Preparation and Characterization of Colon Targeted Beads of Indomethacin Using Chitosan and Chondroitin Sulphate

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Abstract- Targeted therapy is a cutting edge technology that deals with drugs that target some specific pathways in the growth and development of tumors.The cells of this category include polyps, precancerous lesions, premalignancies and other aberrant phenotype which may progress to cancerous state.Some non-steroidal anti-inflammatory drugs (NSAIDs) like Indomethacin have been reported to shrink the polyps by inhibiting proliferation and inducing apoptosis in colorectal cancer cells via a variety of COX-dependent and/or COX-independent mechanisms. It has been reported that indomethacin can inhibit the growth of transplanted human colorectal tumor through inhibition of vascular endothelial growth factor .Effectiveness of Indomethacin to treat colon polyp can be increased if it is targeted directly to colon .So the present study is aimed to prepare colon specific beads of Indomethacin to target directly to colon . An interpolymer complex of polymers like chitosan and Chondroitin sulfate in different ratio are used to prepare the beads.The results confirmed that specific delivery of Indomethacin to colon can sustain the release for a long duration in comparison to marketed product.

Index Terms- Indomethacin, Colon targeted, chitosan, Chondroitin sulfate and beads.

I. INTRODUCTION

High levels of PG's are found in tumoral cells and reduction in 15-PGDH activity, by Indomethacin in tumor cells, may be regarded as a mechanism of tumor suppression[1]. It has been reported that Indomethacin can inhibit the growth of transplanted human colorectal tumor through inhibition of vascular endothelial growth factor[2]. Indomethacin treatment has also been reported to significantly upregulate the expression of tumor suppressor gene PTEN, the MAP kinase phosphatase-3 (MKP-3) and protein tyrosine phosphatase (SHP-2). As a consequence of increased expression of phosphatases and dephosphorylation of kinases, Indomethacin can negatively regulate the EGF/ PDGF pathways in colon cancer[3]. Indomethacin, an anti-inflammatory drug, is a dual-COX inhibitor. Due to COX-1 inhibition various adverse effects are associated with oral delivery of Indomethacin such as upper GIT toxicity. However the adverse effects can be minimized if the drug is directly delivered to the colon. In the light of above facts, it appears that colon-targeted delivery of Indomethacin can reduce the number of polyps and also the dosing frequency. Colonic drug delivery refers to those dosage forms which upon oral administration pass the stomach and small intestine in intact form and release the drug only in the large intestine. Although, designing of orally administered colonic delivery system is very complicated because the colon, located at the end of alimentary canal, is difficult to access .In the recent times, the colon-specific delivery systems are gaining importance for the delivery of proteins and peptide drugs due to unprecedented rapid development of biotechnology and genetic engineering, which results in the availability of these drugs at a reasonable cost. These drugs are destroyed and inactivated in the acidic environment of the stomach and/or by the pancreatic enzymes in the small intestine[4]. Hence, these drugs are usually administered by parental route, which is associated with complications such as thrombophlebitis and tissue necrosis that may lead to poor patient compliance.Colon is considered to be more suitable for delivery of peptide and proteins in comparison to small intestine due to negligible activity of brush-border membrane peptidases and also less activity of pancreatic enzymes[5]. Besides peptide and proteins, colon is also a good site for the absorption of many other drugs which are not stable in the acidic environment of the stomach, cause gastric irritation (e.g. aspirin, iron supplements) or are degraded by small intestinal enzymes. Many such drugs are found to be absorbed from large intestine and can be investigated for better bioavailability through colon-specific delivery. These drugs are glibenclamide[6], diclofenac[7], ibuprofen[8], diltiazem hydrochloride[9], metoprolol[10], theophylline[11] and nifedipine[12].

Local treatment is desirable for conditions such as ulcerative colitis and crohn's disease (anti-inflammatory agents e.g. 5-ASA and its prodrugs glucocorticoids), constipation (stimulant laxative e.g. senna, cascara glycoside), colorectal cancer (antineoplastic drugs), spastic colon (anticholinergics)[13], and ameobiasis (metronidazole)[14]. Drug targeting to colon would prove useful when intentional delayed drug absorption is desired from therapeutic point of view in the treatment of diseases that have peak symptoms in the early morning such as nocturnal asthma, angina and arthritis[15]. Colorectum is the portion of the GI tract most frequently affected by tumors. Colorectal cancer is the 4th most common cancer in the world with approximately 875,000 new cases per year worldwide (WHO 1996) and is the second leading cause of cancer death among US men and women. The majority of colonic tumors are benign epithelial polyps. The most common polyp and the only one that can become an adenocarcinoma are the adenomatous polyps. These polyps are precursors to more than 95% of all colorectal cancer.

Both the size and the degree of villous features are predictive of risk of malignancy within the polyp.

Several studies have been undergone on the basis of the activity of colonic bacteria in polysaccharide based carrier systems. The different polysaccharides that are used under evaluation as carrier for colonic drug delivery includes pectin and its salts, chondroitin sulfate, chitosan, inulin and guar gum etc..This work is aimed to prepare colon specific beads of Indomethacin using chondroitin sulfate and chitosan in different ratio. Oral administration of these beads can provide colon specific delivery of Indomethacin in sustained manner so reduction in dosing frequency.

II. MATERIALS

Indomethacin is 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetic acid. Indomethacin used in this study was a gift sample from Venus Remedies, Panchkula. Chitosan was purchased from Central Institute of Fisheries Technology,Cochin. Chondroitin sulfate is a mucopolysaccharide, which consists of D-glucuoronic acid linked to N-acetyl-D-galactosamide. It was obtained as a gift sample from Panacea Biotech, Lalru.

III. METHODS

1. Interaction study between chitosan and chondroitin sulfate

80 mg of chondroitin sulfate was dissolved in 10 ml of distilled water and 20 mg of chitosan was dissolved in 10 ml of 2% v/v acetic acid. Upon mixing the solutions a solid mass was formed which was subjected to IR spectroscopy.

Similarly, interaction study was done between other ratios of chitosan and chondroitin sulfate used in the study.

2. Preparation of empty beads

200 mg of chitosan was dissolved in 100 ml of acetic acid (2% v/v). Chitosan solution was dropped through a disposable plastic syringe into a gently agitated 100 ml solution of acetone:H_2O :: 3:1 mixture containing 800 mg of chondroitin sulfate. Water used contained 0.05% w/v of NaTPP, the final concentration of NaTTP used was 0.00625% w/v. The beads were prepared at the rate of 30 beads/ minute. The beads were separated after one hour of curing time by decantation and were rinsed twice with distilled water. The beads were dried at room temperature for 24 hours and used for further studies. Different ratio of composition of chondroitin and chitosan are as given in Table 1.1.

3. Preparation of drug loaded beads

For preparation of drug loaded beads 750 mg of Indomethacin was dispersed in chitosan solution. Beads were prepared by same method as described above.

Table 1.1 – Composition of Chondroitin Sulfate and Chitosan Used for Different Batches of Beads

Batch Name	Composition by Ratio Chondroitin : Chitosan
I	4.5 : 0.5
II	4.0 : 1.0
III	3.5 : 1.5
IV	3.0 : 2.0
V	2.5 : 2.5

4. Bead characterization

a. Encapsulation Efficiency

The Indomethacin contents in the beads was calculated from the difference between the total amount of Indomethacin added and the amount lost in the external phase[16]. The concentration of Indomethacin in decanted solution and two washings was determined by measuring the absorbance at 319 nm using Beckman USA DU-640 UV-visible spectrophotometer. Using a series of different concentrations of Indomethacin, in solution of mixture of acetone: water and acetic acid (2% v/v), as standard, the amount of Indomethacin lost in solution was quantified. The encapsulation efficiency (EE) was calculated from the following expression:

$$EE\ (\%) = \frac{\text{Total amount of drug used-Amount lost x100}}{\text{Total amount of drug used}}$$

b. Bead Diameter

Diameter of 20 beads from each batch was determined by using electronic digital caliper.

5. *In vitro* drug release studies

In vitro drug release studies were carried out using USP II (paddle method) dissolution apparatus at 37±0.2°C with constant stirring rate of 50 rev./min. Beads containing Indomethacin (equivalent to 75mg) were tested for drug release firstly in buffer pH 1.2 (900ml) for 2 hours as the average gastric emptying time is about 2 hours. The dissolution medium was then replaced with pH 7.4 PBS (900ml) and the drug release study was carried out for further 3 hours. Finally, the dissolution medium was replaced with phosphate buffer pH 6.8 (900ml) and the dissolution was continued until a maximum amount of incorporated drug was released. A sample volume of 3 ml was withdrawn from each

dissolution vessel at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and 1 ml of filtered sample was diluted suitably with respective dissolution medium. At the end of the study the beads were crushed in the pestle and mortar to determine the residual drug content of beads. Amount of drug released was determined spectrophotometrically at 319 nm. All the dissolution studies were carried out in triplicate and standard deviation was applied.

6. Preparation of rat caecal content medium

Spargue-dawley rats weighing 200-300 g maintained on normal diet were used for the study. Rats were treated with chitosan dispersion and chondroitin sulfate aqueous solution for inducing the enzymes specifically acting on these polymers. The procedure involved oral treatment of rats with 1 ml of 1% (w/v) dispersion of chitosan for five days and on fifth day of treatment 1 ml of 20% (w/v) aqueous solution of chondroitin sulfate. Thirty minutes before the commencement of drug release studies, five rats were killed by spinal traction. The abdomen were cut opened, caecum were ligated at both ends and cut loose. The formed caecal bags were immediately transferred into phosphate buffer pH 6.8, previously bubbled with CO_2. The caecal bags were opened, their contents were weighed, pooled and suspended in phosphate buffer pH 6.8 under CO_2 atmosphere.

7. *In vitro* drug release studies in the presence of rat caecal content

Selected batches were subjected to drug release studies in the presence of rat caecal contents to confirm the susceptibility of chitosan-chondroitin sulfate complex to the colonic bacteria. Drug release studies were carried out in 500 ml phosphate buffer pH 6.8 containing 1.25% w/v caecal contents after completing the first five hours of study in buffer pH 1.2 (900 ml; 2 hours) and PBS pH 7.4 (900ml; 3 hours). Drug release studies were carried out under continuous supply of CO_2 at 37±0.2°C with a constant stirring rate of 50 rev/ minute. Sample (3 ml) was withdrawn at regular intervals and replaced with fresh dissolution medium containing 1.25% w/v rat caecal content maintained under continuous supply of CO_2. The sample was filtered and 1ml sample was diluted suitably with dissolution medium. Amount of drug released was determined spectrophotometrically at 319 nm. All dissolution studies in the presence of rat caecal contents were carried out in triplicate and standard deviation was applied.

Wavelength scanning of 0.0025% w/v solution of Indomethacin, in methanol: 1N HCl (9:1) mixture, in a 1 cm cell from 350 nm to 300 nm exhibited absorbance maxima at 319 nm. Purity of the sample calculated using ∈ value as 0.45 was found to be 99.2% which is within pharmacopoeial limits i. e. 98.5-101.0% (IP 1996).

Standard plots were constructed in pH 1.2, PBS pH 7.4 and phosphate buffer pH 6.8. The mean absorbance value of experiments performed in triplicate for different concentration of Indomethacin in various dissolution media.

Wavelength scan of drug sample (25 μg/ml) solution in 1 cm cell from 350 nm to 300 nm was done in presence of chondroitin sulfate and chitosan to ascertain that these materials do not alter the absorbance maxima of the drug. During this study the sample exhibited absorbance maxima at 319 nm which is same as before (in the absence of chondroitin sulfate and chitosan). Therefore, it can be assumed that these substances do not alter the absorbance maxima of the drug.

IV. Results

1. Confirmation of Complex Formation

In order to confirm the chitosan-chondroitin sulfate interaction samples were analysed by IR spectroscopy. IR spectra of Chitosan showed a sharp peak of strong intensity at 1570.3 cm^{-1} which is the characteristic peak of amino group in Chitosan while in case of chondroitin sulfate sharp peaks in range of 1378.8 cm^{-1} to 1257.9 cm^{-1} indicated the presence of carboxyl groups. Secondary amide in chondroitin sulfate is indicated by broad peak at 1646.9 cm^{-1} due to NH bend. IR spectra of complex showed sharp peaks of strong intensity at 2358.9 cm^{-1} and 1400 cm^{-1}. These peaks could be attributed to $-NH_3^+$ ion and COO^- formed as a result of ionic interaction between amino groups of chitosan and carboxyl groups of chondroitin sulfate. Similarly appearance of absorption band at around 2500 cm^{-1} has been reported due to ionic interaction between amino groups of chitosan and carboxyl groups of sodium hyaluronate[17].

2. Optimization of Bead Formation

Dropwise addition of chitosan solution into chondroitin sulfate solution resulted into lump formation as reaction between chitosan and chondroitin sulfate was rapid. To control the rate, addition of water miscible organic solvent i.e. acetone was done[18]. The use of mixture of acetone and water in ratio of 3: 1 lead to bead formation but still it was found that after curing time of 1 hour the beads started bursting so a cross-linker NaTPP (0.00625% w/v) was added to get intact beads.

3. Bead Characterization

The encapsulation efficiency, expressed as a percentage of the amount of Indomethacin entrapped in the beads, of the formulated batches was found to vary from 94.54% to 90.68%. The mean values are tabulated in Table 1.2.

The beads produced all had good spherical geometry. There was no significant variation in bead diameter among the different batches of beads, the mean diameter varied from 1.83 ± 0.15 to 1.99 ± 0.13(n = 20). Table 1.3 summarizes the mean diameter of beads in each batch.

4. *In Vitro* Dissolution Studies

Beads of Indomethacin were prepared as described before. In preliminary dissolution studies, the amount of drug released from each batch over a predetermined period of time was followed through dissolution studies in pH progression medium, pH 1.2 for 2 hours, PBS pH 7.4 for 3 hours and phosphate buffer pH 6.8 till end of the study. Comparative data obtained from dissolution studies of different batches using pH progression medium, and drug release profiles are depicted in Fig 1.1, 1.2, 1.3 & 1.4 and Table 1.4.

In Batches I to IV drug release started after 1 hour while in case of Batch V drug release started after 30 minutes. The batch releasing minimum amount of drug in the pH 1.2 and PBS pH 7.4 and at the same time releasing maximum amount of incorporated drug was selected as best batch. Batch I and II were selected according to this criterion (Fig 1.4).

To simulate *in vivo* enzyme activity of colonic flora, *in vitro* dissolution studies of selected batches (I and II) were

carried out in the presence of rat caecal contents (1.25% w/v in 500 ml of phosphate buffer pH 6.8). Out of these two batches, Batch II releasing only 3.78% and 7.15% of drug in pH 1.2 and PBS pH 7.4 respectively and also 98.45% of drug after a total 24 hours of study was selected as the best batch. (Fig 1.4).

In vitro dissolution profile of marketed sustained release capsules of Indomethacin shows that drug release starts within thirty minutes and a large amount of drug released in pH 1.2 (2 hours) and PBS pH 7.4 (3 hours) and also sustained drug release only for 13.5 hours (Fig 1.5). So these results confirmed that prepared beads can sustain the release for a long duration upto 23 hours in comparison to marketed product which release only upto 13.5 hours.

The Batch II was selected as best batch as it released minimum amount of incorporated drug in dissolution medium pH 1.2 and pH 7.4 PBS. In case of Batch I large amount of drug got released in dissolution medium pH 1.2 and pH 7.4 PBS as compared to Batch II. It might be assumed that in case of Batch I, chitosan available for complex formation with chondroitin sulfate was less then optimum and the unreacted chondroitin sulfate remained dissolved in solution.

Table 1.2 – Encapsulation Efficiency of Beads

Sr. No.	Batch Name	Encapsulation Efficiency (%)
1.	I	94.54±0.54
2.	II	92.23±0.08
3.	III	92.35±0.15
4.	IV	91.85±0.54
5.	V	90.68±0.61

Each chondroitin sulfate, chitosan value represents Mean ± S.D. (n=3)

Table 1.3 – Diameter of Bead

Sr. No.	Batch Name	Diameter (mm)
1.	I	1.96 ± 0.11
2.	II	1.83 ± 0.15
3.	III	1.92 ± 0.17
4.	IV	1.99 ± 0.13
5.	V	1.92 ± 0.16

Each value represents Mean ± S.D. (n=3)

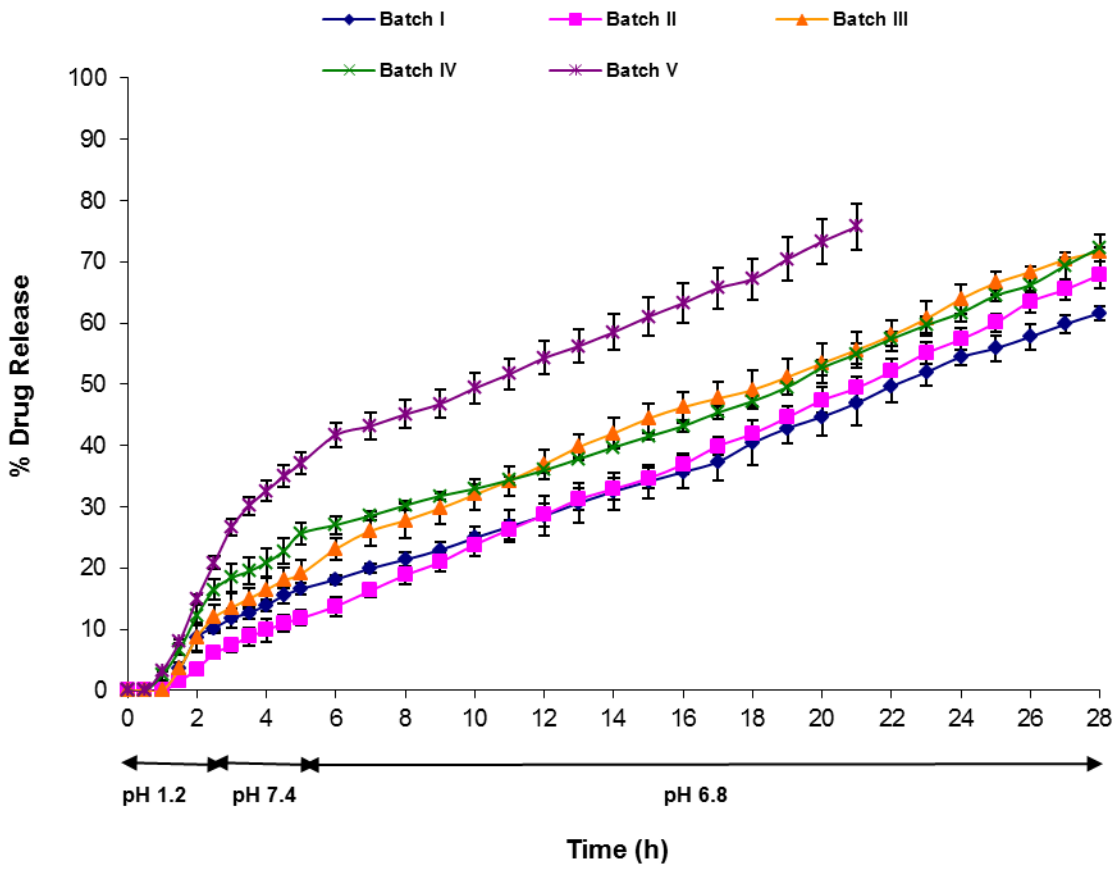

Figure 1.1 – Percent of Indomethacin Released from Investigational Batch I, II, III, IV and V in pH Progression Media. Each Value Represents Mean ± S.D. (n=3)

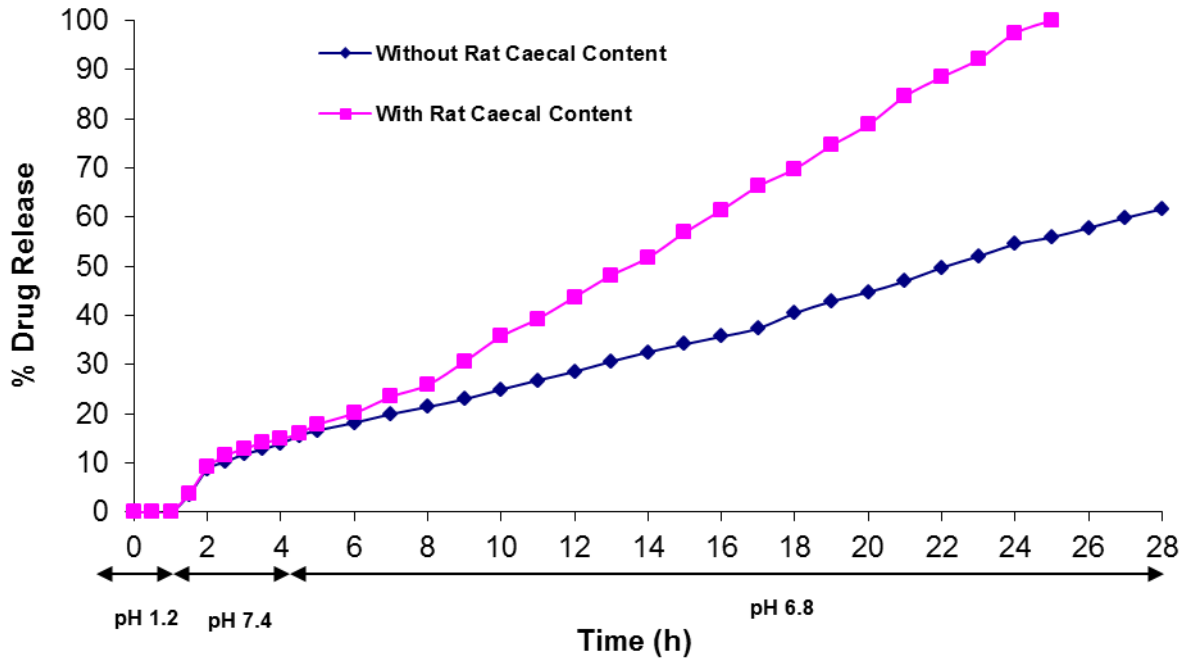

Figure1.2– Percent of Indomethacin Released from Investigational Batch I with and without Rat Caecal Content. Each Value Represents Mean ± S.D. (n=3)

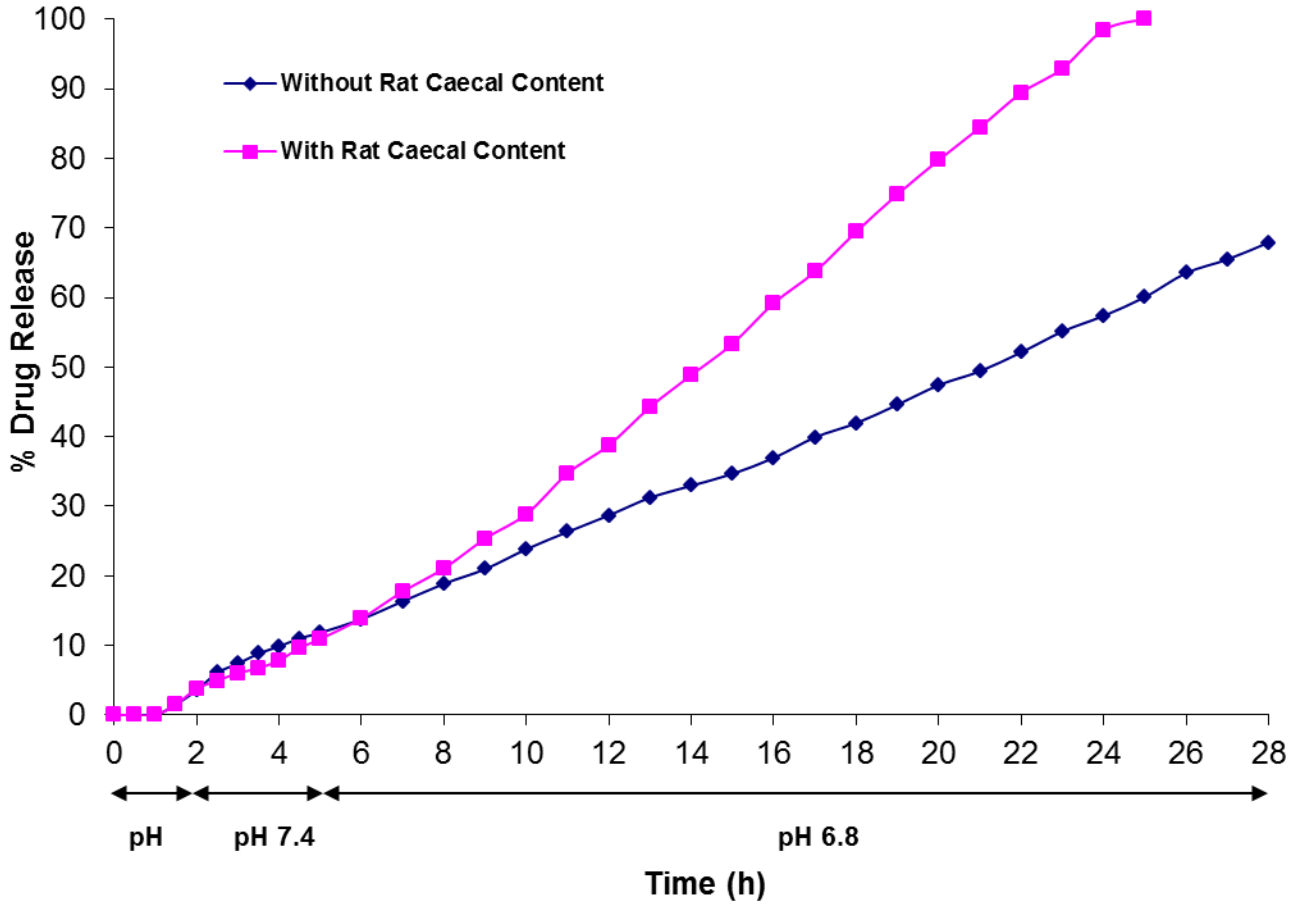

Figure 1.3 – Percent of Indomethacin Released from Investigational Batch II with and without Rat Caecal Content. Each Value Represents Mean ± S.D. (n=3)

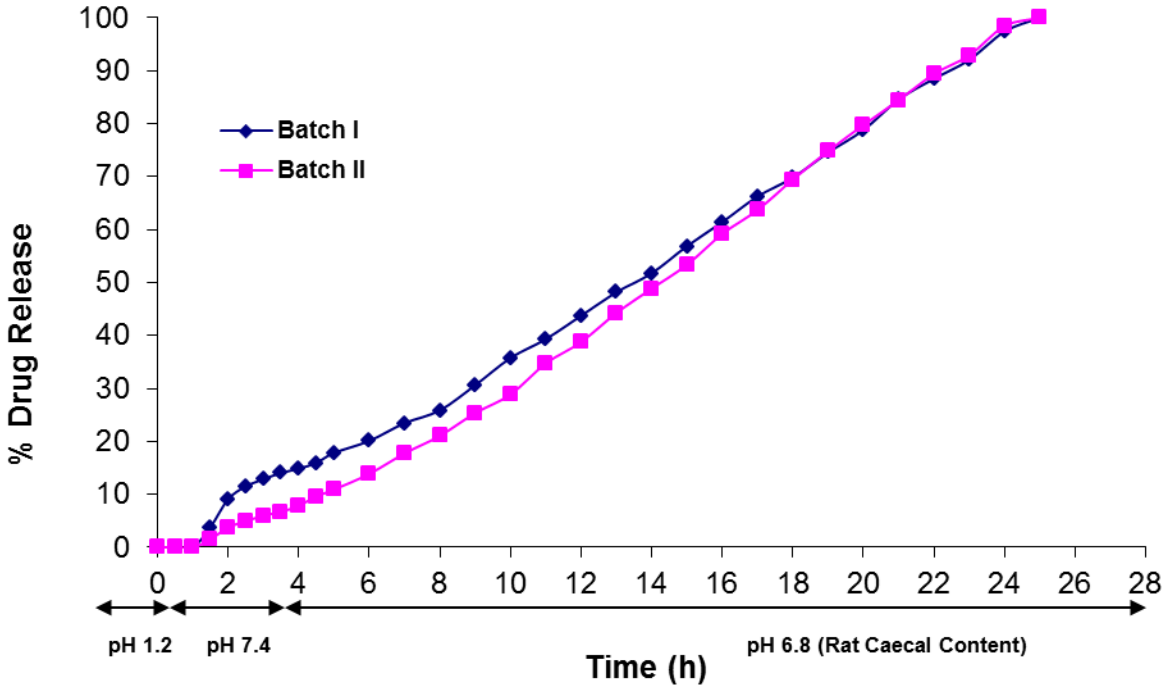

Figure1.4– Percent of Indomethacin Released from Investigational Batch I and Batch II with Rat Caecal Content. Each Value Represents Mean ± S.D. (n=3)

Table 1.4 – Selection of the Best Batch

Sr. No.	Batch Name	% Drug Released			Total % age of drug released
		pH 1.2 (For 2 hr.)	pH 7.4 PBS (For 3 hr.)	pH 6.8 (Till end of study)	
Without rat caecal contents					
1.	I	8.70	7.91 (16.61 in 5h)	45.04 (In 23 hours)	61.65
2.	II	3.55	8.28 (11.83 in 5h)	56.08 (In 23 hours)	67.91
3.	III	8.77	10.30 (19.07 in 5h)	52.60 (In 23 hours)	71.67
4.	IV	12.33	13.34 (25.66 in 5h)	46.65 (In 23 hours)	72.31
5.	V	14.97	22.17 (37.14 in 5h)	38.64 (In 16 hours)	75.76
With rat caecal contents					
6.	I	9.09	8.75 (17.84 in 5h)	79.57 (In 20 hours)	97.41
7.	II	3.78	7.15 (10.93 in 5h)	87.52 (In 20 hours)	98.45

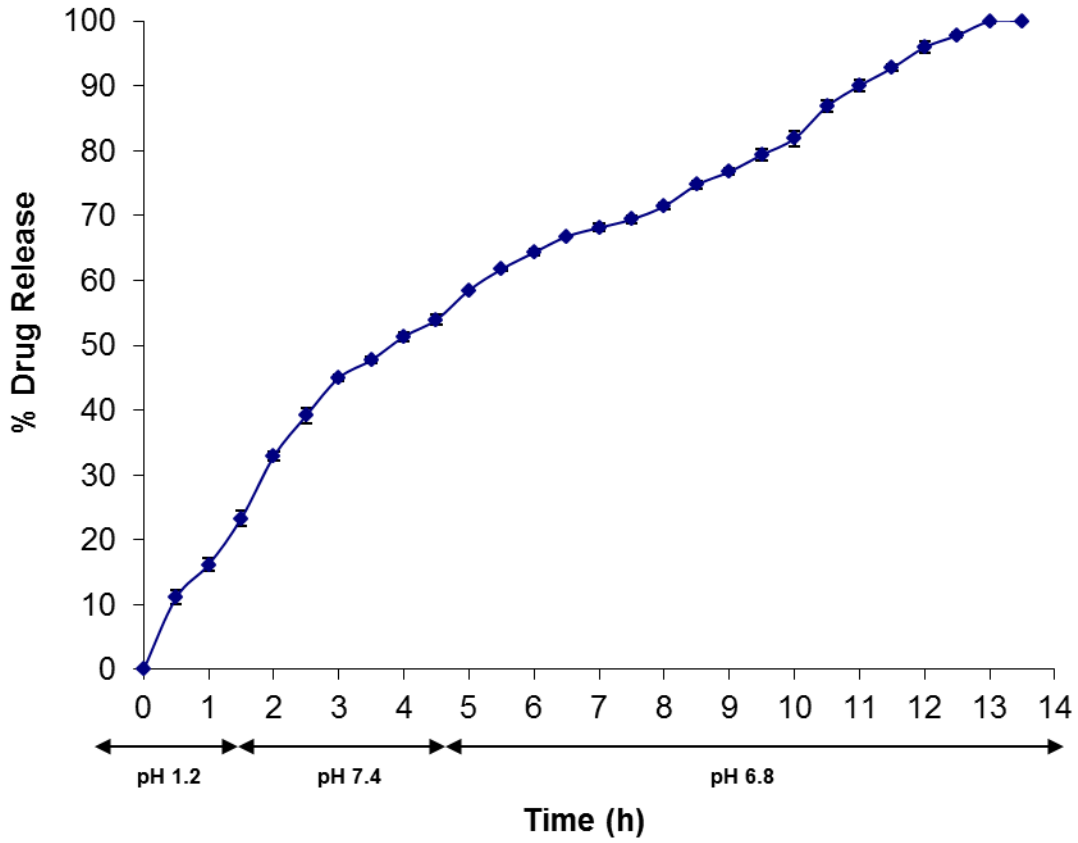

Figure 1.5 – Percent of Indomethacin Released from Marketed Sustained Release Capsules in pH Progression Medium. Each Value Represents Mean ± S.D. (n=3)

Mechanism of Drug Release

To determine the mechanism of drug release from different formulations the data were treated according to power model equation[19]. The equation is

$M_t/M_\infty = Kt^n$ (eq. 1)

Where,

M_t = amount of drug release

t = release time

M_∞ = amount of drug release after infinite time

K = kinetic constant incorporating structural and geomatric characteristics of the formulation

n = diffusinal exponent indicative of the mechanism of drug release.

To clarify the release exponent for different batches of beads, the log value of percentage drug dissolved was plotted against the log time for each batch according to equation 2[20], which is derived from equation 1[21].

$\text{Log}\ (M_t/M_\infty) = \text{Log}\ k + n\ \text{Log}\ t$ (eq. 2)

Where M_t / M_∞ is the percent of drug dissolved.

Value of the kinetic constant (k) and the release exponent (n) were determined from the graph between log percentage drug release Vs log time. Value of release exponent (n) determines the mechanism of drug release from different batches (Table 1.5)

Table 1.5: Effect of polymers ratio on the drug release and the release rate from the formulation.

Batches	Rate constant (Log k)	Release exponent (n)	r^2	Order of Release
I	0.6890	0.7372	0.9951	Non-fickian
II	0.2936	1.0678	0.9972	Super case II
III	0.7352	0.7732	0.9985	Non-fickian

IV	0.9112	0.6273	0.9923	Non-fickian
V	1.0791	0.6097	0.9794	Non-fickian
Marketed Formulation	1.3721	0.5537	0.9961	Non-fickian
In presence of rat ceacal contents				
I	0.5834	0.9927	.09923	Non-fickian
II	0.1101	1.3621	0.9981	Super case II

The value of release exponent n ranged between 0.5537 to 0.7732 except Batch II, for which the value was 1.3621. As all these n values are greater than 0.45, the mechanism of drug releases distinctly non-fickian diffusion for Batch I, II, IV and V while mechanism of drug release follow super case II transport for Batch II. Release mechanism for marketed formulation followed non-fickian diffusion as n value for that was 0.5537.

V. Discussions

Chondroitin sulfate is a polysaccharide that is used for colon-specific delivery system. However, chondroitan sulfate is water soluble and therefore it is unable to protect the drug release in upper GI tract. Chitosan is also a very promising polysaccharide for colon-specific delivery, but it is soluble in the acidic environment of the stomach, hence it cannot be used alone for colon targeting.

In the present study an interpolymer complex of chitosan and Chondroitin sulfate were prepared using different ratio of polymers. Interpolymer complexation was confirmed by IR spectroscopy which revealed an ionic interaction between anionic (chondroitin sulfate) and cationic (chitosan) polymer.

Indomethacin, a NSAID has the potential to reduce the number and size of polyps in colorectal cancer by upregulating the expression of tumor suppressor genes (PTEN, MAP-kinase phosphatase-3 and protein tyrosine phosphatase) and by induction of 15-LOX and 15-PGDH. Therefore colon-specific Indomethacin beads were formulated using inter-polymer complex of chondroitin sulfate and chitosan as a carrier.

In vitro dissolution studies of Indomethacin beads were carried out in pH progression media i.e. pH 1.2 for 2 hrs, pH 7.4 PBS for 3 hrs and pH 6.8 till end of the study. Best batch was selected on the basis of minimum drug release in pH 1.2 and pH 7.4 PBS and maximum amount of drug release at the end of study. Batch 1 (chondroitin sulfate: chitosan:: 4.5:0.5) and batch 2 (chondroitin sulfate: chitosan :: 4.0:1.0) were selected as best batches. Batch I released 8.70% drug in pH 1.2 for 2 hours, 16.61% pH in 7.4 PBS till end of 5 hours and 61.65% in pH 6.8 till end of the study (23 hours). Batch II released 3.55% of drug in pH 1.2, 11.83% in pH 7.4 PBS till end of 5 hours and 67.91% in pH 6.8 till end of the study (23 hours).

Batch I and batch II were then subjected to *in vitro* dissolution studies in the presence of rat caecal contents. Here, the same criterion was used for the selection of the best batch. Batch II was selected as best batch as it was releasing 3.78% and 7.51% of drug in pH 1.2 and pH 7.4 PBS respectively and a total drug release of 98.45% at the end of the study. Release mechanism of all the batches was determined by using Peppas equation. The best batch selected i.e. batch II showed the Super case II transport of release mechanism.

In vitro dissolution study of marketed formulation of Indomethacin in pH progression media revealed a large amount of drug release i.e. 32.86% in pH 1.2 (2 hours) and 58.49% in pH 7.4 PBS (till end of 5 hours) and it sustained drug release only for 13.5 hours.

VI. Conclusions

Thus it can be concluded from the study that interpolymer complex formed between chondroitin sulfate and chitosan was able to overcome the problems associated with both of the polymers i.e. solubility in upper GI tract. *In vitro* dissolution studies of batch II, prepared by using chondroitin sulfate and chitosan in the ratio of 4.5: 0.5 released minimum amount of drug in the upper GI tract, indicated the optimum ratio of polymers for complex formation. *In vitro* dissolution studies in the presence of rat caecal contents revealed that colonic enzymes can still act upon chondroitin sulfate and chitosan and degrade them despite the cross linking of both of the polymers. The drug release was 67.91% and 98.45% in absence and presence of rat caecal contents respectively in case of batch II.

In vitro dissolution data (in presence and absence of rat caecal content) demonstrated that batch II is able to carry the drug to the colon with minimum release in upper GI tract and upon reaching the colon, maximum drug is released.

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