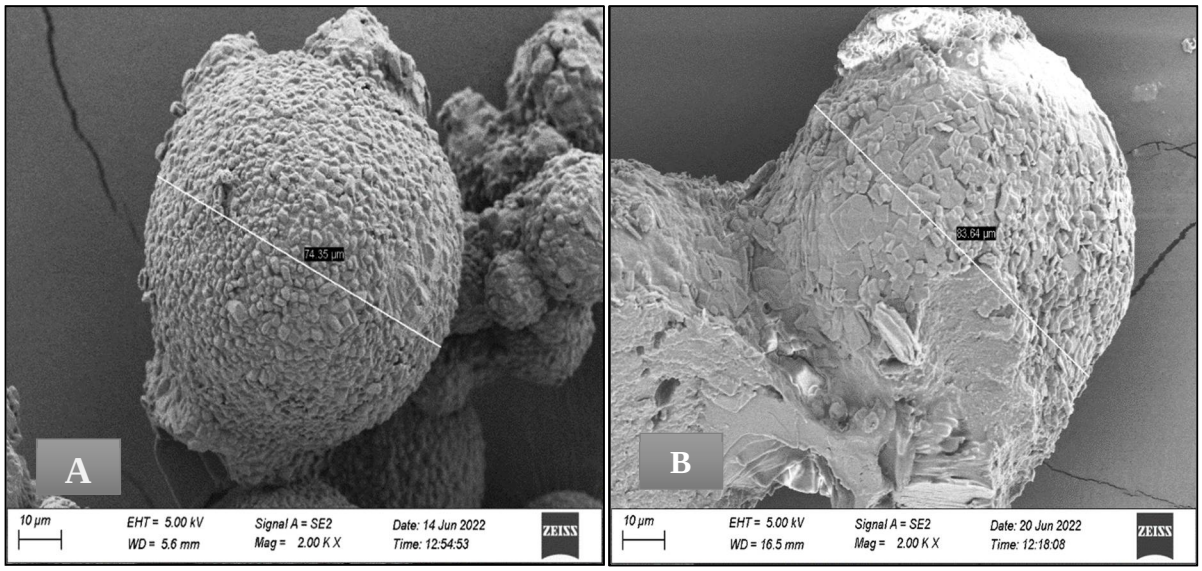

Figure 3. SEM image of 5-FU microsphere containing (A) only pectin and (B) RS-pectin at 2000X magnification.

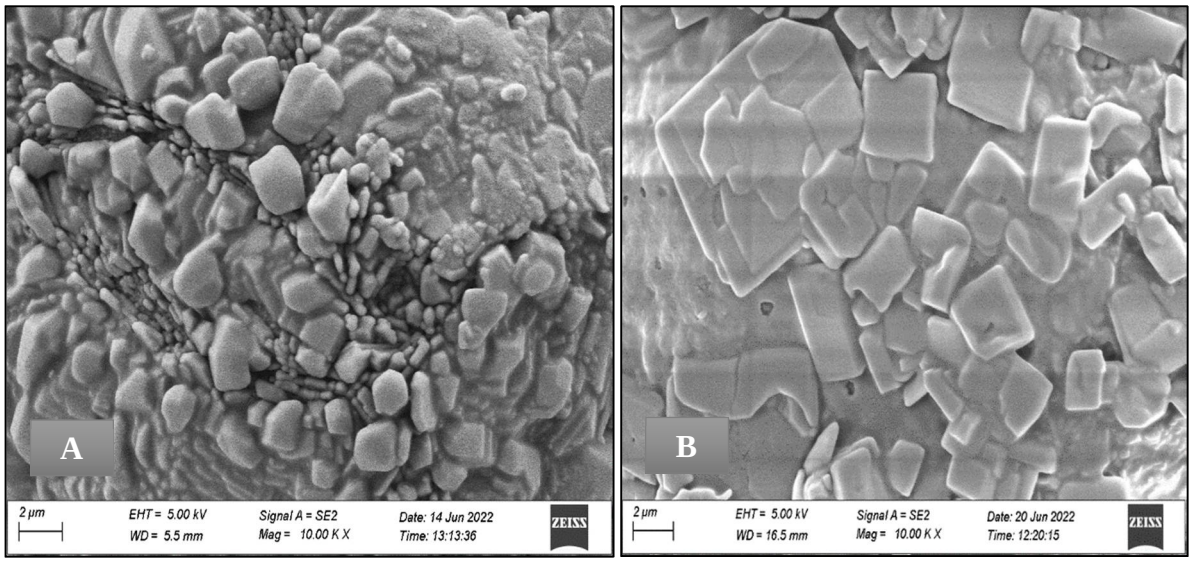

Figure 4. SEM image of 5-FU microsphere containing (A) only pectin and (B) RS-pectin at 10,000X magnification

Encapsulation efficiency: Percent encapsulation efficiency of different formulations is presented in table 2. The encapsulation efficiency was found to be dependent on the nature and composition of polymer used in the formulation. The highest percent encapsulation efficiency was observed at 90.08%. High encapsulation efficiency might be spotted because when the polymer was highly concentrated, it precipitated more rapidly on the external surface of the dispersed phase and hindered drug diffusion

through the phase boundary (Jyothi *et al.*, 2010). The solution became more viscous due to the high concentration, which also slowed down drug dispersion within the polymer droplets (Jyothi *et al.*, 2010).

Table 2. Actual & theoretical drug loading percentages and encapsulation efficiency of prepared microspheres.

Polymer drug ratio	Theoretical drug loading	Actual drug loading (%)	Encapsulation efficiency (%)
Pectin and 5-FU			
1:3	25%	20.39	81.56
1:1	50%	35.47	70.94
3:1	75%	56.4	75.2
Pectin, RS and 5-FU			
1:3	25%	22.52	90.08
1:1	50%	35.27	70.54
3:1	75%	58.04	77.39

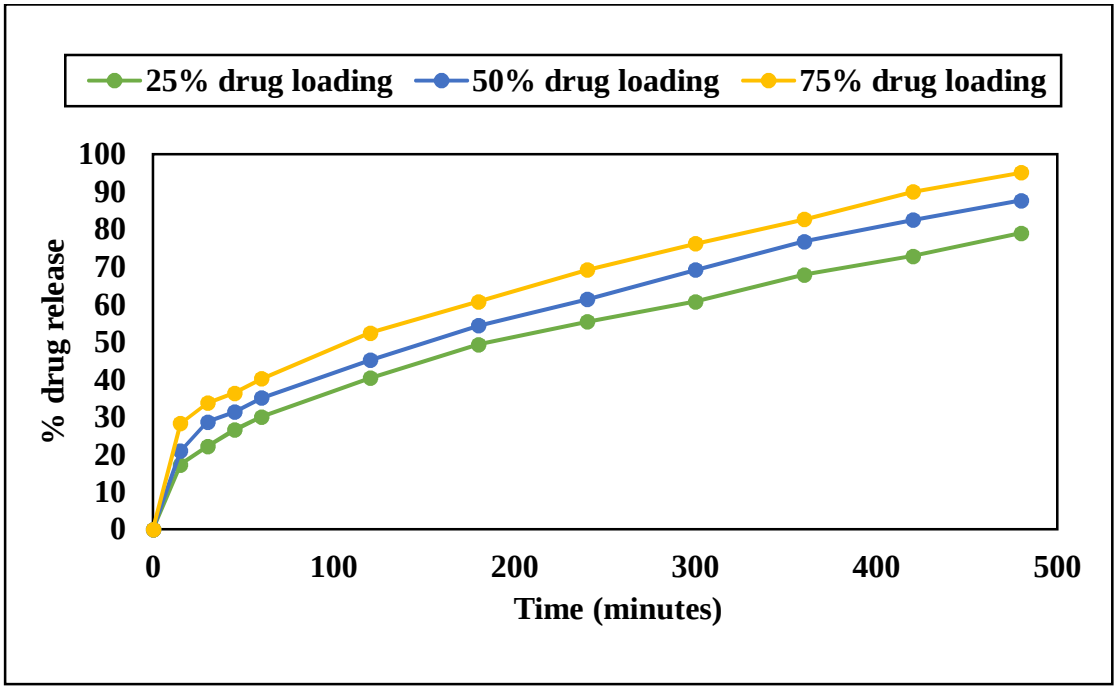

Figure 5. *In vitro* drug release percent of microspheres containing only pectin and 5-FU

The percent release in both cases was decreased with the increasing polymer percentage. It was because a higher proportion of polymer compacted the drug more firmly than a lower proportion of polymer did, and increased the distance that the drug traveled through the microspheres' surface. Drugs that would otherwise be quickly degraded by the body were protected by controlled drug release, and infrequent (or one-time) dosage would replace the repeated ones, boosting patient comfort and compliance (Varde and Pack, 2004).

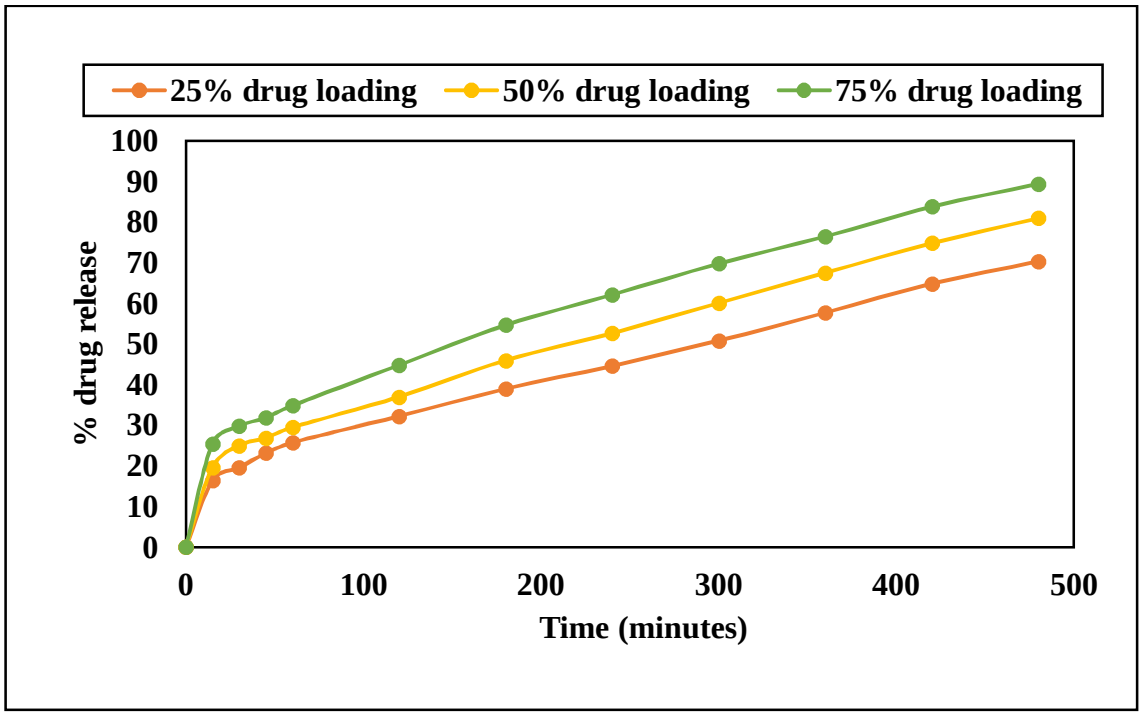

Figure 6. *In vitro* drug release percent of microspheres containing pectin, RS & 5-FU

Conclusion

According to the findings, resistant starch and pectin are potential drug carriers in oral drug delivery to the colon. The study also shows that colon-specific delivery of 5-Fluorouracil might be a valid alternative to the conventional dosage form. By using the solvent evaporation process, microspheres were successfully prepared. Studies on the characteristics of the prepared microspheres such as their shape, drug loading, and encapsulation efficiency and *in vitro* drug release profile of the microspheres explain why they are suitable for targeted drug delivery. The *in vitro* drug release study clarified the drug release profile's dependence on pH. When colonic circumstances were mimicked, a significant amount of the drug was released at pH 6.8 and pH 7.4, but a small amount was released in an acidic medium. The current investigation's findings can confirm the precise formulation ratio that will be able to prevent premature swelling and increase release yield in the colonic pH. However, additional work will be required to make this 5-Fluorouracil colonic delivery method commercially viable.

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