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pH and Microbially Trigerred Release of Aceclofenac to large intestine for the treatment of Rheumatoid Arthritis

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ABSTRACT

The aim of the present study was to develop colon targeted drug delivery system of Aceclofenac to treat the early morning symptoms of Rheumatoid arthritis using chondroitin sulphate. Four formulae of Aceclofenac chondroitin sulfate pellets (CSP_1 to CSP_4)were prepared by powder layering method using nonpareil seeds and fluidized bed coating apparatus and were evaluated for micrometric properties such as bulk density (0.432 to 0.623), tapped density (0.518 to 0.803), Hausner's ratio (1.20 to 1.289), carr's index (20.12 to 28.9) and angle of repose ranged from 18°59′ to 28°47′ for CSP_1 to CSP_4 respectively. The drug release was conducted in SGF (simulated gastric fluid), SIF (simulated intestinal fluid), SCF (simulated colonic fluid -control -without rat cecal content) and SCF with 4% w/v rat cecal content. In SGF less than 10 % of the drug was released at the end of 2 hrs. In SIF the cumulative % drug release ranged from 26.7 ±0.76 % to 10.04 ± 0.82% at the end of 6^{th} hour from CSP_1 to CSP_4. The cumulative % drug release in SCF with enzyme was found to be 80.64 ± 0.72, 42.4 ± 0.62, 32.4 ± 0.62, 18.46 ± 1.04 % w/v at 8^{th} h from CSP_1, CSP_2, CSP_3 and CSP_4. The optimized formula (CSP_1) was given a special pH dependent coating with 1:4 of eudragit L100 and eudragit S100 10% to prevent drug release in the upper GI tract. Thus it was proved that the CSP_1 pellets made with 2 % w/w of chondroitin sulfate and coated with eudragit was found to be potential in targeting Aceclofenac to colon for the treatment of early morning symptoms of Rheumatoid arthritis.

Keywords: Aceclofenac, eudragit coating, chondroitin sulfate pellets, chronotherapy and colon targeting

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INTRODUCTION

Research in chronopharmacotherapy has demonstrated the importance of biological rhythms in drug therapy. The present study was to develop pH and microbially trigerred colon targeted drug delivery system of Aceclofenac using chondroitin sulphate as a microbially degradable polymer and eudragit as a pH dependent polymer to target the drug in the colon to synchronize the early morning symptoms of Rheumatoid arthritis. Here the drug is administered at least 4 to 6 h before the pain reaches its peak. It will be more helpful if arthritis patients take the NSAIDs before bed time if they experience a particularly high level of discomfort in the morning [1]

pH Sensitive Drug Delivery Systems (PSDDS) are gaining importance as these systems deliver the drug at specific time as per the pathophysiological need of the disease, resulting in improved patient therapeutic efficacy and compliance. The specific time that patients take their medication is very important as it has significant impact on treatment success. Optimal clinical outcome cannot be achieved if drug plasma concentrations are constant [2]. If symptoms of a disease display circadian variation, drug release should also vary over time. Drug pharmacokinetics can also be pH-sensitive; therefore, variations both in a disease state and in drug plasma concentration need to be taken into consideration in developing drug delivery systems intended for the treatment of disease with adequate dose. PSDDS wherein the drug release is controlled primarily by the delivery system, stimuli induced PSDDS in which release is controlled by the stimuli, such as the pH present in the intestinal tract. These systems are useful to several problems encountered during the development of a pharmaceutical dosage form [3].

Controlled drug delivery systems, which are intended to deliver drugs at predetermined rates for predefined periods of time, have been used to overcome the shortcomings of conventional drug formulations. Although, significant progress has been made in the controlled drug delivery area, more advances are yet to be made for treating many clinical disorders, such as diabetes and rhythmic heart disorders [4]. In these cases, the drug has to be delivered in response to pH in the body. In fact, it would be most desirable if the drugs could be administered in a manner that pre- cisely matches physiological needs at proper times (temporal modulation) and/or at the proper site (site-specific targeting). In addition, the controlled drug delivery area needs further development of techniques for delivery of peptide and protein drugs. In the body, the appearance of numerous bioactive peptides is tightly controlled to maintain a normal metabolic balance via a feedback system called 'homeostasis' [5]. It would be highly beneficial if the active agents were delivered by a system that sensed the signal caused by disease, judged the magnitude of signal and then acted to release the right amount of drug in response. Such a system would require coupling of the drug delivery rate with the physiological need by means of some feedback mechanism [6].

The pH range of fluids in various segments of the gastrointestinal tract may provide environmental stimuli for responsive drug release. Studies have been performed on polymers containing weakly acidic or basic groups in the polymeric backbone. The charge density of the

polymers depends on pH and ionic composition of the outer solution (the solution into which the polymer is exposed) [7]. Altering the pH of the solution will cause swelling or deswelling of the polymer. Thus, drug release from devices made from these polymers will display release rates that are pH sensitive. Polyacidic polymers will be unswollen at low pH, because the acidic groups will be protonated and hence unionized. With increasing pH, Polyacidic polymers will swell [8]. The opposite holds for polybasic polymers, because the ionization of the basic groups will increase with decreasing pH. By combining the knowledge of polymers and their solubility at different pH environments, delivery systems have been designed to deliver drugs at the target site [9].

MATERIALS AND METHODOLOGY

Aceclofenac sodium was a gift sample from Torrent pharmaceutical Ltd, Ghandhinagar, Chondroitin sulphate was from Ozone international, Mumbai, 2.5% Kollidon, Eudragit L100 were obtained from SD Fine chem. Ltd, Mumbai. All other chemicals were of analytical grade.

Preparation of Aceclofenac Chondroitin sulphate pellets using different ratios following materials by using powdered layered method

Table1: Formulation of Aceclofenac Chondroitin sulphate pellets

Ingredients	CSP_1	CSP_2	CSP_3	CSP_4
(i) Cores- non-pareils (%)	50	50	50	50
(ii) Layering powder (%)	50	50	50	50
Aceclofenac (%)	40	40	40	40
Chondroitin sulphate (%)	2	3	4	1
Polysaccharide	2	4	6	8
Aerosil	1	1	1	1
Starch powder (%)	7	5	3	1
(ii) Binder solution 2.5 % kollidon	QS	QS	QS	QS
Water	QS	QS	QS	QS

Procedure:

Pellets were prepared by powder layering of Aceclofenac on nonpareils (nuclei) in a 35 cm diameter, conventional coating pan (Erweka, Germany). The composition of the pellets are listed in table 2 [10].

All excipients of the powder layering composition were sieved through a 200 µm screen and mixed for 15 min in a double cone mixer.

After mixing, the powder mixture was again sieved through a 400 µm screen and mixed again for 10 min. Binder solution was prepared by dissolving Polyvinyl pyrolidone (kollidon 25) in water with magnetic stirring. Binder solution was continuously sprayed on the moving non-

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pareils by means of a peristaltic pump and a walther Bingo type spray nozzle with a 1-mm orifice. The powder addition was started after a 2-min lag time of the binder solution [11]. At fixed intervals, a fixed amount of the powder composition was layered onto the particles. The drug loaded pellets were dried in an oven at 40°C for 24 h after which sieve analysis was done and the fraction of 0.5 - 1.0 mm was separated for coating [12].

Layering condition for the preparations of Aceclofenac pellets in a conventional coating pan.

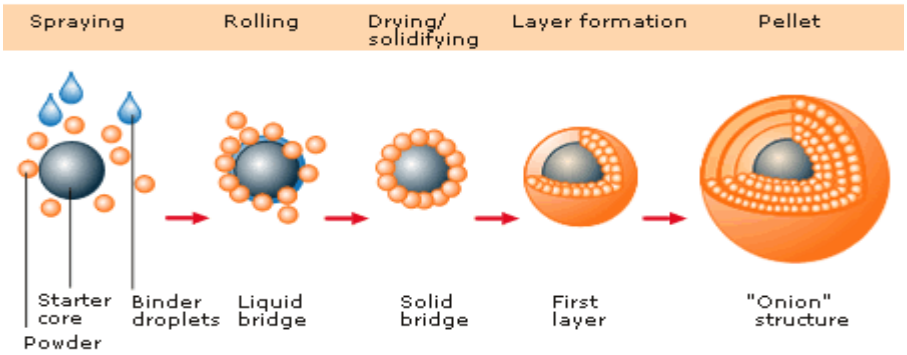

Figure 1: POWDER LAYER METHOD

Coating of pellets:

Table 2: Composition of coating solution

Ingredient	Amount
Eudragit L 100	1 part
Eudragit S 100	4 parts
Glyceryl mono stearate	0.5 %
Trimethyl citrate	1 % w/v
Water	QS

Procedure for 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 [12]. 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, guar gum, chitosan, chondroitin sulphate and xanthan gum. Then the pH dependent polymeric coated pellets were tested for drug release studies [13].

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Evaluations:

Prepared pellets were evaluated for micromeritic properties, FT-IR spectral studies, DSC, and for drug release studies.

Micromeritic properties of Chondroitin sulphate pellets:

- **Angle of repose:** The angle of repose was determined by funnel method was calculated by using the following equation

$$\text{Tan } \theta = h/r$$

Where,
h = height of the powder pile
r = radius of the powder pile

- **Bulk density**: Bulk density was calculated by using the following formula.

$$\text{Bulk density} = m/V_0$$

Where,
M = Mass of the blend
V_0 = Untapped volume.

- **Tapped density:** Tapped density was calculated by using the following formula.

$$\text{Tapped density} = m / V_1$$

Where,
m = Mass of the blend
V_1 = Tapped volume

- **Compressibility index**: carr's compressibility index was determined by using following formula

$$\text{Carr's index}(\%) = \text{Tapped density- Bulk density/ Tapped density} \times 100$$

- **Hausner ratio:** it is another method to find the flow of powder. It was calculated by the formula,

$$\text{Hausner ratio} = \text{Tapped density / Bulk density}$$

1) Interaction studies:

Differential scanning calorimeter (DSC) and FT-IR studies were performed to characterize formulation for excipient compatibility. FT –IR spectra were recorded by using KBr

disc as references on FT- IR spectrophotometer (FTIR-8300, Shimadzu Co., Kyoto, Japan). The scanning range was 750-4000 cm^{-1} and the resolution was 1cm [17]. The FT- IR spectra were shown in fig 8, 9, 10, 1. DSC studies were carried out using DSC – Shimadzu 60 with TDA trend line software [21]. Thermograms were recorded for pure drug and formulation were shown in fig 11,12 and fig 13.

2) In vitro drug release study [16]:

Cumulative percentage drug release in SGF (pH 1.2 buffer), cumulative percentage drug release in SIF (pH 7.4 buffer). Cumulative percentage drug release in SCF (Ph 7.4 buffer with 4% rat caecal contents). In vitro dissolution studies in SGF, SIF and SCF for CSP_1 pellets were conducted and In vitro release kinetics were found out [15].

In vitro dissolution studies were performed for selected coated pellets. The following conditions were maintained for the dissolution process:

Instrument: Electro lab- USP Dissolution test apparatus.

Apparatus: Paddle type.

Temperature: 37±0.1^{0}C

Speed : 75 rpm

Dissolution medium: Distilled water.

Volume of medium: 900 ml.

Sampling intervals: Every 30 min

Sample volume: 5 ml withdrawn and replaced with 5 ml of distilled water

RESULTS AND DISCUSSIONS

Table 3: Micromeritic properties of chondroitin sulphate pellets

Test	CSP_1	CSP_2	CSP_3	CSP_4
Bulk density(gm/cc)	0.432	0.488	0.558	0.623
Tapped density(gm/cc)	0.518	0.606	0.709	0.803
Hausner's ratio	1.20	1.24	1.27	1.289
Carr's index	20.12	24.96	27.04	28.9
Angle of repose(^{0})	18°59′	22°56′	26°89′	28°47′

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The coated pellets of different developed formulations were evaluated for Angle of Repose, Bulk density, Tapped density, Compressibility Index, Hausner ratio. The results of Angle of Repose, Compressibility Index and Hausner ratio ranged from $18^0 59'$ to $28^0 47'$, 20.12 to 28.9, and 1.20 to 1.289 respectively. The results of bulk density and tapped density values ranged from 0.432 to 0.623 respectively.

The following graphs are the standard plots of Aceclofenac at different pH buffers

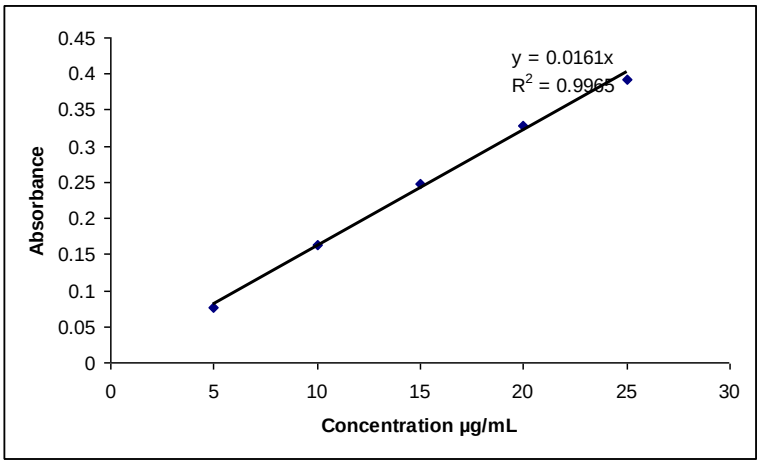

Fig 2: Standard plot of Aceclofenac in pH 1.2 buffer

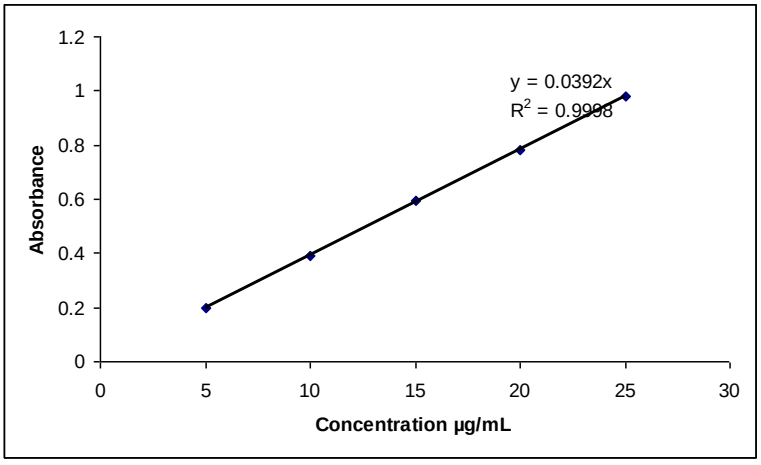

Fig 3: Standard plot of Aceclofenac in pH 7.4 Phosphate buffer

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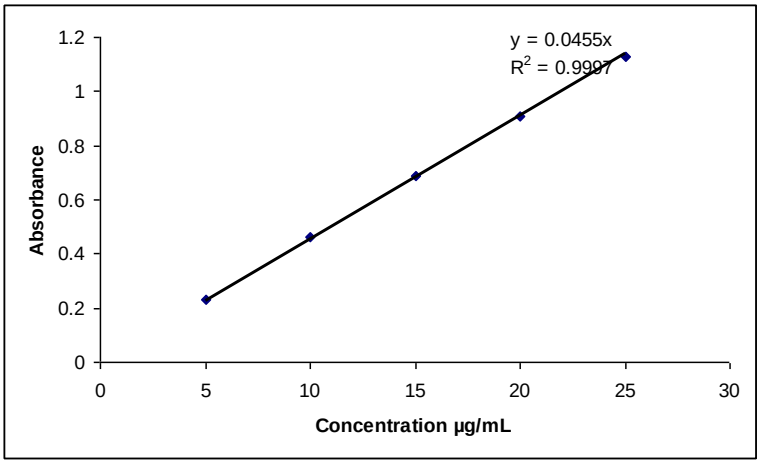

Fig 4: Standard plot of Aceclofenac in pH 7.0 phosphate buffer

Table 4: Cumulative percentage drug release of CSP in SCF

Time (h)	CSP $_1$	CSP $_2$	CSP $_3$	CSP $_4$
0.5	4.63 ± 1.2	3.1 ± 0.62	1.62 ± 0.72	0.98 ± 0.16
1	8.40 ± 0.46	5.30 ± 0.72	3.6 ± 0.82	1.62 ± 0.82
2	15.62 ± 0.9	10.12 ± 0.86	6.4 ± 0.12	3.24 ± 0.16
3	26.48 ± 0.84	21.41 ± 0.92	13.0 ± 0.65	8.12 ± 0.92
4	48.4 ± 0.62	26.2 ± 0.12	18.0 ± 0.72	10.14 ± 1.24
6	69.82 ± 0.02	38.6 ± 0.82	28.0 ± 0.61	14.26 ± 0.82
8	80.64 ± 0.72	42.4 ± 0.62	32.4 ± 0.62	18.46 ± 1.04
10	88.72 ± 0.12	56.6 ± 0.72	42.12 ± 0.12	26.12 ± 0.92
12	94.62 ± 0.64	65.2 ± 0.84	56.18 ± 0.18	39.40 ± 0.62
16	99.12 ± 0.98	70.6 ± 0.12	62.26 ± 0.24	51.20 ± 0.26
24	101.24 ± 0.94	76.40 ± 0.16	70.14 ± 0.16	60.18 ± 0.32

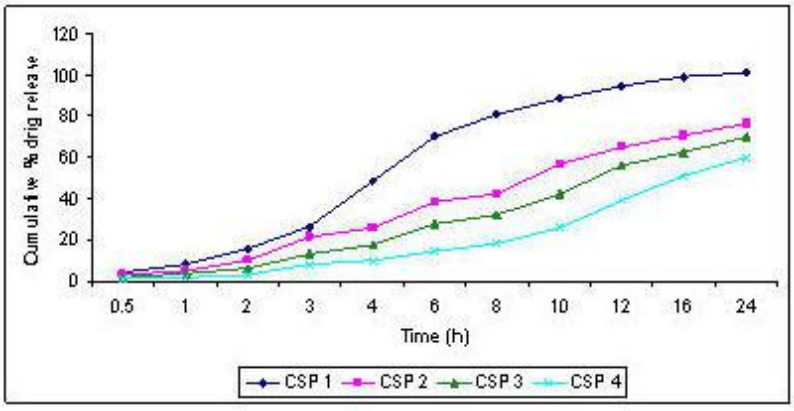

Fig 5: Cumulative percentage drug release of CSP in SCF

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Table 5: Cumulative percentage drug release of CSP in SIF

Time (h)	CSP $_1$	CSP $_2$	CSP $_3$	CSP $_4$
0.5	2.10 ± 0.98	1.56 ± 0.72	1.02 ± 0.25	0.98 ± 0.42
1	5.6 ± 0.64	4.82 ± 0.62	3.45 ± 0.74	1.6 ± 0.65
2	10.2 ± 0.82	6.21 ± 0.74	5.12 ± 0.18	3.8 ± 0.82
3	13 ± 0.92	7.12 ± 0.65	6.5 ± 0.92	5.0 ± 0.42
4	17.5 ± 0.64	9.15 ± 0.06	8.12 ± 0.82	6.12 ± 0.82
5	22.6 ± 0.56	12.16 ± 0.58	10.16 ± 0.96	9.24 ± 0.64
6	26.7 ± 0.76	14.18 ± 0.92	12.14 ± 0.24	10.04 ± 0.82

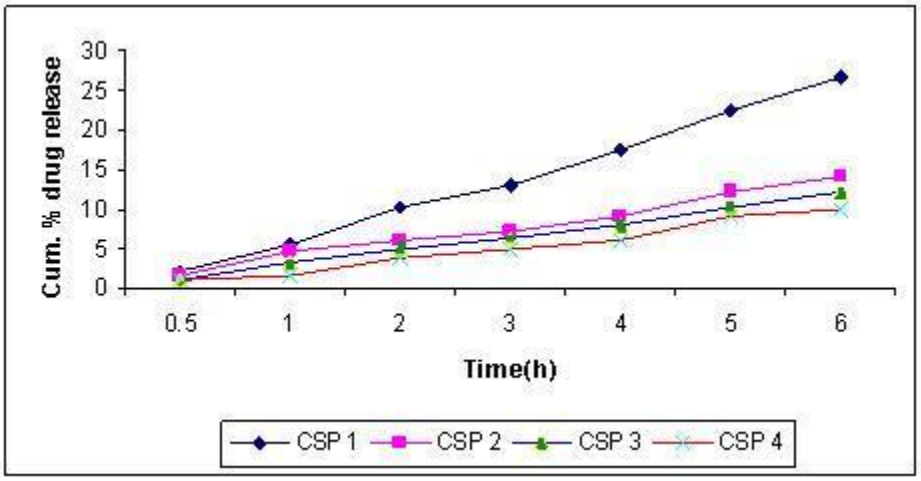

Fig 6: Cumulative percentage drug release of CSP in SIF

Cumulative percentage drug release in SIF and SCF (control and enzyme)

In SIF, the highest percentage release was 26.7 ± 0.76 (CSP_1) at the end of 6th h and the same formula released about 80.64 ± 0.72% of drug at the 8th h in SCF. This indicated that, though the polysaccharide is soluble in water, it needs the colonic enzymes to get degraded and thus released a maximum percentage of drug in the presence of SCF with 4 % w/v of rat cecal contents. The release was about 14.18 ± 0.92 to 10.04 ± 0.82 % for the batches CSP_2 to CSP_4 respectively in SIF. Their corresponding percentage release in the SCF medium was 38.6 ± 0.82 to 14.26 ± 0.82 at the 6th h for CSP_2 to CSP_4 respectively [17].

The same release trend observed in the eudragit coated pellets in the SIF and SCF, as observed for uncoated pellets in SIF and SCF. At the 8th h maximum release of 78.19 ± 0.62% took place in the presence of enzyme induction. Chondroitin sulphate pellets were prepared by powder layering method using kollidon as a binder. Though the polymer was very soluble in water, it did not show much release in SIF. This may be due to the binding effect of kollidon-25 which made the pellets more intact. So, the water uptake was very slow and there by release was also low in SIF. But in the SCF, in the presence of rat cecal contents (4 % w/v) the polymer

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degraded fastly and released a maximum 78.19 ± 0.62 % at the 8th h, which was necessarily to be achieved as per the aim of the study to synchronize the chronobiological symptoms [18]. The results of cumulative percentage drug release of CSP_1 in SIF and SCF were given in table 6 and fig. 7.

Table 6: Cumulative percentage drug release of CSP_1 in SIF and SCF

Time (h)	SIF	SCF
0.5	0.92 ± 0.72	5.02 ± 0.82
1	1.8 ± 0.12	10.20 ± 0.21
2	5.2 ± 0.62	16.72 ± 0.64
3	11.24 ± 0.15	25.8 ± 0.72
4	13.98 ± 0.92	49.4 ± 0.68
5	20.18 ± 0.12	55.5±0.96
6	24.16 ± 0.72	63.5 ± 0.72
8	-	78.19 ± 0.62
10	-	88.72 ± 0.14
12	-	93.12 ± 0.64
16	-	98.26 ± 0.62
24	-	102.22 ± 0.52

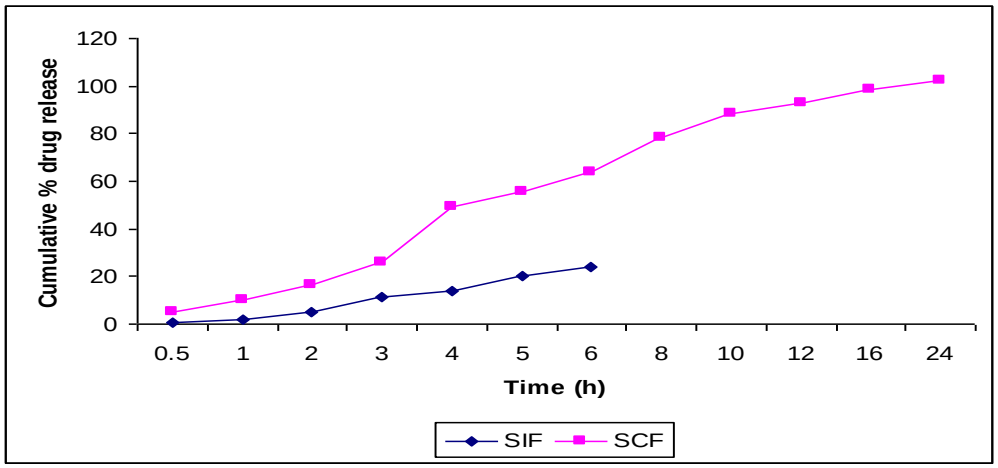

Fig: 7 Cumulative percentage drug release of CSP_1 in SIF and SCF

Table 7: Release kinetics for pellets

Formulation code	Zero order		First order		Higuchi		Korsemeyar peppas		Mode of diffusion
	K	R^2	K	R^2	K	R^2	N	R^2	
CSP_1	25.36	0.9739	0.1142	0.570	64.22	0.898	0.8903	0.9428	Non-fickian case II

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IR STUDIES

Infrared (IR) Spectroscopic Analysis

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 [19].

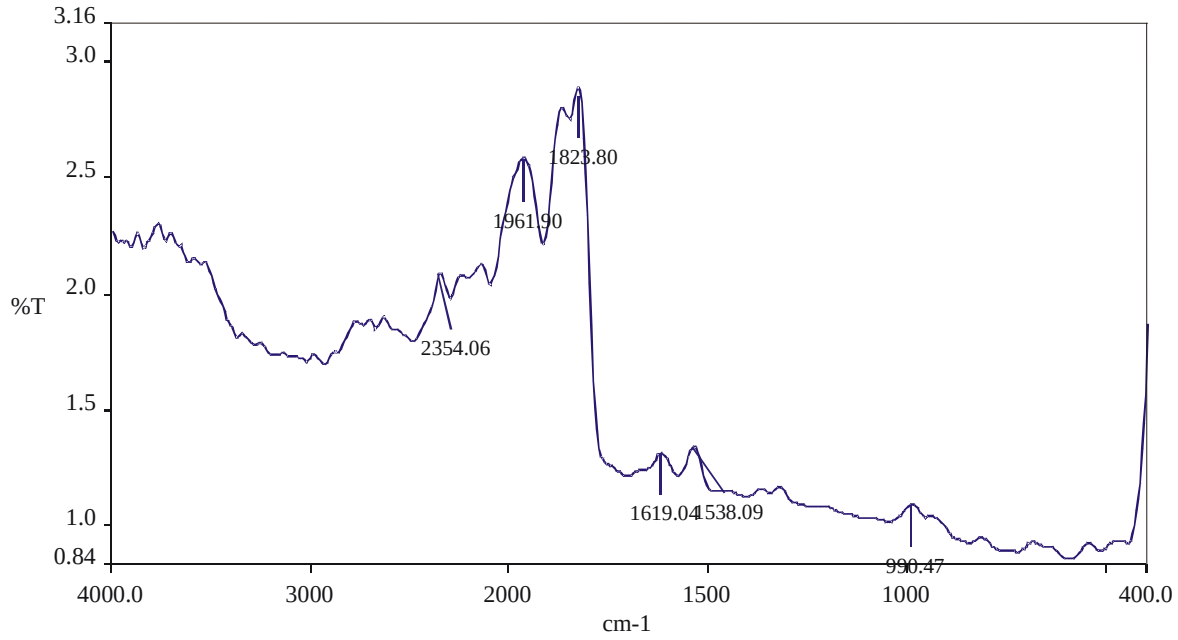

Fig 8: IR OF ACECLOFENAC

The FTIR of chondroitin sulphate 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.

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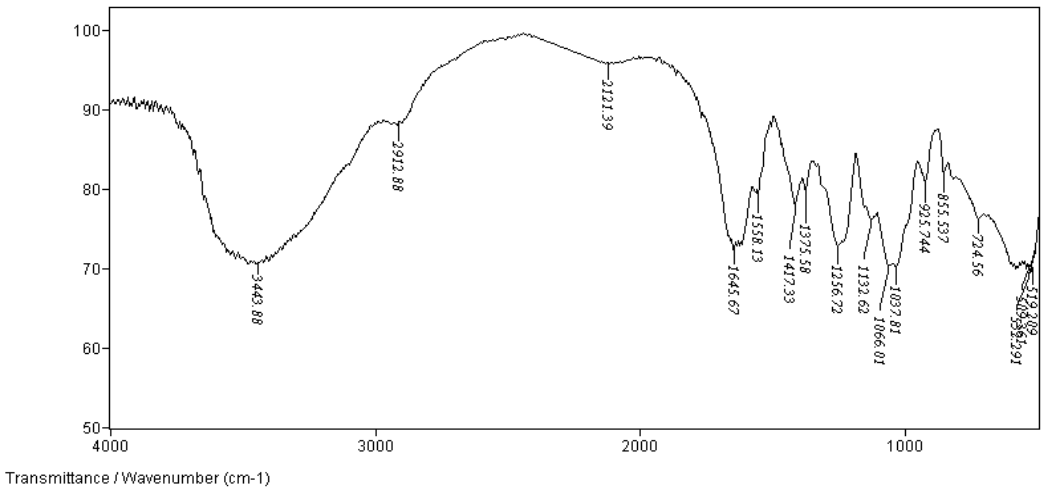

Fig 9: IR OF CHONDROITIN SULPHATE

The peaks observed in FTIR of physical mixture of 1771.62 cm^{-1}, 1716.76 cm^{-1}, 1589.84 cm^{-1}, 1055.88 cm-1 for chondroitin sulphate and Aceclofenac mixture. From the above interpretation it is understood that there is no major shifting in the frequencies of above said functional groups of Aceclofenac which indicates that there is no chemical interaction between Aceclofenac and polymers which were used in the formulations [20].

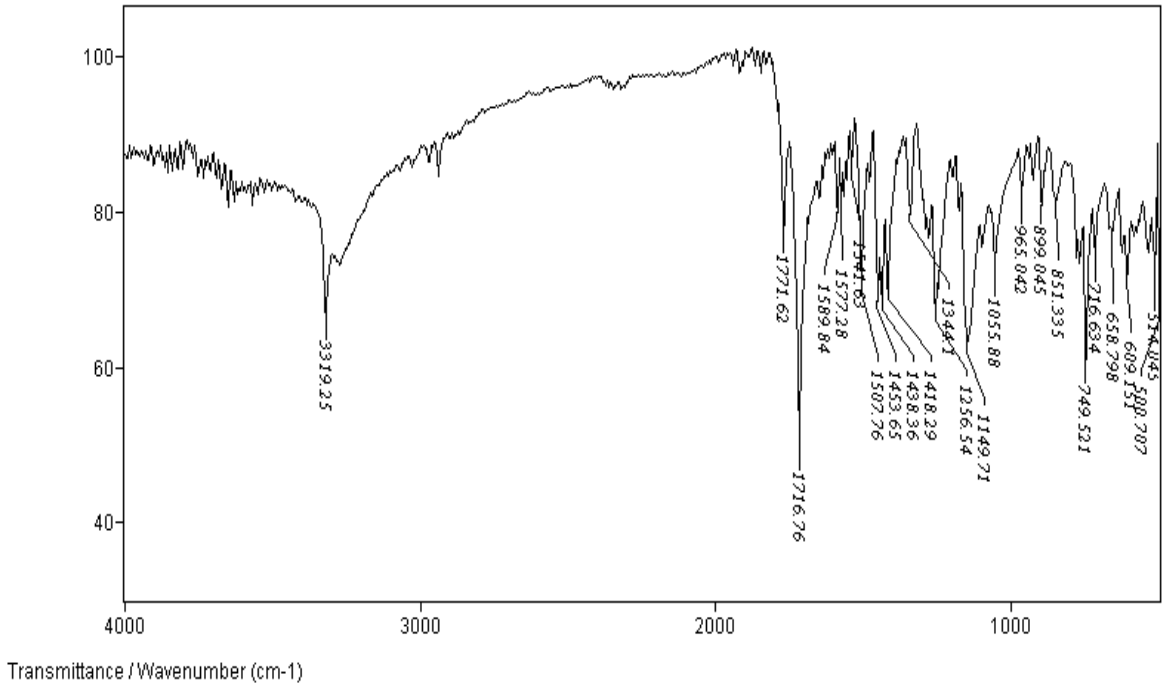

Fig 10: IR OF ACECLOFENAC and CHONDROITIN SULFATE

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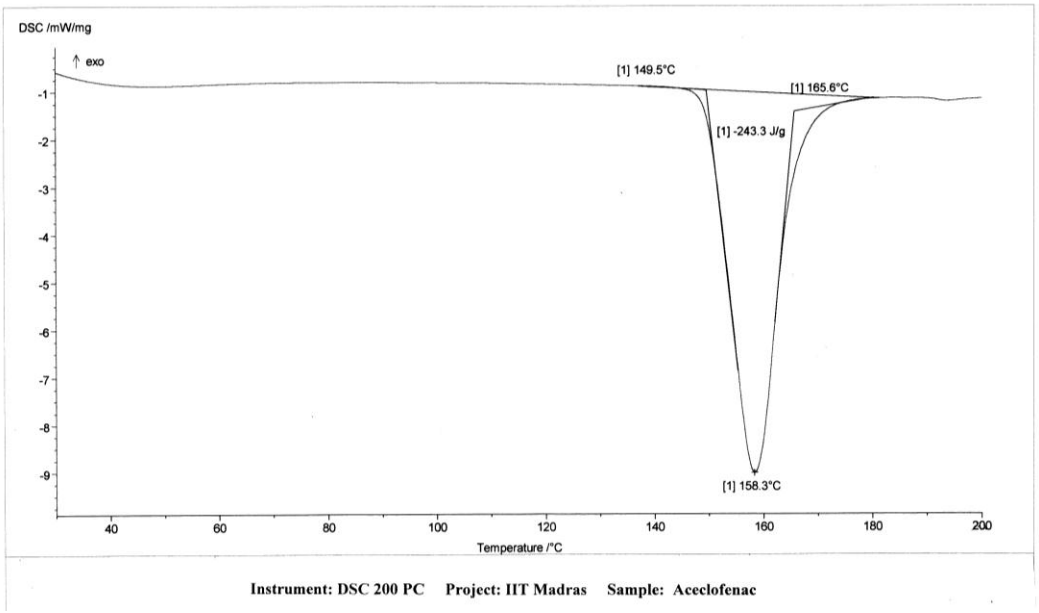
DSC STUDIES

Differential Scanning Calorimetry (DSC) Analysis

The output of a DSC is a plot of heat flux (rate) versus temperature at a specified temperature rate. 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[22]. While the thermogram of physical mixture of Aceclofenac and chondroitin sulphate was found at 158.0ºC , 162.4ºC for eudragit L 100 and Aceclofenac mixture and 155.3ºC for eudragit S 100 and Aceclofenac drug.

However a broad endothermic peak observed for chondroitin sulphate (i.e 123.8ºC), for eudragit L 100 and 80.5ºC for eudragit S 100.

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 polymers which were employed in the formulations and thus showed the peaks corresponding to the crystalline drug molecules when present in the mixture [23]. The DSC graphs are given below.



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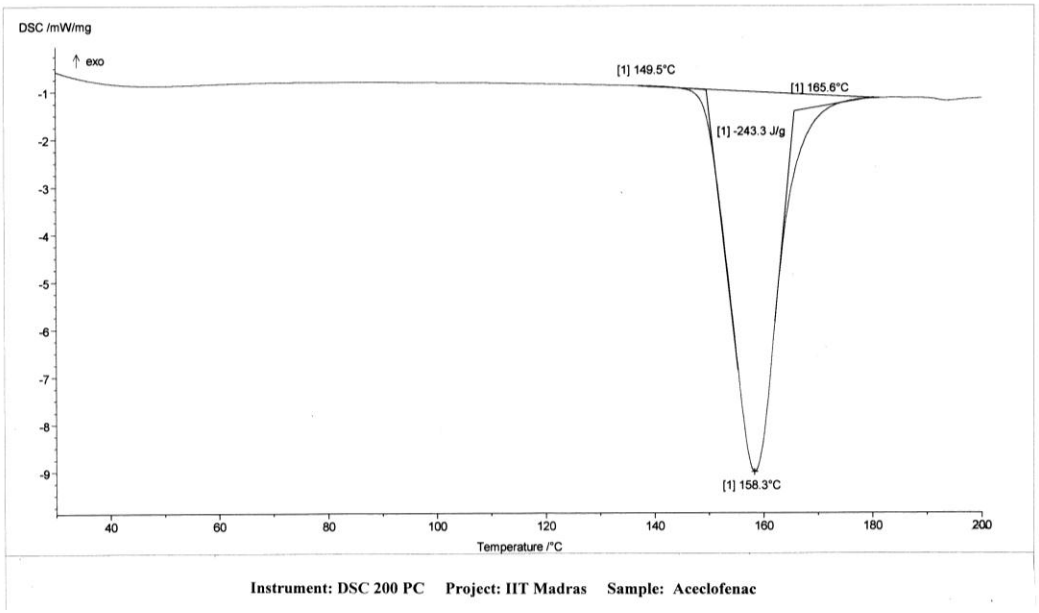

Fig 11: DSC OF ACECLOFENAC

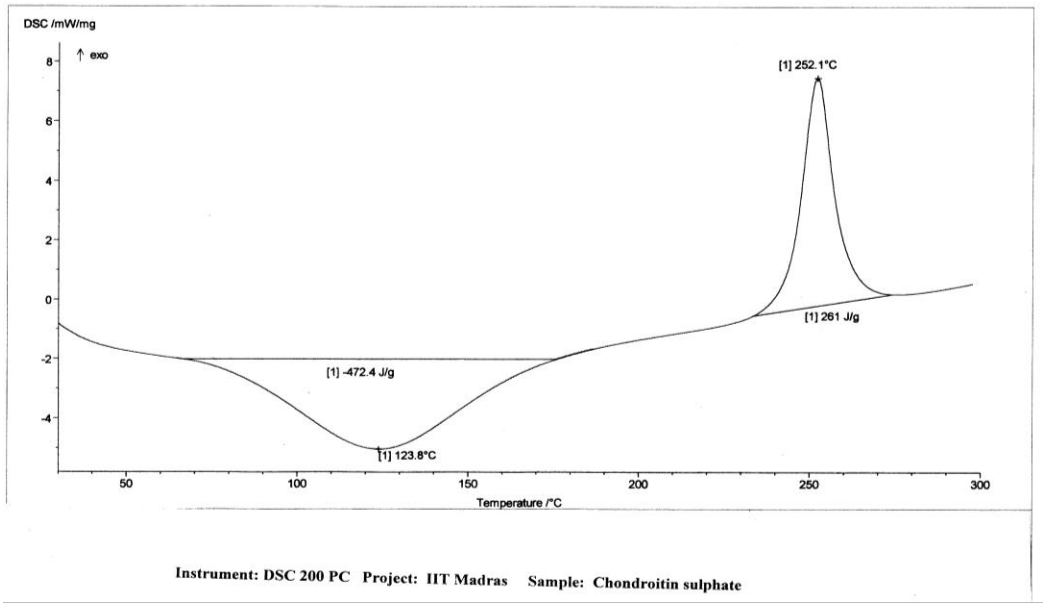

Fig 12: DSC OF CHONDROITIN SULPHATE

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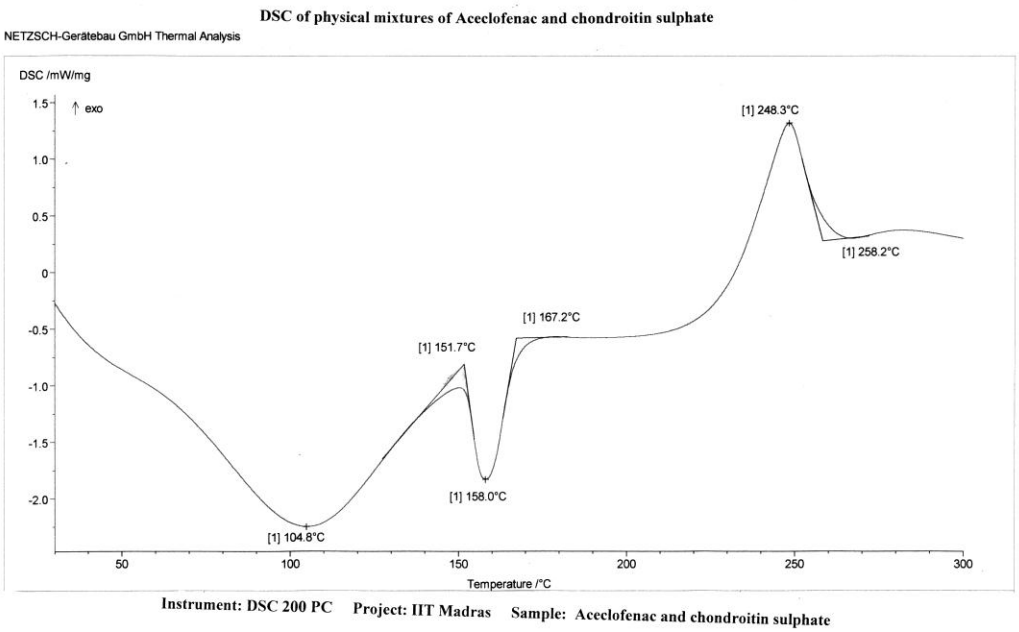

Fig 13: DSC OF ACECLOFENAC AND CHONDROITIN SULFATE

CONCLUSION

The present study was also aimed to examine how multiparticulate drug delivery systems influences colon targeting and also achieving the circadian rhythm dependent release of the drug (Aceclofenac) in rheumatoid arthritis [24]. The in vitro dissolution profile shows that the polymer concentration reduced the percent drug release as the concentration of the polymer increased in SGF over a period of 2 h. The reduction in the release behaviours of the drug is possibly depending on the thickness of the swelling layer around the drug. Accordingly the formulations namely CSP_1 were coated with the mixture of eudragit L 100 and S 100 in a ratio of 1:4 with a view to minimize the drug release in the upper gastrointestinal tract [25]. Since the polymers dissolve pH dependently. The eudragit coating reduced the percent drug release in respect of CSP1 in SCF at the end of 8^{th} h (78.19 ± 0.62 %), though the release pattern was sustained. In respect of other formulation the eudragit coat resulted in sustained release of the drug and the maximum release reaching about 90 % at the end of 8^{th} h. Although, chondroitin sulphate is equally enzymatically degradable in the colon as other polymers, the exact mechanism for the reduction in the percent drug release (78.19 ± 0.62 %) at the end of 8^{th} h is not clearly understood [26]. All the pellets formulations showed zero order release kinetics and it follows non – fickian diffusion mechanism. Thus it was proved that the CSP_1 pellets made with 2 % w/w of chondroitin sulphate and coated with eudragit was found to be potential in targeting Aceclofenac for the chronotherapy of Rheumatoid arthritis.

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