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Preparation and characterization of pectin pellets of Aceclofenac for colon targeted drug delivery

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Abstract

The present study objective was to develop novel colon specific drug delivery systems for aceclofenac using pectin as a microbially degradable polymeric carrier and to coat the optimized batches with a pH dependent polymeric coating solution containing Eudragit L 100 and S 100 (1:4). Pellets containing four proportions of pectin were prepared. The pellets were evaluated for physicochemical properties, drug content, dissolution, water uptake & erosion characteristics, in vitro drug release studies. The amount of aceclofenac released from the pectin pellets at different time intervals was estimated by UV spectrophotometric method at 275nm. Eudragit coated pectin pellets prevented release of the aceclofenac in the physiological environment of stomach and small intestine depending on the proportion of pectin used in the formulation. The dissolution profile and *in vitro* release kinetics showed that pectin pellets were promising for controlled delivery of the drug. The findings of the present study conclusively state that pectin pellets are promising for colon targeting of aceclofenac to synchronize the chronobiological symptoms for effective treatment of rheumatoid arthritis.

Key Words: Aceclofenac, pectin, pellets, eudragit coating, targeted delivery, colon targeting.

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) and rheumatoid arthritis

and can be effectively treated by the local delivery of drugs to the large intestine. Targeting of drugs to the colon via the oral route can be achieved by different approaches including different formulation systems, for which the drug release is controlled by different pH conditions, transit time, and intestinal microbial flora [1]. Specific targeting of drugs to the colon is recognized to have several therapeutic advantages. The colon is an ideal site for both systemic and local delivery of drugs [2]. To reach the colon and absorb the drug there the dosage forms must be formulated taking into account the obstacles of the gastrointestinal tract (GIT). Various strategies have been developed to achieve this goal, such as, use of specific characteristics of the organ, for example, pH, microbial flora, enzymes, reducing medium, and transit time [3]. A number of serious diseases of the colon, for example, colorectal cancer, ulcerative colitis, and other inflammatory conditions could be treated more effectively if drugs are targeted to the colon[4],[5]. The colonic site is being investigated as a potential site for the delivery of proteins, peptides, vaccines, and other drugs such as nifedipine, theophylline, and isosorbide [6], [7]. Due to a comparatively longer transit time than in the stomach, colonic absorption of poorly absorbed drugs can be improved[8]. Methods for drug delivery to the colon have recently been discussed [9].

The presence of certain enzymes in the colon, which have been involved to specifically cleave certain types of drugs attached to another molecule or a polymer, has been studied by a number of authors [10]. Colon cancer, one of the serious diseases, can also be treated by means of effective targeting of anticancer drugs to the colonic region [11]. The pH of the GI tract gradually increases as it moves down the GI tract from the stomach (pH 1.5-3) to the terminal ileum (pH 7-8). Khan *et al*. developed a single coating system for mesalazine based on the combination of polymers [12]. Optimization of two factors (coating composition and thickness) is useful to achieve the best pH-dependent colonic drug delivery [13].

The utilization of enzymes produced by the bacteria residing exclusively in the colon is a means of obtaining site specific delivery to this region. Dosage forms have been prepared from [14], or coated with [15] selected polysaccharides to achieve this aim. The polysaccharide, pectin, has been extensively investigated [16]. To overcome the problem of dissolution of pectin in the upper gastrointestinal tract (GIT), relatively thick compression coats have been used [17] or the pectin has been combined with an insoluble polymer, such as ethylcellulose, to produce a film coat [18]. Site-specific targeting of drugs to the colon has been attempted by several different approaches [19]. Of these, utilisation of the bacterial population, existing almost exclusively in the colon, as a means of targeting offers considerable promise [20]. Multiple-unit systems have been shown to spread out on entry to the colon [21] and this may give improvements in drug absorption and local treatment. Additionally, the higher surface area of multiple unit systems should lead to a more rapid release of drug due to more rapid bacterial breakdown. The transit of dosage forms in the upper gastrointestinal tract is also a consideration in colonic delivery as delays expose the material to longer periods of time in a harsh environment. In this regard also, multiple-unit systems may empty from the stomach and traverse the ileo-caecal junction in a more reproducible manner than single units.

The potential of pectin as a carrier for colonic drug delivery has been demonstrated previously. The use of high-methoxy pectin or cross-linking with calcium have been investigated as methods

for reducing the inherent solubility. An alternative approach is to form an interpolymer complex with pectin in a similar manner to that investigated by Meshali and Gabr [22].

Aceclofenac, a non steroidal anti-inflammatory drug used for the treatment of rheumatoid arthritis is selected as a model drug. The aim of the study was to design a novel colon specific drug delivery systems containing pectin pellets coated with pH dependent polymers (Eudragit L 100 and S 100 in the ratio of 1:4). The goal in drug delivery research is to develop formulations to meet therapeutic needs related to particular pathological conditions. Variation of physiological and pathophysiological functions at a particular time of a day has brought a new approach to the development of drug delivery systems [23]. Rheumatoid arthritis (RA) is traditionally considered a chronic, inflammatory autoimmune disorder that causes the immune system to attack the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction [24].

Multiparticulate approaches tried for colonic delivery include formulations in the form of pellets, granules, microparticles and nanoparticles. The multiparticulate drug delivery systems are used in preference to single unit dosage forms for colon targeting. The multiparticulate systems enabled the drug to reach the colon quickly .The purpose of designing multiparticulate dosage form is to develop a reliable formulation that has all the advantages of a single unit formulations [25].

Materials and Methods

Materials

Materials used included aceclofenac, which was kindly provided as a gift sample by Restek Pharma, Pondicherry. pectin was purchased from Indian Research Products, Chennai. Eudragit S 100 and Eudragit L 100 were purchased from Loba chemicals, Mumbai. All other chemicals were of analytical grade.

Preparation of pellets

Pellets were prepared using a power-consumption-controlled, twin-screw extruder (type ZE25x18D, Berstorff AG, Hannover, Germany) at a power consumption of 180 W [26]. The extruder had 48 dies 1 mm in diameter and 2.5 mm in length. The powder feed rate was 25 g to 26 g depending on the formulation. The extruded mass was rounded in a spheronizer (Type S-320, Nica, Molndal, Sweden) with a crosshatched plate 320 mm in diameter at 800 rpm for 5 minutes. The pellets were dried in a fluid-bed dryer at 50°C for 30 minutes (Glatt, Binzen, Germany). The four batches of pectin pellets(PP1,PP2,PP3,PP4) were prepared with increasing concentration of polymer pectin of 22%, 35%, 45% and 51% relative to total tablet weight

Step II

Coating of pellets

The pellets were coated with a combination of Eudragit L-100 and S100 in a fluidized bed coating apparatus. (Walther ‘bingo’ Air spray gun). In-process samples at various coating levels 10, 25 % w/w (% polymeric weight gain) were taken to check the morphology of coating to do dissolution studies in SGF fluid. Coating was continued until complete polymer weight gain was

achieved. After the coating, the pellets were gently fluidized for about 5 min after which they were cured in an oven for 24 h at 40°C. A 25 % w/w increase in the coating level was selected as an optimum coating percentage level for all the pellets namely, pectin. Then the pH dependent polymeric coated pellets were tested for drug release studies as described above in SGF, SIF and SCF separately [27].

Preformulation studies

Differential scanning calorimetry

In this technique the difference in energy inputs into a substance and reference material is measured as a function of temperature as the specimens are subjected to controlled temperature program.

Fourier transforms Infrared spectroscopy

The Fourier transform infra-red analysis was conducted for the structural characterization. FTIR of pure drug, polymers, and their physical mixtures were recorded. Samples were taken in a KBr pellet using BOMEN MB SERIES FTIR instrument.

Standard plot of aceclofenac in pH 7.0 phosphate buffer saline

100 mg of aceclofenac (standard drug) was accurately weighed and dissolved using pH 7.0 phosphate buffer saline(PBS) solution in 100 ml standard flask and 5, 10, 15, 20 and 25 µg/ml were prepared by suitably diluting the stock solutions with pH 7.0 PBS, each sample was then analysed spectrophotometrically at 275 nm using ELICO SL -159 double beam UV Visible spectrophotometer.

Evaluation of granules

Determination of bulk density and tapped density

An accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume (Vo) was measured. Then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 tabs and after that, the volume (V_f) was measured and continued operation till the two consecutive readings were equal. The bulk density, and tapped density were calculated using the formulae

$$\textbf{Bulk density} = W / V_0$$

$$\textbf{Tapped density} = W / V_f$$

Where,

W= Weight of the powder

V_0 = Final volume

Compressibility index (Cars indices)

Compressibility index was calculated by using the formula

$$Ci = \frac{(V_0 - V_f)}{V0} \times 100$$

Ci < 15 % shows good flow property
Ci > 25 % shows poor flow property
Ci >50 % shows great potential problems.
Ci 20 %- 40 % shows reasonable flow property.

Hausner's Ratio
Hausner's ratio was measured by the ratio of tapped density to bulk density [28].
Hausner's ratio = Tapped density/ Bulk density

***In Vitro* Drug Release Studies in Simulated Fluids**
Pellets were evaluated for the in vitro drug release in simulated GI fluids (SGF). The drug dissolution test of pellets was performed by the paddle method specified in USP XXIII. Pellets were dissolved over the surface of 500 mL of dissolution medium (SGF). 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 hours then, the pH of the dissolution medium was adjusted to 7.4 and maintained up to 24 hours. The samples were withdrawn from the dissolution medium at various time intervals using a pipette fitted with a microfilter. The receptor volume was maintained constant by replacing equivalent amount of SGF and SIF. The concentration of aceclofenac in the samples was calculated based on average calibration curves (n = 6). All dissolution studies were performed in triplicate [29].

***In Vitro* Drug Release Study in the Presence of Rat Cecal Content**
Rat cecal content was prepared by the method reported by Van den Mooter et al. Four albino rats of uniform body weight (150-200 g) with no prior drug treatment were used for all the present *in vivo* studies. They were weighed, maintained on normal diet, and administered 1 mL of 2% dispersion of pectin in water, and this treatment was continued for 7 days for polymer induction to animals. Thirty minutes before starting the study, each rat was humanely killed and the abdomen was opened. The cecal were traced, legated at both ends, dissected, and immediately transferred into phosphate buffered saline (PBS) pH 6.8, which was previously bubbled with CO_2. The cecal bag was opened, the contents were weighed, homogenized and then suspended in PBS (pH 7.4) to give the desired concentration (2%) of cecal content, which was used as simulated colonic fluid. The suspension was filtered through cotton wool and ultrasonicated for 10 minutes in an ice bath at 40% voltage frequency using a probe sonicator (Soniweld, Imeco Ultrasonics, Mumbai, India) at 4°C to disrupt the bacterial cells. After sonication, the mixture was centrifuged (Remi) at 2000 rpm for 20 minutes.

Pellets were placed in 200 mL of dissolution media (PBS, pH 7.4) containing 2% w/v rat cecal content. The experiment was performed with continuous CO_2 supply into the dissolution medium. At different time intervals, the samples were withdrawn and replaced with fresh PBS. The experiment was continued up to 24 hours. The withdrawn samples were pipetted into a series of 10 mL volumetric flasks, and volumes were made up to the mark with PBS and centrifuged. The supernatant was filtered through 0.45-µm membrane filter and the filtrate

analyzed for aceclofenac content at 275 nm using UV spectrophotometer method. All the experiments were performed in triplicate [30].

Stability studies

The selected formulation of pellets were stored in amber-colored glass bottles at 45ºC+75% RH for a period of 3 months and observed for any change in colour, odour, and percentage drug content and entrapment efficiency [31].

***In vitro* release kinetics**

The *in vitro* drug release data were fitted to various release kinetic models. viz. first order, zero order model, Higuchi and Korsemeyer - Peppas equation. The goodness of fit was found out from the above mathematic models [32].

Results

DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and polymers in formulations. According to the thermograms, aceclofenac presented a sharp endothermic peak at 158.3ºC corresponding to the melting point of the drug in the crystalline form. While the thermogram of physical mixture of aceclofenac and Pectin was 160ºC.

However a endothermic peak observed for Pectin at 91.5ºC. Thus the thermograms of physical mixture showed that drug was in its crystalline form and also there is no interaction between the aceclofenac and the polymer .The DSC graphs are given in fig. 1-3.

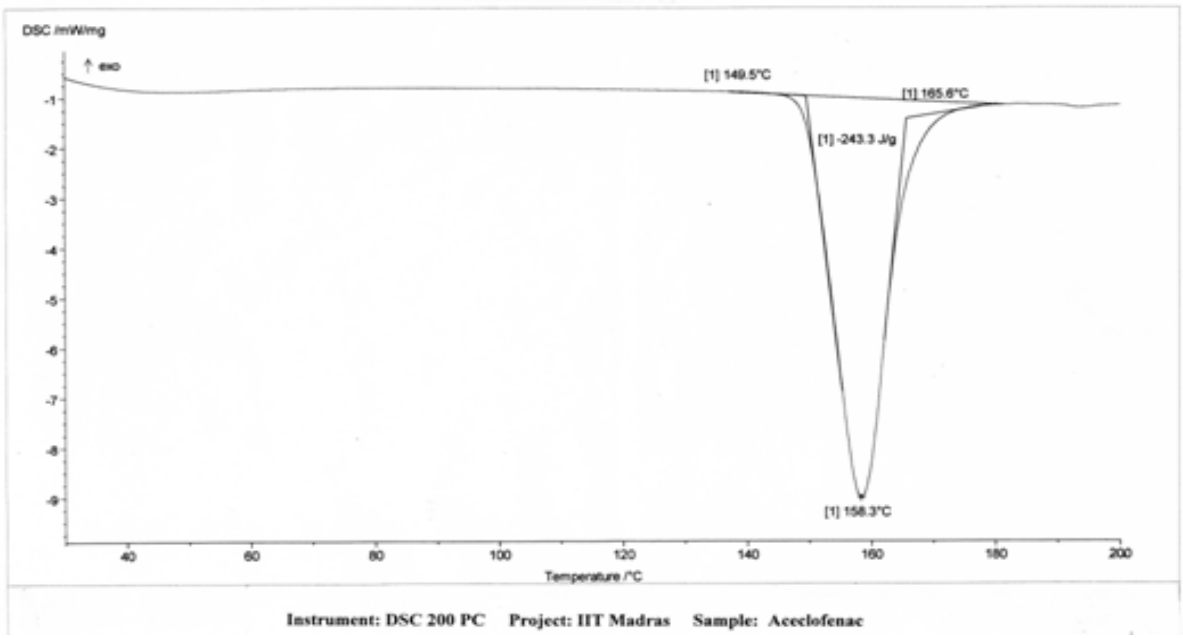

Fig.1 DSC of Aceclofenac

IR studies

FTIR spectroscopy was used to ensure that no chemical interaction between the drugs and polymers had occurred. From the FTIR spectral interpretation the following result were obtained. The FTIR of aceclofenac show intense band at 1771.47 cm^{-1}, 1716.89 cm^{-1} , 1589.53 cm^{-1} and 1055.9 cm^{-1} corresponding to the functional groups C=O, COOH, NH and OH bending. The peaks observed in FTIR of physical mixture of aceclofenac and pectin was found to be at 1771.62 cm^{-1}, 1716.76 cm^{-1}, 1589.84 cm^{-1}, 1055.88 cm-1 respectively. The IR graphs were given in fig.4-6.

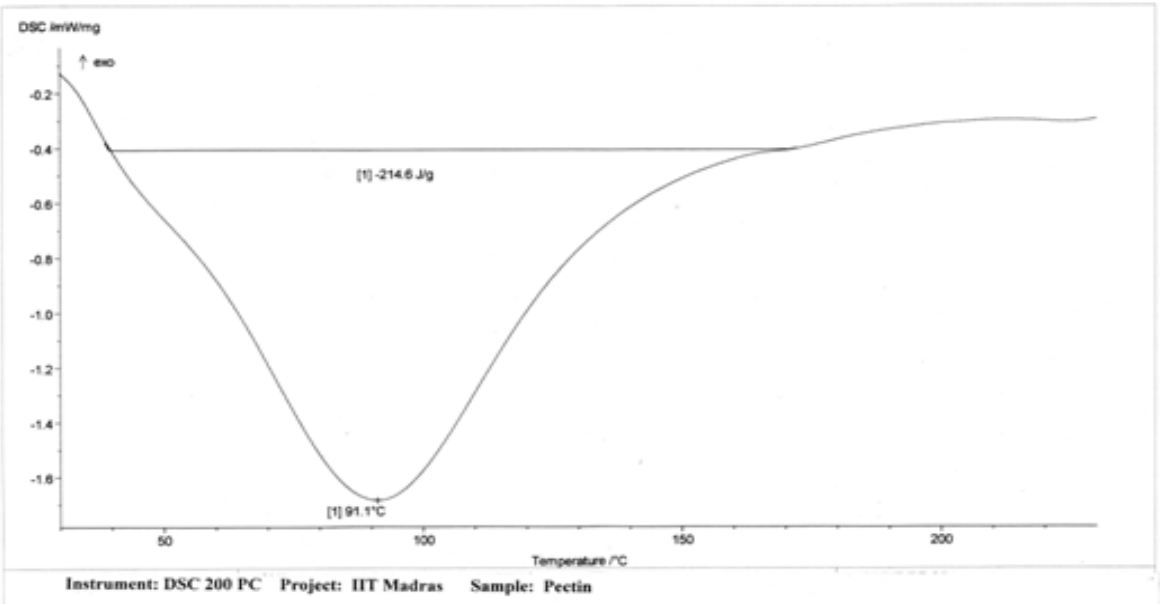

Fig.2 DSC of pectin

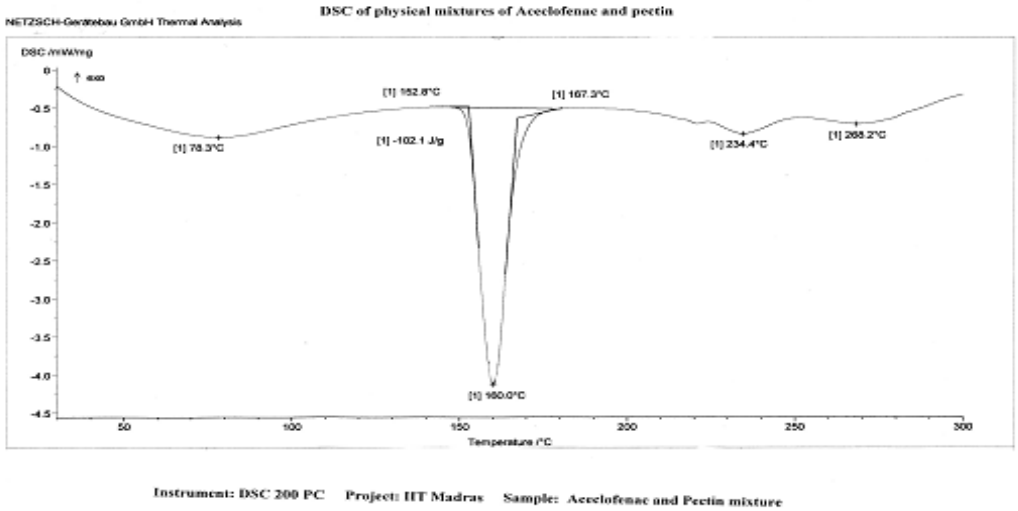

Fig.3 DSC of Aceclofenac and Pectin mixture

From the above interpretation it is understood that there is no major shifting in the frequencies of above said functional groups of aceclofenac was identified which indicates that there is no chemical interaction between aceclofenac and polymers which were used in the formulations.

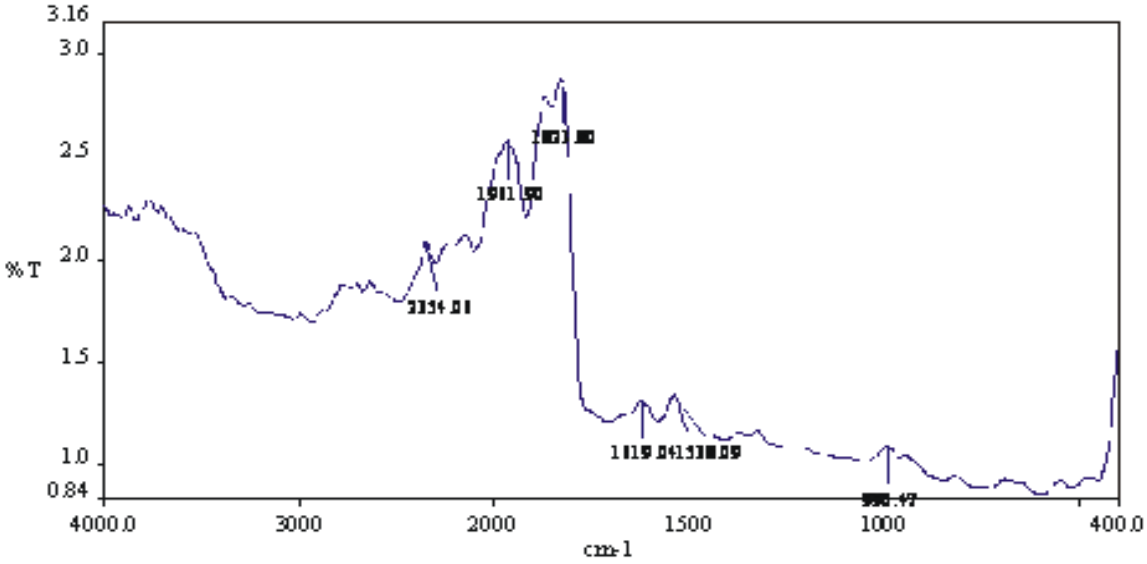

Fig 4 IR of Aceclofenac

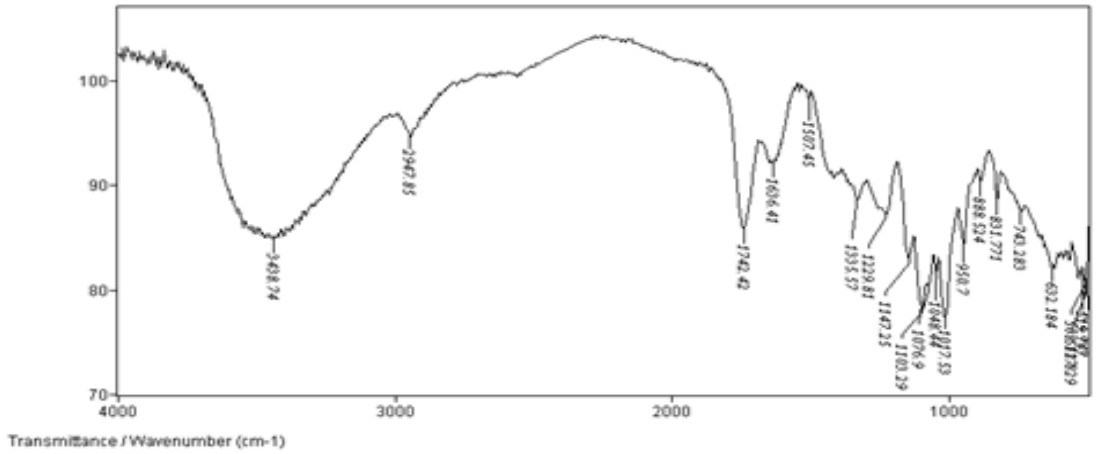

Fig.5 IR of pectin

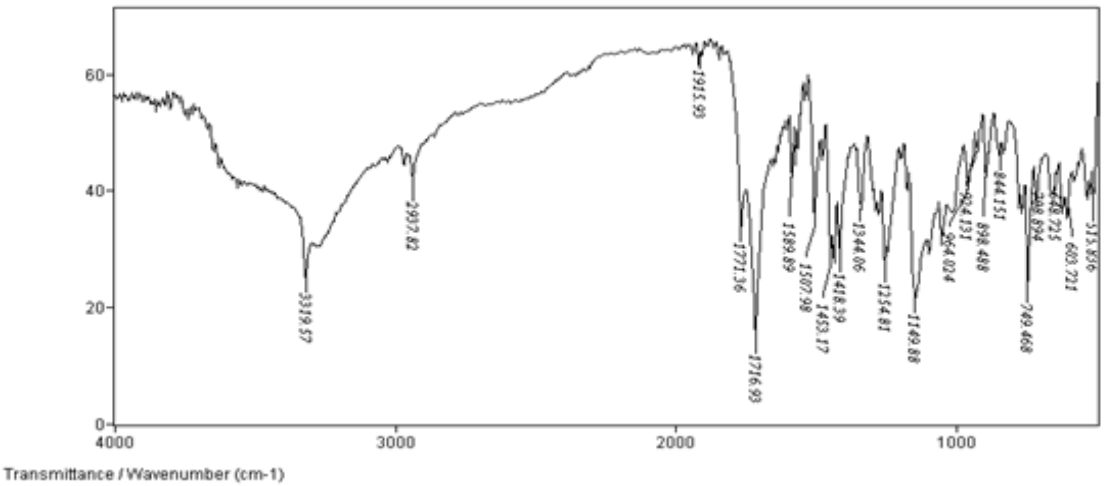

Fig.6 IR of Aceclofenac and Pectin

Micromeritic properties of pectin pellets

The micromeritic properties of the Pectin pellets were analysed and found that the bulk density of the PP_1 was 0.458 g/cm^3 and it increased as the polymer concentration increased so that the bulk density was 0.626 g/cm^3 for PP_4 and similarly Hausner's ratio also increased from 1.19 to 1.27 and so is the Carr's index.

Cumulative percentage drug release in SGF (pH 1.2 buffer)

The percentage release of the drug was analysed in a SGF medium. The percent release of drug was found to be 2.42 ± 0.86% for PP_1 at 15 min where it decreased according to increase in the polymer concentration and thus it was 0.56 ± 0.2% for the PP_4. The release rate increased with the prolongation of the time and thus at the 120 min the release was 20.07 ± 1.2% for PP_1 batch and it decreased towards the final formulation and finally the release was 3.23 ± 0.16% for PP_4 batch for the same time period. The results for cumulative percentage drug release in SGF are given in fig.7.

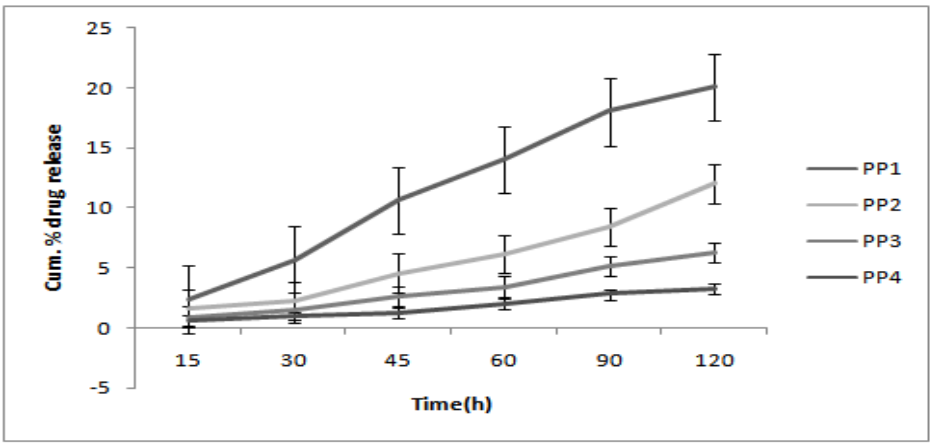

Fig.7 Cumulative percentage drug release in SGF

Cumulative percentage drug release in SIF (pH 7.4 buffer)

The percentage release of the drug was analysed in a SIF medium. The percent release of drug was found to be 6.0 ± 1.2% for PP_1 at 30 mins where it decreased according to increase in the polymer concentration and thus it was 0.56 ± 0.16% for the PP_4.

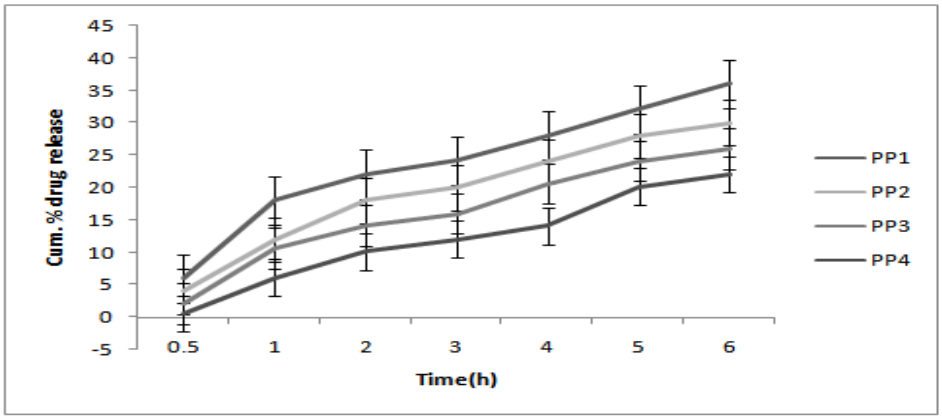

Fig. 8 Cumulative percentage drug release in SIF

The release rate increased with the prolongation of the time and thus at the 6th hour the release was 36.02 ± 1.4%. For PP_1 batch and it decreased towards the final formulation and finally the release was 22.04 ± 0.56% for PP_4 batch for the same time period. The results for cumulative percentage drug release in SIF are given in fig.8.

Cumulative percentage drug release in SCF (pH 7.4 buffer with 4% rat caecal contents)

The percentage release of the drug was analysed in a SCF medium. The percent release of drug was found to be 5.06 ±1.02% for PP_1 at 30 min where it decreased according to increase in the polymer concentration and thus it was 0.92 ± 0.52% for the PP_4. The release rate increased with the prolongation of the time and thus at the 8th hour the release was 102.14 ± 0.92%. For PP_1 batch and it decreased towards the final formulation and finally the release was 28 ± 1.02% for PP_4 batch for the same time period. The enhanced release took place in SCF medium in the presence of the enzyme induction due to the action of enzyme on the polymer as the later degraded the complex structure thus leave with the more release . As the PP_1 batch released maximum amount of drug (102.14 ± 0.92) at the 8th hour which was the prerequisite for the treatment of chronobiological symptoms of rheumatoid arthritis. Thus this batch was regarded as the optimal batch. The results for cumulative percentage drug release in SCF are given in fig.9.

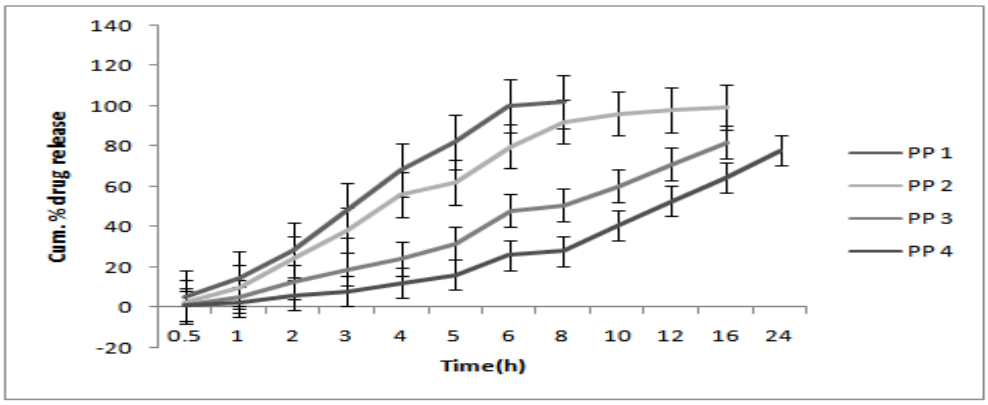

Fig. 9 Cumulative percentage drug release in SCF

Step II

The PP_1 batch was optimized one and was given the pH dependent polymeric coating. The coating was described under the general methodology.

Sieve Analysis

The particle size of coated pellets were determined by sieve analysis and found that maximum percent of particles (63.3%) lying in the sieve size between 710-600 µ.

***In vitro* dissolution studies in SGF, SIF and SCF for E-PP_1 pellets**

In vitro dissolution studies were conducted in SGF, SIF and SCF. There was no absorbance obtained in pH 1.2 buffer for 2 hours. This revealed that the coating has protected the drug release in the acidic medium.In SIF, the eudragit coated pectin pellets released about 34.02 ± 0.62 % at the 6^{th} hour and about 101.92 ± 0.42 % at 8^{th} hour in SCF. The release was more or less same as that of uncoated pellets. The pectin pellets of aceclofenac can sense as a promising mode of targeting aceclofenac into colon for synchronizing the circadian rhythm of rheumatoid arthritis.

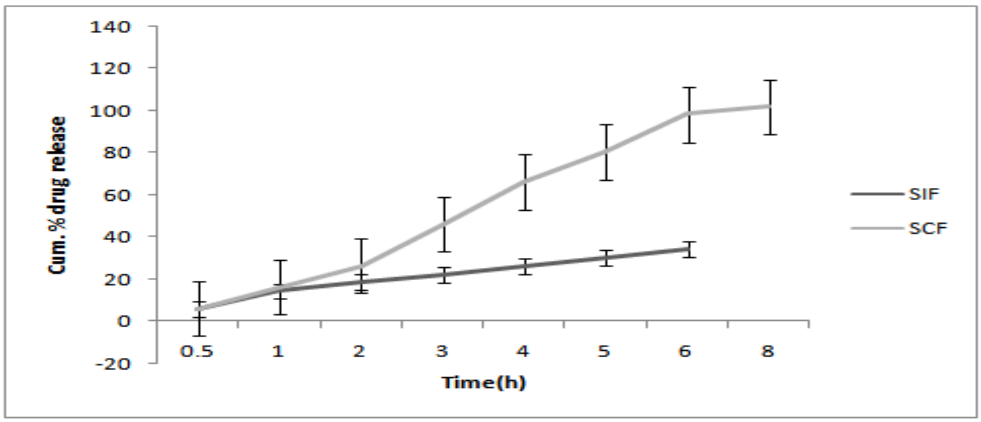

Fig. 10 Cumulative percentage drug release of E-PP1 pellets in SIF and SCF

In SIF, the eudragit coated pectin pellets released about 34.02 ± 0.62 % at the 6th hour and about 101.92 ± 0.42 % at 8th hour in SCF. The release was more or less same as that of uncoated pellets. The pectin pellets of aceclofenac can sense as a promising mode of targeting aceclofenac into colon for synchronizing the circadian rhythm of rheumatoid arthritis. The results for cumulative percentage drug release in SIF and SCF are given in fig.10.

***In vitro* release kinetics**

The release data of the optimized batch were fitted with various kinetic equations and the results revealed that the PP_1 batch followed zero order kinetics as the R^2 value was 0.9471 and R^2 value for Higuchi plot is 0.9719. And 'n' value of Korsmeyer Peppas plot was 1.1205 which indicated that the release of drug followed zero order kinetics with super case II mechanism of diffusion.

Stability studies

Stability studies also revealed that there was no significant change in organoleptic characteristics and percentage drug content at a raised temperature of 45ºC and 75 % RH for 3 months.

Discussion

The objective of the present study was to develop a controlled release colon targeted drug delivery system of aceclofenac, the non steroidal anti-inflammatory drug to approximate the chronobiology of rheumatoid arthritis. The system has a potential value when a delay in absorption is desired from a therapeutic point of view in the treatment of disease such as rheumatoid arthritis which have peak symptoms in early morning. Rheumatoid arthritis is a chronic inflammatory auto immuno disorder that causes the immune system to attack the joints. The disease displays circadian variations and so drug release should also vary over time. Research in this so called chronopharmacological field has assumed significance in developing drug delivery systems that demonstrate the important of biological rhythms in drug therapy. Pectin in all concentrations (22%, 35%, 45% and 51% relative to total tablet weight) did not show significant change in the physical characteristics of the formulations including bulk density, tapped density, Carr's index, Hausner's ratio, hardness and weight variation, however the swelling index increased with increase in concentration of polymers whereas the percentage erosion decreases as concentration of polymer increased. The results show that the physical characteristics of the tablet appear to be polymer concentration independent. Increase in concentration in polymer where as the percentage erosion decreased as the concentration of polymer increased. Among the pectin-aceclofenac pellets, the one with the minimal concentration (22%) of the polymer (PP_1) showed zero order release followed by non-fickian diffusion mechanism. The release profile shows that there was about 43% drug release in the SIF, however the drug release was sustained over a period of 8 hours in SCF. PP_1 showed 100.28 ± 0.78 % drug release as compared to 88.02 ± 0.87 %, 56.02 ± 0.69 % and 48.22 ± 0.64 % for PP_2, PP_3 and PP_4 respectively at the end of 8 hours. Taking into consideration, the percentage release profile of the drug over a period of 8 hours the PP_1 seems to be promising for controlled colon specific delivery and also for meeting the requirement of circadian rhythm related drug release in rheumatoid arthritis. As the morning stiffness is one of the diagnostic criteria of rheumatoid arthritis and this symptom is greatest on awakening and in the early morning hours

thus displaying circadian variations. Considering this factor, the tablet formulation was aimed to deliver the drug maximum in the early morning to relieve pain over a period of 8 hours.

The release profile characteristics show that PP_1 is found to be the optimized formulation as it obeyed zero order release kinetics over a period of 8 hours. Conversely, the optimized formulation PP_1 also released about 43% of drug in the simulated intestinal fluid over a period of 6 hours which seems to nullify the objective of releasing maximum percentage of the drug in the colon. With a view to minimize the release of drug in SIF the PP_1 was coated with a mixture of Eudragit L 100 and S 100 in a ratio of 1:4. It has been documented that these polymers in combination in different proportion can minimize the release of the drug in fluids less than pH 7.0, whereas maximize the drug release above pH 7.0 which is simulating the pH of the colonic environment. The dissolution profile of Eudragit coated PP_1 shows that the drug release followed zero order kinetics with non –fickian diffusion mechanism. There was no significant difference in the dissolution profile of the plain PP_1 in SCF. This finding indicates that the pH dependent release characteristic of Eudragit polymers has not influenced the enzymatic degradation of the pectin in SCF. From the overall results on the behavior of Pectin-aceclofenac pellets, it appears promising, since the drug release could be a result of the combination of fine dependent hydration of Pectin and enzymatic degradation by colonic bacteria.

The present study focused on viability of pellets of aceclofenac with polymer Pectin for controlled and colon specific delivery in chronotherapy of rheumatoid arthritis. The *in vitro* studies suggest that a Pectin tablet of aceclofenac is promising for therapy of rheumatoid arthritis. A further detailed study in human subjects will through more light on their efficacy and compliance.

Conclusion

The present study focussed on viability of pellets of aceclofenac, the anti-inflammatory drug prepared with polymer pectin was examined for controlled and colon specific delivery in chronotherapy of rheumatoid arthritis. The *in vitro* studies suggest that pectin pellets of aceclofenac are promising for therapy of rheumatoid arthritis. A further detailed study in human subjects will through more light on their efficacy and compliance.

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