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Development and characterization of pectin-prednisolone microspheres for colon targeted delivery

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Abstract: The purpose of the present study was to obtain a novel microparticulate formulation of prednisolone, which was adequate for the treatment of ulcerative colitis.The formulations prepared were evaluated *in vitro*. Two types of pectin microspheres containing prednisolone named, pectin-prednisolone microspheres (PPMS)and pectin prednisolone eudragit microspheres (PPEMS), were prepared by an emulsion-dehydration technique and o/o solvent evaporation method respectively with some modifications. Various process variables as stirring speed, stirring time, as well as formulation variables i.e. polymer concentration and emulsifier concentration were optimized to get small uniform and spherical discrete microspheres. In vitro drug release studies were performed in presence of simulated gastric fluid, simulated gastric and intestinal fluid, and simulated intestinal fluid respectively, in presence or absence of rat caecal content. By coating the microspheres with eudragit S100 pH dependent release profiles were obtained The cumulative percent drug release of prednisolone from pectin microspheres in SGF and SIF after 4 hrs were varied from 30- 45% and from eudragit coated microspheres after 4 hrs it varied from 6.25 to 8.95% respectively. Further, the release of drug was observed higher in the presence of rat caecal contents, indicating the susceptibility of pectin to colonic enzymes released from rat caecal content.
Key words : colon targeted drug delivery, prednisolone, ulcerative colitis

Introduction:

In the controlled release area, biodegradable microspheres are one of the most useful devices to deliver materials in an effective, prolonged and safe manner. 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 heterosaccharide derived from the cell wall of plants used as a gelling agent for canning purpose. In the experiment pectin is used as a core polymer for drug entrapment. Inflammatory bowel disease (IBD), Ulcerative colitis, colon cancer are severe and chronic. The major cause of IBD is considered to include autoimmune disease and an imbalance of microorganisms. (Kleessen et al.,2002; Pallone et al.,2003). Non steroidal and steroidal anti-inflammatory drugs are frequently used for the treatment of IBD. However, when these drugs are administered in simple conventional oral dosage forms, they are absorbed systemically to a large extent and not delivered efficiently to the diseased site in the gastrointestinal tract. Therefore, various approaches have been examined in an attempt to achieve the efficient delivery of such drugs to the target site. Other novel dosage forms such as bacteria degradable capsules and biodegradable matrix systems have been investigated to achieve a more effective delivery to the colonic region. (Larouche et al.,1995;Tozaki et al.,2002). Pectin is less susceptible to degradation in the gastrointestinal tract than alginate. 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.(Rubinstein et al.1993, Ashford et al.,1994).Thus in the present study, simple pectin microspheres containing prednisolone were prepared by emulsion-dehydration technique and pectin-prednisolone microspheres coated with eudragit S100 were prepared by solvent evaporation method. These two types of microspheres were compared and evaluated. In vitro drug-release studies were performed in conditions simulating stomach to colon transit in presence or absence of rat caecal contents. By the study of release profile of various pectin microspheres and Eudragit coated pectin microspheres it

was observed that these formulations exhibits almost similar release patterns and release rate in the dissolution medium. A very negligible amount of drug was released at acidic pH. However it was observed that where Eudragit S100 starts solubilizing there was continuous release of drug from the formulation. Further it was observed that rate of release was quite higher in presence of rat caecal contents, showing the fact that due to the presence of various anaerobic bacteria in the caecum and they are responsible for digestion/degradation of pectin in order to release drug from microspheres.

Materials:

Pectin was procured from Himedia Laboratories Pvt. Ltd., Mumbai. Span 85, Acetone and isooctane were procured from CDH, Mumbai. The drug Prednisolone was purchased from Otto Kemi, Mumbai.

Method:

The pectin microspheres were prepared by emulsion-dehydration technique as reported by Esposito et al. Pectin and Prednisolone were dissolved in 20 ml of water and stirred overnight to solubilize completely. Drug-Polymer ratio (w/v) was used as 1:3, 1:4, 1:5 and 1:6. This drug-polymer solution was dispersed in 50 ml isooctane containing 1.25% w/v of span 85 and stirred continuously to assume stable emulsion. The solution was rapidly cooled at 15˚C and then 50 ml of acetone were added in order to dehydrate the pectin droplets. This system was maintained under mechanical agitation with propeller stirrer at 1000 rpm at room temperature for 20-40 minutes to allow the complete solvent evaporation. The microspheres were washed with acetone, collected and freeze dried overnight and kept in airtight container for further studies.

Optimization:

Various formulation variables e.g. drug concentration, polymer concentration, emulsifier concentration and process variables viz. stirring speed, stirring time, which would affect the preparation and properties of microspheres were identified and studied. The composition of formulation code of designed formulae of pectin microspheres are given in table 1.

Optimization of formulation variables:

Various formulation variables were tried to prepare microspheres viz. drug concentration: 0%, 1%, 2%, and 3% (table 1), pectin concentrations: 3%, 4%, 5%, 6% (table 1), and emulsifier concentrations: 0.75%, 1%, 1.25%, 1.5% (table 1) were optimized. The effects of drug concentration, pectin concentration and emulsifier concentration on the particle size, shape, size distribution and total drug loading efficiency are reported in table 1 and figure 1.

Optimization of process variables:

Various process variables that could affect the preparation and properties of final preparations were optimized i.e. stirring speed: 500, 1000, 1500 and 2000 rpm (table 1) and stirring time: 20, 30, and 40 minutes (table 1). Effects of these variables were observed on final particle size, size distribution and shape of microspheres and total drug loading efficiency are reported in fig 2, 3 & 4.

Drug Content

The amount of Prednisolone associated with the microspheres was analyzed in terms of surface adsorbed drug and entrapped drug.

Estimation of surface drug in microspheres:

100 mg of microspheres was dispersed in 10 ml of PBS (pH 7.4) and shaken vigorously for 10 minutes and supernatant was kept aside. Similarly, the sediment was again treated in the same manner and second supernatant was mixed with first supernatant and analyzed for prednisolone content spectrophotometrically as described previously. The amount of prednisolone in the mixed washings gave the amount of drug adsorbed on the surface of the microspheres.

Estimation of entrapped drug in microspheres:

The microspheres obtained after two washings were digested in 10 ml of pectinase solution (4% w/w) for 12 hr. the digested homogenate was assayed for prednisolone, spectrophotometrically. The percent drug entrapped was calculated and reported in table 1.

Eudragit coating of Pectin Microspheres

The coating of Eudragit S100 on pectin microspheres was done by oil-in-oil solvent evaporation method as reported by Lorenzo-Lomora *et al.*

50 mg of pectin microspheres were dispersed in minimum amount of organic solvent (acetone and ethanol) in which Eudragit S100 was previously dissolved to give 10:1 coat: core ratio. This organic phase was then poured in light liquid paraffin containing 1% w/v Span 85. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-Hexane and dried for 24 hrs. All prepared pectin microspheres were coated with Eudragit S100.

Optimization

The core: coat ratio was optimized in terms of particle size, shape and size distribution. The core: coat ratio was varied from 1:5 to 1:10. The results are given in table 1. and graphically presented in fig 5 – 8.

Characterization of prepared Microspheres

The prepared microspheres were characterized for shape morphology, size and size distribution, percent drug loading and total drug loading efficiency, swellability, invitro digestion and in vitro drug release.

1. Shape and surface morphology

Microspheres were suspended in water; a drop was placed on a glass slide, covered with a cover slip and viewed under the optical microscope to examine their shape. The photomicrographs of different microspheres were taken as shown in photograph 1.

In order to examine the surface morphology, the formulations were viewed under scanning electron microscope. The samples for SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to thickness of about 300 Å using a sputter coater. The photomicrographs were taken with the help of SEM analyzer.

2. Size and Size Distribution

Microspheres were studied microscopically for their size and size distribution using calibrated ocular eyepiece. Effect of drug concentration, polymer concentration, emulsifier concentration, stirring rate and stirring time on particle size, shape and size distribution were studied on pectin microspheres. The observations are recorded in the table 1. Effect of core: coat ratio was also studied foe Eudragit coating on pectin microspheres.

3. Swellability / Degree of Swelling

As pectin is soluble in water, may swell and dissolve in aqueous GIT fluids in the upper part of GIT and may release the drug there before reaching the colon. Therefore pectin microspheres were coated with Eudragit S100 to prevent the dissolution of pectin with consequent drug release in upper GIT. Therefore the effect of Eudragit coating on swellability of microspheres was studied. The swelling ability of the microspheres on physiological media was determined by suspending them in the PBS buffer (pH 7.4). Accurately weighed amount of microspheres was immersed in a little excess of PBS (pH 7.4) and allowed to swell up to constant weight.

The swelling of pectin microspheres is influenced by the extent of cross-linking. The formula used for calculation of swelling of various microspheres is as follows:

$\alpha = [\omega_g - \omega_o]/\omega_o$

where, α = degree of swelling

ω_g = initial weight of microspheres

ω_o = final weight of microspheres

4. In Vitro Digestion Study of Microspheres

This was carried out to ensure 100% delivery of drug in colon. This was done by incubating 200 mg of microspheres in 100 ml of simulated colonic fluid containing 2% of rat caecal content for 48 hrs. Two ml sample was withdrawn, diluted appropriately and estimated spectroscopically for the amount of Prednisolone released.

5. In Vitro Drug Release Studies in Simulated Gastrointestinal Fluids of Different pH

All formulations of pectin and eudragit coated pectin microspheres were evaluated for the in vitro drug release study. The drug dissolution test of microspheres was carried out by the paddle method specified in USP XXIII. 100 mg of microspheres equivalent to 13.78 mg Prednisolone was weighed accurately and gently spread over the surface of 900 ml of dissolution medium as specified in the IP 1996. The content was rotated at 100 rpm and thermostatically controlled at 37±.5˚C. Perfect sink condition was prevailed during the drug dissolution.

The effect of different a) polymer concentrations, b) emulsifier concentrations, c) stirring speed and d) stirring time on the drug release was studied in simulated gastrointestinal fluids of different pH in the following sequence, in order to mimic mouth-to-colon transit:

- In simulated gastric fluid (pH 1.2) – 2^{nd} hr,
- Mixture of simulated gastric and intestinal fluid (pH 4.5) 3^{rd}-4^{th} hr,
- Simulated intestinal fluid (pH 7.5)/ simulated colonic fluid (pH 7.0) 5^{th}-8^{th} hr.

The medium was filtered through Whatmann filter paper after 2 and 4 hours and the residue on filter paper was added to the next medium immediately. The dissolution study was continued further and samples were withdrawn at suitable time intervals from the dissolution vessel and assayed spectrophotometrically at 265.6 nm for prednisolone.

6. In Vitro drug Release Study from microspheres (coated and uncoated) in the presence of Rat Caecal Content

To overcome the limitations of conventional dissolution testing for evaluating the performance of colon specific drug delivery systems triggered by colon specific bacteria, rat caecal contents has been utilized as an alternative dissolution medium, called rat caecal content medium or simulated colonic fluid.

This medium was prepared by the method as reported by Van den Mooter et al. (1994). Rats weighing 150-200 g were weighed, maintained on normal diet and administered 1 ml of 2% dispersion of pectin/Eudragit S100 in water and this treatment was continue for 7days for polymer induction to animals. Thirty minutes before starting the study, the rat was sacrificed, the abdomen was opened, ligature was made before and after the caecum, and the caecum was removed. Subsequently under anaerobic conditions, the caecum was isolated and immediately transferred into PBS (pH 7.4), which was previously bubbled with CO_2. the caecum bag was opened, contents were weighed and homogenized and then suspended in PBS (pH 7.0) to give the desired concentration (1%, 2%, 3%, and 4%) of caecal contents and was used as simulated colonic fluid. The suspension was filtered through cotton wool and sonicated (50 watt) for 20min at 4˚C to disrupt the bacterial cells. After sonication, the mixture was centrifuged at 2000 rpm for 20 min. As the environment in caecum is naturally

anaerobic, all the operations were carried out under CO_2 atmosphere.

Formulations PPMS-P2, PPMS-E4, PPMS-R2, PPMS-T1 of pectin and EPMS-P2, EPMS-E4, EPMS-R2 and EPMS-T2 of Eudragit coated microspheres were selected for drug release study in the presence of caecal content. The drug release study was carried out in sealed glass vials at 37±0.5˚C. The 100 mg weighed amount of microspheres was placed in the 20 ml dissolution media (PBS of pH 7.0 containing 1%, 2%, 3% and 4% rat caecal contents). The PBS (pH 7.0) containing same concentration of caecal contents with placebo microspheres served as blank. The vials were shaken and then samples (0.2 ml) were withdrawn after a fixed time interval of 1, 2, 3…..8 and 24 hrs and volume of dissolution medium was replaced with PBS (pH 7.0). The withdrawn samples were centrifuged at 2000 rpm for 10 min, the supernatant was filtered through Whatmann filter paper and filtrate was analyzed for prednisolone content spectrophotometrically. The percent drug released was calculated and reported graphically fig 5-8.

Table 1 : Effect of various parameters on particle shape, size, size distribution and total drug loading efficiency of microspheres .

Formulation	Average Diameter (μm)	Shape	Entrapped Drug (mg)	Total drug loading efficiency (%)
Drug concentration				
PPMS-D0 (0:4)	25.10	Spherical	0	0
PPMS-D1 (1:4)	25.31	Spherical	13.34	68.93
PPMS-D2 (2:4)	26.31	Spherical	19.67	64.14
PPMS-D3 (3:4)	30.44	Spherical	21.42	52.29
Polymer concentration				
PPMS-P1 (1:3)	25.11	Spherical	13.60	70.15
PPMS-P2 (1:4)	26.64	Spherical	14.48	74.85
PPMS-P3 (1:5)	29.17	Spherical	14.25	73.55
PPMS-P4 (1:6)	29.47	Spherical	14.14	72.95
Emulsifier Concentration				
PPMS-E1 (0.75%)	30.30	Spherical	13.69	70.65
PPMS-E2 (1.00%)	28.77	Spherical	13.62	70.35
PPMS-E3 (1.25%)	25.24	Spherical	13.51	69.80
PPMS-E4 (1.50%)	24.70	Spherical	13.47	69.40
Stirring Speed				
PPMS-R1 (500 rpm)	31.83	Irregular	14.13	72.95
PPMS-R2 (1000 rpm)	27.71	Spherical	14.18	73.35
PPMS-R3 (1500 rpm)	27.30	Spherical	13.64	70.15
PPMS-R4 (2000 rpm)	25.11	Spherical	13.42	69.15
Stirring Time				
PPMS-T1	28.37	Irregular	13.97	72.10
PPMS-T2	25.51	Spherical	13.88	71.60
PPMS-T3	27.04	Spherical	13.68	70.35
Core-Coat Ratio				
EPMS-1 (1:5)	162.90	Coating insufficient	-	-
EPMS-2 (1:10)	168.49	Uniform coating	-	-

Result and Discussion:

Pectin microspheres of prednisolone were successfully prepared by emulsion dehydration technique as reported by Esposito et al., (2001). The microspheres produced were generally spherical, discrete upon dispersions in an aqueous medium and having uniform size ranges from 25 to 32 µm. The photomicrographs of microspheres are shown in photograph 1, 2 & 3.

Solution of drug and polymer in distilled water was finally dispersed into isooctane to form discrete droplets, thereby forming a w/o emulsion. Pectin has the disadvantage of swelling and dissolving rather rapidly in aqueous environment, making difficult the use of this polymer for the production of long-acting delivery systems.

The pectin microspheres were coated with Eudragit S100 oil-in-oil solvent evaporation method as reported by Lorenzo-Lamosa et. al., (1998). Different core: coat ratios were taken to optimize the final formulation. Te final product was finally dried for 24 hours. The photomicrographs of coated microspheres are shown in photograph 3

The effect of various process variables viz. stirring time and stirring speed and formulation variables e.g. drug concentration, polymer concentration and emulsifier concentration were studied. The results suggested that these variables influence the shape, size, size distribution, swellability, total drug loading efficiency and in vitro drug release of the final preparation. Hence these parameters were optimized to prepare microspheres of small size with narrow size distribution, good drug loading efficiency and good drug release at the colonic pH.

For the total drug loading efficiency, the microspheres were digested with pectinase enzyme and subsequently prednisolone was extracted with PBS (pH 7.4) and drug extract was determined spectroscopically.

The microscopic examination revealed that the mean diameter of pectin microspheres varied from 25.10 µm to 30.44 µm with varying concentration of prednisolone from 0% w/v to 1% w/v.

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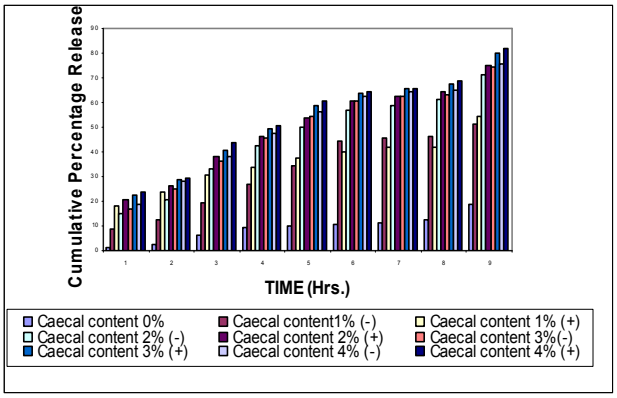


Fig.1 comparative cumulative percentage release with different concentration of caecal content

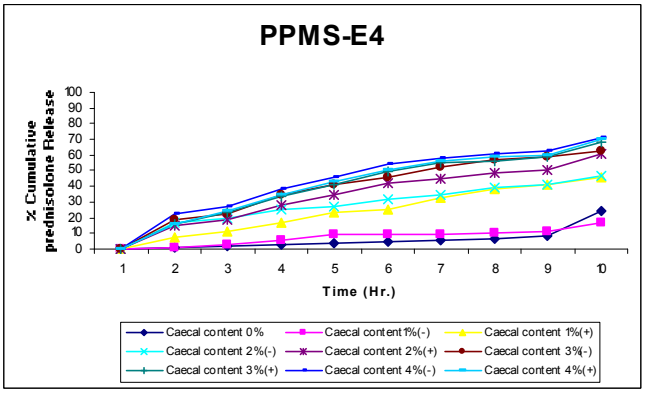


Fig.2 comparative cumulative percentage release with optimum emulsifier concentration and different percentage of caecal content

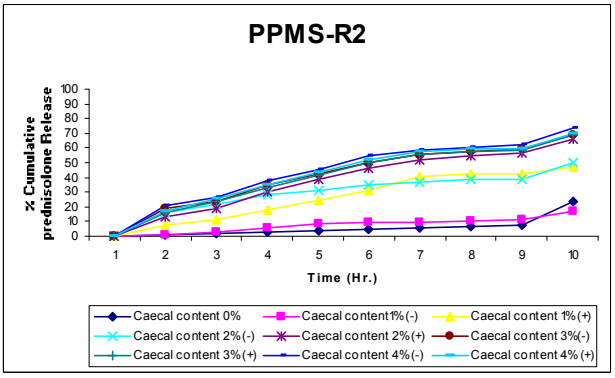


Fig.3 comparative cumulative percentage release with optimum Stirring Speed and different percentage of caecal content

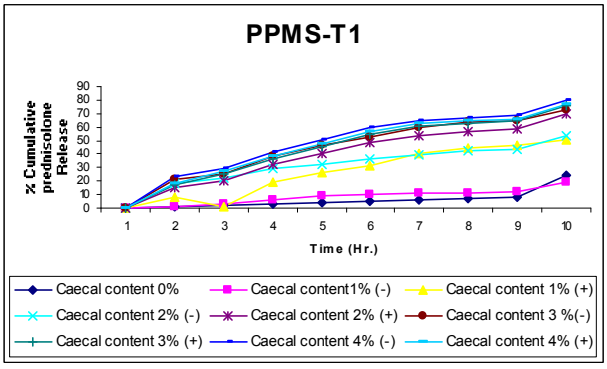


Fig.4 comparative cumulative percentage release with optimum Stirring Time and different percentage of caecal content

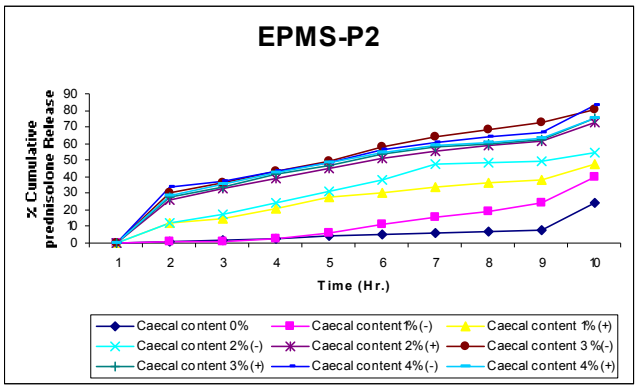


Fig.5 comparative cumulative percentage release of Eudragit coated Pectin microspheres with optimum polymer concentration and different percentage of caecal content

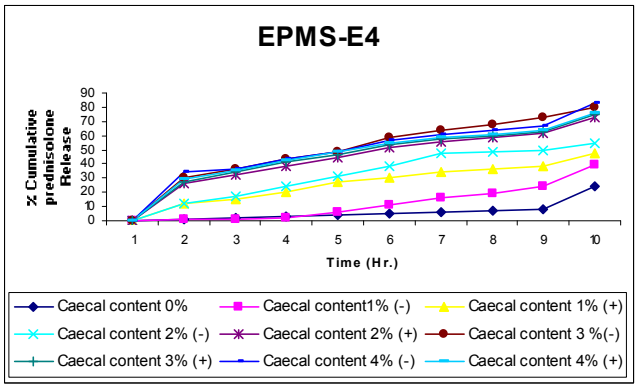


Fig.6 comparative cumulative percentage release of Eudragit coated Pectin microspheres with optimum emulsifier concentration and different percentage of caecal content

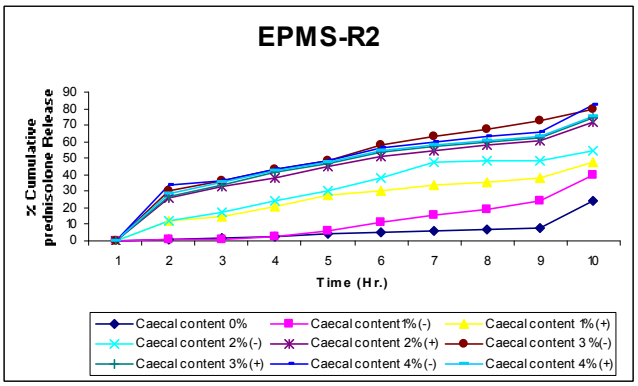


Fig.7 comparative cumulative percentage release of Eudragit coated Pectin microspheres with optimum Stirring Speed and different percentage of caecal content

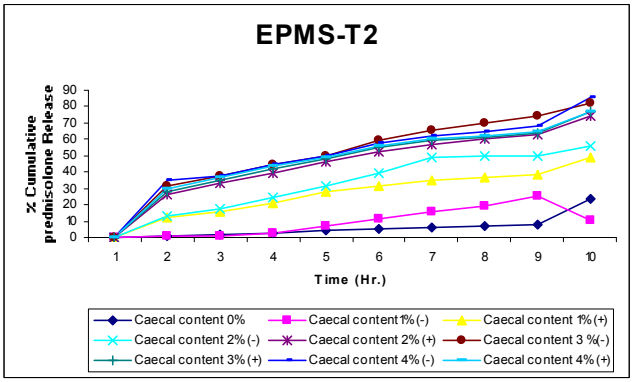
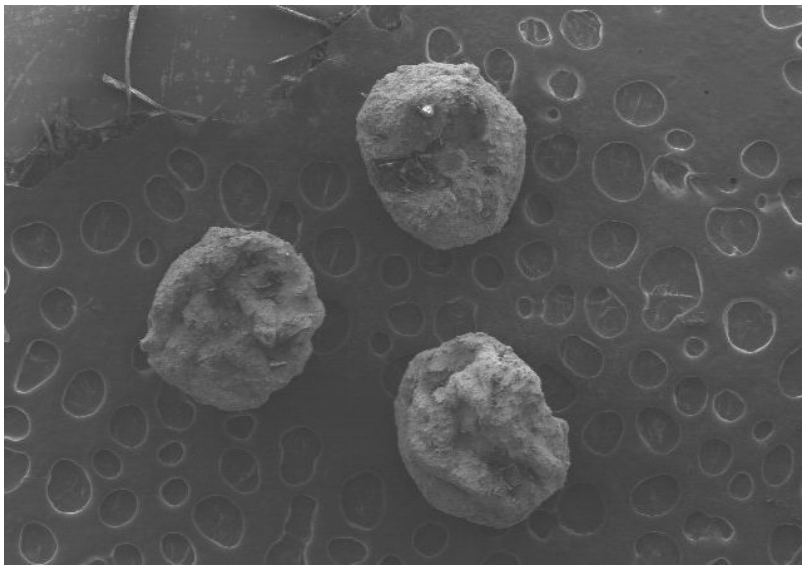
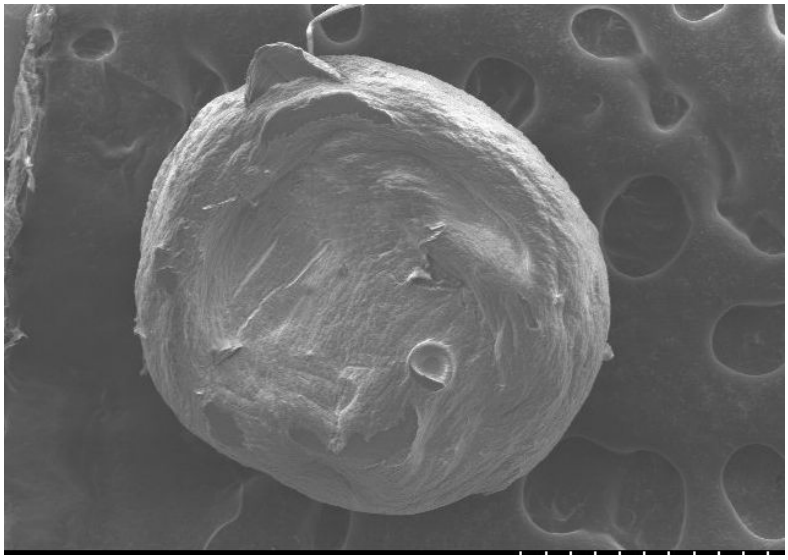


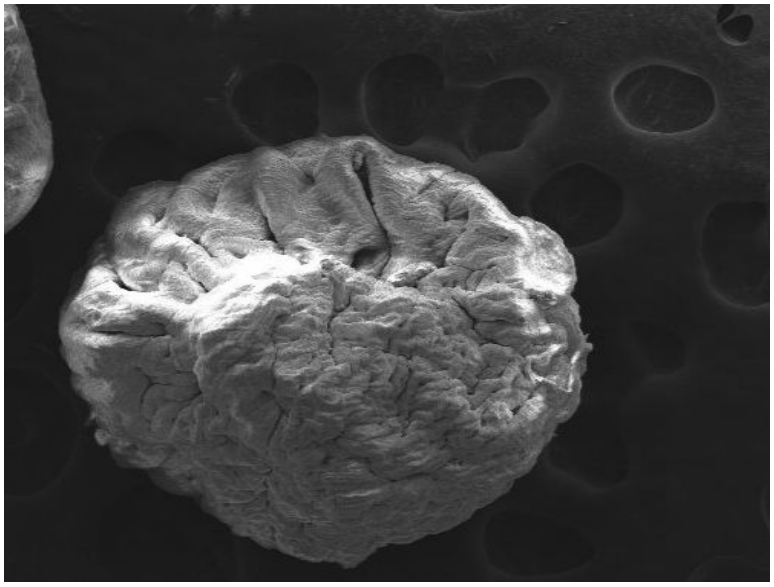
Fig.8 comparative cumulative percentage release of Eudragit coated Pectin microspheres with optimum Stirring time and different percentage of caecal content



Photograph 1



Photograph 2



Photograph 3

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