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Original Article

DEVELOPMENT AND *IN-VITRO* DRUG RELEASE STUDIES OF SATRANIDAZOLE CAPSULES FOR COLON SPECIFIC DRUG DELIVERY

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ABSTRACT

Objective: The aim of the present study is to formulate and evaluate carbopol based enteric capsules for site specific drug delivery of Satranidazole to colon which was used in the treatment of amoebiasis.

Methodology: 10 different formulations of carbopol based Satranidazole capsules were prepared and coated with different ratios of HPMC and Eudragit S-100. The capsules were evaluated for various physic chemical parameters. The formulations were capable of delaying drug release in the time period of 3-5 h in the simulated physiologic environment of upper gastrointestinal tracts depending on coating ratios of HPMC and Eudragit S-100. Dissolution studies of all the formulations were performed and the cumulative percentage drug release for Satranidazole was calculated.

Results: Dissolution studies demonstrate that, these polymeric coated formulations were gastro resistance for 2 h at 0.1 N Hcl and further for 3 h at pH 6.8; since they released only 7-9% of drug in physiological environment of stomach and small intestine. They showed better drug release in colonic region (pH 7.4) only. Bio-adhesive studies and Rheological studies reveal that carbopol is effective in pH 7.4 than 0.1 N Hcl and pH 6.8 buffers. Diffusion studies and Histopathological studies show that the drug can easily penetrate though the mucosal membrane. DSC and IR analysis shows no possibility of interaction between drug and polymers used in the study.

Conclusion: Studies demonstrated that the developed system can be a promising device for targeting of Satranidazole to colonic region.

Keywords: Colon specific drug delivery, Histopathology, pH sensitive polymer, Satranidazole.

INTRODUCTION

During the last decade there has been an increased interest in the development of site specific formulations for targeting drug delivery to colon. The colon is a site for local and systemic drug delivery. Colon targeting is useful for

1. Promising site for drug delivery
2. Local disorders
3. Systemic absorption
4. Drugs unstable in upper GIT
5. Drugs poorly absorbed from GIT
6. Drugs that necessitate targeting at site

The colon is beneficial for local treatment of number of pathologies such as colorectal cancer, Chon's disease, IBD and amebiasis.

In recent times colon targeting drug delivery systems have gained importance for the systemic delivery of protein and peptide drugs. This is because the peptide and protein drugs get destroyed or inactivated in acidic environment of the stomach or by pancreatic enzymes in small intestine. Drug targeting to colon is also useful when a delay in drug absorption is desired from therapeutic point of view, such as treatment of diseases that have peak symptoms in the early morning like nocturnal asthma, angina and arthitis[1].

Drug targeting to colon offers many advantages by delivery of intact drug molecules as possible up to the target site, able to cut down the dose size and frequency, reduced incidence of side effects, improved patient compliances. Colon is attracting interest as a site where these poorly absorbed drug molecules may have an improved bioavailability. Additionally the colon has longer residence time and appears highly responsive to agents that enhance the absorption of poorly absorbable drugs. Conventional dosage forms were inefficient for delivery drugs to colon in appropriate concentration due to the reason of being absorption or degradation in the hostile upper GIT.

It provides a friendlier environment for drug candidates including those of proteins and peptides, oligo-nucleotides, vaccines, growth hormones etc. than the hostile upper GIT.

The site specific delivery of drugs to the target receptor sites has the potential to reduce the side effects and improve the pharmacological response. However, for successful colonic drug delivery many physiological barriers must be overcome. The major one being absorption or degradation of active drug in the upper part of the GIT.

Irrespective of therapy desired for local (colonic) or systemic delivery of drug, the development and aim of the drug delivery to colon, remains same. Firstly, the drug must not be absorbed from other regions of the GIT. Secondly, the release of the drug in the colon should be at quantitatively controlled rate and the released drug should be absorbed from the lumen of the large intestine without any appreciable degradation in the lumen. Thirdly, it should only suffer negligible degradation in the small intestine lumen [2-3].

Various approaches have been utilized to achieve oral delivery of drugs in order to achieve colon specific drug delivery which includes pH sensitive polymer coatings, Biodegradable polymer systems or microbially triggered enzymatic degradation by colonic bacteria, Bioadhesive polymer systems, Biodegradable Matrix and Hydrogel Systems, Systems Based On Particulate Ion Lining, Redox Sensitive Polymer Systems, Time dependent delivery, Pressure controlled release systems, and prodrug approach based delivery. Of all the systems developed, the use of pH dependent systems (Enteric coated systems) found to be more practical significance for colon specific drug delivery [4].

Carbomer is a generic name for a family of polymers known as Carbopol®. Carbopols® were first used in the mid 1950s. Carbopol polymers are polymers of acrylic acid cross-linked with poly alkenyl ethers or divinyl glycol [5]. They provide numerous benefits in bioadhesive formulations. They improve bioavailability of certain drugs, enhance patient compliance (fewer doses are needed per

day), Lower concentrations of the active ingredients can be used and they Provide excellent adhesion forces [6-16]. Carbomer has been investigated extensively by the pharmaceutical researchers because of its high viscosity at low concentration and low toxicity. *In-Vitro* experiment has proved that carbomer-934 have good bioadhesion with the gastrointestinal mucus. It prolongs gastric residence, since it binds very strongly to mucus via non-covalent bonds.

EUDRAGIT® polymers are used worldwide as functional excipients in the manufacturing of sophisticated pharmaceutical dosage forms. Eudragit S-100 is traditionally used as a coating polymer for site specific drug delivery (pH 6.0-7.5)[17].

Hypromellose (INN), short for hydroxyl propyl methyl cellulose (HPMC), is a semisynthetic, inert, viscoelastic polymer used as an ophthalmic lubricant, as well as an excipient and controlled-delivery component in oral medicaments, found in a variety of commercial products [18-19]. HPMC is a versatile pharmaceutical excipient available as different grades and used in oral and topical formulations [20].

The formulation consist of hard gelatin capsule (00 size) filled with drug and carbopol slug and it was coated with hydrophilic swellable HPMC (inner layer) and an Enteric coating layer of Eudragit S-100(outer layer) to avoid the gastric emptying variables. The enteric layers eroded when capsule enters the higher pH region of dissolution fluids. In contrast to gelatin the HPMC has a rough surface, which provides good adhesion to the coating and increase the water permeability to reduce the lag time (3-5 h). When capsule enters the small intestinal conditions (pH 6.8) the enteric coating gets slightly eroded and carbopol matrix was so adjusted to sustain the drug release during the lag time of 3-5 h, thereafter enteric coating completely eroded and carbopol adheres to the colonic mucosa and releases the drug completely in colonic region.

The proposed system, carbopol based enteric coated capsules, combines the pH sensitive property of enteric polymer as well as the bioadhesion of carbopol in the colon for targeted delivery of Satranidazole for the treatment of amoebiasis.

MATERIALS AND METHODS

Materials:

Satranidazole, was a gift sample from Alkem (Mumbai, India). Carbopol-934, Hydroxyl Propyl Methyl Cellulose, Triethanolamine and Di butyl phthalate were purchased from LOBA Chemie Laboratory Reagents & Fine Chemicals Ltd., (Mumbai, India). Eudragit S-100, was purchased from Zydus Cadila Healthcare Ltd., (Ahmedabad, India). All other chemicals were of analytical/pharmacopoeial grade from commercial suppliers and were used as received without further purifications.

Eqipment:

Dissolution Apparatus

UV Spectrophotometer

pH-Meter

Electronic Balance

Disintegration Apparatus

Franz Diffusion Cell

Brook Field Viscometer

Objective: The objective of the present study was to formulate carbopol based enteric coated capsules of satranidazole to reveal its protective effect against amebiosis.

Methods:

Preparation and Coating of Colon Targeting Delivery Capsules: (CTDC)

All the formulations consist of 20 mg drug and 100 mg carbopol mixed with triethanolamine. This slug was coated with HPMC, Dichloromethane mixture to avoid the adhering of carbopol slug to the capsule shell. This was filled in capsule (size 00). In all cases the drug mixture content was maintained at 120 mg but F_{10} formulation contains 140 mg that is 20 mg drug, 100 mg carbopol and 20 mg acacia as binder. The joint of the capsule was sealed with a small amount of 5% w/v ethanolic solution of ethyl cellulose. Each batch of the capsules was coated with HPMC (hydrophilic layer) as inner coating and outer coating by Eudragit S-100 (enteric layer) using dip coating method. The elasticity of Eudragit S-100 film was enhanced using 1.25% of dibutyl phthalate as plasticizer in coating solution. For each polymeric solution coating of capsule formulations was made with different thickness ratios of HPMC : Eudragit S-100 into F_1 (3:1)CTDC, F_2 (3:4) CTDC, F_3 (2:4) CTDC, F_4 (4:4) CTDC, F_5 (3:4 Acacia) CTDC, F_6 (4:2) CTDC, F_7 (3:2) CTDC, F_8 (4:3) CTDC, F_9 (1:3) CTDC, F_{10} (2:3) CTDC respectively by dipping twice, thrice & four times respectively in each coating solution at room temperature. The film was allowed to dry in air and stored in well-closed container for further studies [21-22]. The compositions for polymeric solutions were mentioned in table I.

Table I: Composition of coating solutions and standard operating conditions

Coating layer	Inner layer (Hydrophilic polymer layer)	Outer layer (Enteric polymer layer)
Composition of Coating solution (W/w %)	HPMC (4.5%) Ethanol (23%) H_2o (71.5%)	Eudragit S-100 (15%) Ethanol (93.75%) Dibutylpthalate (1.25%)
Operating condition	Simple stirring or by shaking on the shaking table at room temperature (25^0c)	

Construction of Standard plot of Satranidazole:

Satranidazole 100 mg was accurately weighed and dissolved in phosphate buffer saline pH 7.4 and the volume was made up to 100ml with the buffer. Further dilutions were made from the range of 1 µg/ml to 10 µg/ml using buffer. The samples were then scanned using Double beam UV-Visible Spectrophotometer for absorbance at 320 nm. A standard curve was calibrated by plotting the absorbance vs. concentration of drug taken [23]. The results were shown in fig I.

Drug Content Determination: (Assay)

Drug content in capsule was determined as mentioned in I.P. The capsules were crushed and dissolved in methanolic PBS solution (pH 7.4) and volume made up to 100 ml in volumetric flask. A 0.1 ml aliquot was taken out and volume made up to 10 ml with methanolic PBS (pH 7.4) solution and filtered though what man No.1 filter paper. The absorbance and % drug content of the filtrate was recorded at λmax of 320 nm with the help of Double beam UV-Visible Spectrophotometer [2-3] and the results were tabulated in table II.

Weight Variation Test:

The weight variation test defined by USP XX is a sequential test, in which 20 intact capsules are individually weighed and the average weight is determined. The test requirements are met if none of the individual weights are less than 90%, or more than 110%, of the average. If the original 20 do not meet these criteria, the individual net weights are determined. These are averaged, and differences are determined between individual net content and the average. The test requirements are met (1) if not more than two of the individual differences are greater than 10% of the average, or (2) if in no case any difference is greater than 25%.

If more than 2 but less than 6 net weights determined by the test deviate by more than 10% but less than 25%, the net contents are determined for an additional 40 capsules, and the average is calculated for the entire 60 capsules. 60 deviations from the new average are calculated. The requirements are met (1) if the difference does not exceed 10% of the average in more than 6 of the 60 capsules, and (2) if in no case any difference exceeds 25% [24] and the results were tabulated in table III.

Resistance of Coated Capsules:

The capsules were rubbed on a sheet of paper. The film remaining intact, they were resilient and tough [25].

Determination of Film Surface Characteristics:

Visual inspections were done by viewing the capsule though dissection microscope to define capsule coat quality.

***In-Vitro* Drug Release Studies:**

The *In-Vitro* drug release studies of the 10 batches of Satranidazole colon targeted capsule formulations were performed using USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37±0.5^{0}C) for the first 2 h in 0.1 N Hydrochloric acid (900 ml). The dissolution medium was changed with 900 ml of SIF (pH 6.8 phosphate buffer); dissolution was continued for 3 h. At predetermined time intervals 5ml of samples were withdrawn and replaced by an equal volume of fresh medium and test were continued in SCF (pH 7.4 phosphate buffers) for up to 24 h. Samples were collected at 1,2,3,4,5,6,7,8,9,10,12,23 & 24 h, filtered and analyzed at each interval for satranidazole content released at λmax of 320 nm using Double beam UV-Vis Spectrophotometer in SGF, SIF and SCF media, respectively[2-3]. The results were shown in fig II.

Release kinetics:

The analysis of drug release mechanism from a pharmaceutical dosage form is an important but complicated process and it is particularly evident in the case of colon targeted drug delivery. As a model dependent approach, the dissolution data are fitted to four popular release models such as zero-order, first- order, diffusion and erosion equations, which have been described in the literature. The order of release from matrix systems was described by using zero-order kinetics or first- order kinetics. The mechanism of drug release from colon targeted capsules was studied by Higuchi equation and erosion equation.

Zero-order release kinetics:

It defines a linear relationship between the fractions of drug released versus time.

$Q = k_o t$

Where Q is the fraction of drug released at time t

k_o is the zero-order release rate constant.

A plot of the fraction of drug released against time will be linear if the release obeys zero-order release kinetics. The results were shown in fig III (a).

First-order release kinetics:

Wagner assuming that the exposed surface area of a tablet decreased exponentially with time during dissolution process suggested that drug release from most slow release tablets could be described adequately by apparent first-order kinetics. The equation used to describe first-order release kinetics is

$\ln (1-Q) = -k_1 t$

Where Q is the fraction of drug released at time t and

k_1 is the first-order release rate constant.

Thus a plot of the logarithm of the fraction of drug remained against time will be linear the release obeys first-order release kinetics. The results were shown in fig III (b).

Higuchi (Diffusion) equation:

It defines a linear dependence of the active fraction released per unit of surface (Q) on the square root of time.

$Q = k_2 t^{1/2}$

Where k_2 is the release rate constant.

A plot of the fraction of drug released against square root of the time will be linear if the release obeys Higuchi equation. This equation describes drug release as a diffusion process based on the Fick's law, square root time dependent. The results were shown in fig III(c).

Hixson Crowell (Erosion) equation:

This equation defines the drug release based on tablet erosion alone.

$Q = 1-(1-k_3 t)^3$

Where Q is the fraction of drug released at time t and

K_3 is the release rate constant.

Thus a plot between $(1-Q)^{1/3}$ against time will be linear if the release obeys erosion equation. The results were shown in fig III (d).

Bioadhesion Studies:

Mucoadhesion Testing by *In-Vitro* Wash-Off Test:

The mucoadhesive property of the bioadhesive material that is carbopol was evaluated by an *In-Vitro* adhesion testing method known as the wash-off method [6]. Freshly excised pieces of colonic mucosa (2 × 2 cm) from *Ovis aries* were tied onto the glass slides (3 × 1 inch) using thread. Two glass slides were connected with a suitable support. Some amount of carbopol slugs were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the test fluid at 37°C contained in a 1 l vessel of the machine. At the end of 30 min, at the end of 1 h, and at hourly intervals up to 12 h, the machine was stopped and the amount of carbopol still adhering to the tissue was weighed. The test was performed at colonic pH (pH 7.4) [26] and the results were tabulated in table IV.

***Ex-Vivo* Residence Time:**

The *ex-vivo* mucoadhesion time was studied after application of carbopol slug on freshly cut *Ovis aries* colonic mucosa (2 × 2 cm). The fresh *Ovis aries* colonic mucosa was tied to the dissolution basket with the help of thread. Some amount of carbopol slug was fixed onto the wet rinsed tissue specimen by applying a light force with a fingertip for 30 s. 900 ml of phosphate buffer (pH 7.4) was taken as dissolution medium and was kept at 100 rpm, 37±0.5^{0}C. Carbopol adhesion was monitored for 12 h. The time required for the carbopol to detach from the *Ovis aries* colonic mucosa was recorded as the mucoadhesion time [27].

***Ex-Vivo* Mucoadhesive Strength (Bioadhesion strength):**

Fresh *Ovis aries* colonic mucosa was obtained from a local slaughterhouse and used within 2 h of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer (pH 7.4) at 37^{0}C. The bioadhesion strength of the bioadhesive tablets was determined by using the device shown in the figure IV (a). The device was mainly composed of a two-arm balance. The left arm of the balance was replaced by a vial containing buffer solution. At the same side, another vial is fixed at the bottom in order to fix the model mucosal membrane. The vials were filled with 0.1 N Hcl and intestinal membrane was tightly tied to the vials. The carbopol slug was placed on lower vial on that upper vial was placed and wait for 10 min for hydrating the tablet. Sand was added slowly on the right hand side until the vial detaches from the tablet and sand was weighed and bioadhesion force was calculated. Similarly the experiment was repeated for pH 6.8, pH 7.4 and bioadhesion strength was determined [27]. The results were shown in fig IV (b).

Peel adhesion Strength:

The peel adhesion strength of the carbopol was determined by using the device shown in the figure. A flow device was constructed of plexi glass (Fig V(a)) had a length, l, of 29.9 cm, width, w, of 4 cm, height, h, of 0.4 cm. In the middle of the bottom base there was a cavity of 7.62 cm length by 5 cm width by 0.8 cm depth for the placement of the polymer gel. A pulley was attached in order to add the weights. The polymer solution (100 mg carbopol in 100 ml buffer) was added in the cavity that was present and the upper portion was placed on the solution and allows it for 30 s. Then sand was slowly added until the upper plate starts moving and the weight was noted. The experiment was repeated for three times in 3 buffers (0.1 N Hcl, pH 6.8 and pH 7.4) [28]. The results were shown in fig V (b).

Degree of Swelling:

Accurately weighed amount of carbopol slugs were separately immersed in little excess of 0.1 N Hcl, pH 6.8 Buffer and pH 7.4 Buffer for 24 h and washed. The degree of swelling in each solution was calculated by [29]

$\alpha = Ws\text{-}Wo/Wo$

Thumb Test:

The thumb test is a simple test method which can be used to identify mucoadhesiveness. The adhesiveness is quantitatively measured by the difficulty of pulling the thumb from the adhesive as a function of pressure and the contact time. It is most likely that any mucoadhesive system is adhesive to fingers, since most mucoadhesives are non-specific and not mucin specific. Like mucin the skin has many hydroxyl groups. Although the thumb test may not be conclusive, it provides useful information on mucoadhesive potential [30].

Rheological Studies:

Viscosity Studies:

100 mg carbopol slugs were placed in glass beakers containing 100 ml of different buffers (0.1 N Hcl, pH 6.8, pH 7.4) and 2 ml samples were withdrawn at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8 & 24 h. The viscosity of the samples was measured by using Brookfield- LVDV-II + Pro Viscometer with spindle No. 42 at 0.5, 1, 2, 2.5, 4 & 5 rpm. The results were shown in fig VI.

Thixotropic Studies:

100 mg carbopol slugs were placed in glass beakers containing 100 ml of buffer solutions, allowed the contents for complete swelling of the carbopol and to get polymeric solutions. Viscosity, shear stress, shear rate of the solutions was measured by using Brookfield- LVDV-II + Pro Viscometer with spindle No. 42 at varying speeds i.e. 0.5, 1, 2, 2.5, 4, 5, 4, 2.5, 2, 1, 0.5 rpm at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8 & 24 h[31].

Drug Penetration though Mucosal Membrane :(Diffusion Studies)

Fresh *Ovis aries* colonic mucosa was obtained from a local slaughterhouse and used within 2 h of slaughter. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with phosphate buffer (pH 7.4) at 37^{0}C. An open ended test tube was taken and the membrane was tied to one end of the test tube and polymer-drug mixture was placed inside the test tube. This test tube was placed in beaker containing 100 ml of pH 7.4 buffer. The contents were continuously stirred by placing it on magnetic stirrer. 5 ml samples were withdrawn at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8, 23 & 24 h. Sink conditions were maintained by replacing 5 ml fresh pH 7.4 buffer. The samples were analyzed at 320 nm by using UV-Vis spectrophotometer [32]. The results were shown in fig VII.

Histopathological Studies:

The histopathological studies were performed on the sheep colon by Haematoxylin and Eosin staining. The photographs were taken by using Zeiss Binocular Microscope with cannon digital camera at 400X magnification [33]. The results were shown in fig VIII (a), VIII (b), VIII(c) and VIII (d).

Compatibility Studies:

Differential Scanning Calorimetry: (DSC)

DSC analysis was performed for pure drug and drug excipient in order to predict the possible interaction or compatibility between them. Samples (2-8 mg) were weighed and hermetically sealed in flat bottomed aluminium pan. The temperatures were increased at the rate of 20^{0}C/min from room temp to 600^{0}C under nitrogen atmosphere (50 ml/min). The formulations assayed were: (a) satranidazole (drug) (b) carbopol (c) satranidazole and carbopol (d) satranidazole and triethanolamine (e) satranidazole, carbopol and triethanolamine[2-3]. The results were shown in fig IX (a), IX (b) and IX(c).

Infrared Spectrometry (IR):

An infrared spectrum was recorded with IR spectrophotometer. The formulations assayed were: (a) satranidazole (drug) (b) satranidazole and carbopol(V. Ravi et al, 2008). The results were shown in fig X (a) and X (b).

RESULTS & DISCUSSION:

Colon targeted capsules were being formulated by various methods. The present study focused on the formulation of colon targeted capsules by using pH sensitive polymer Eudragit S-100 and HPMC and to evaluate its efficacy in reducing the amebiasis.

The colon targeted capsules were characterized for their weight variation, drug content determination, *In-Vitro* dissolution studies, bioadhesion studies and rheological studies for carbopol, IR and DSC studies for any incompatibility, Histopathological studies, and drug penetration though mucosal membrane.

Calibration Curve for Satranidazole:

The standard plot as per the dilutions of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml mentioned in experimental procedure for Satranidazole was done.

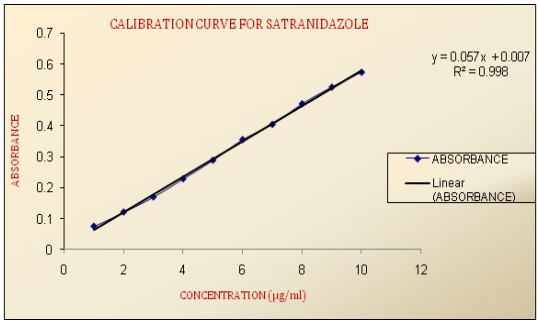


Fig I: Calibration Curve for Satranidazole

Drug Content Determination:

The assay of the different formulations F_1 (3:1) CTDC, F_2 (3:4) CTDC, F_3 (2:4) CTDC, F_4 (4:4) CTDC, F_5 (3:4 Acacia) CTDC, F_6 (4:2) CTDC, F_7 (3:2) CTDC, F_8 (4:3) CTDC, F_9 (1:3) CTDC, F_{10} (2:3) CTDC were determined as given in the experimental methods.

The assay or drug content in the formulations ranged between 90-97%. The assay value of F_1 (3:1) CTDC was found to be higher compared to the other formulations.

Table II: Drug content determination

S.No	Formulations	Assay
1	F1 (3:1)CTDC	96.95 % ± 0.75%
2	F2 (3:4) CTDC	90.72% ± 0.89%
3	F3 (2:4) CTDC	95.97% ± 0.67%
4	F4 (4:4) CTDC	96.32% ± 0.55%
5	F5 (3:4	94.79% ± 0.47%

	Acacia) CTDC	
6	F6 (4:2) CTDC	91.75% ± 0.64%
7	F7(3:2) CTDC	95.80% ± 0.53%
8	F8 (4:3) CTDC	96.14% ± 0.35%
9	F9 (1:3) CTDC	90.88% ± 0.86%
10	F10 (2:3) CTDC	93.73% ± 0.41%

Weight Variation Test

No of capsules taken= 20

Limit of % deviation = 90%-110%

Table III: Weight variation test

S.No	Formulation	% weight
1	F1	96.8 % ± 1.24%
2	F2	100.2 % ± 1.58%
3	F3	99.8 % ± 2.87%
4	F4	101.3 % ± 1.93%
5	F5	104.8 % ± 0.88%
6	F6	103.6 % ± 2.33%
7	F7	98.3 % ± 1.85%
8	F8	93.7 % ± 1.52%
9	F9	104.2 % ± 1.72%
10	F10	100.8 % ± 2.56%

Resistance of Coated Capsules:

All the capsules were rubbed on a sheet of paper. The capsules films were remaining intact, they were resilient and tough.

***In-Vitro* Studies:**

The *In-Vitro* release studies were performed as mentioned in the experimental methods. The result of the *In-Vitro* drug release studies carried out with different layer coated capsules in SGF (0.1 N Hcl, 2 h), SIF (pH 6.8, 3 h) and in SCF(pH 7.4) for up to 24 h in order to investigate the potential of formulations to withstand the adverse environment of upper gastrointestinal tracts. During 5 h study the formulation F_2 (3:4) CTDC released 6.898% of drug, F_3 (2:4) CTDC released 6.259% of drug, F_5 (Acacia3:4) CTDC released 7.29% of drug, F_9 (1:3) CTDC released 7.681% of drug and F_{10} (2:3) CTDC released 7.29% of drug in the dissolution medium as increased outer enteric coating layers when compared to inner hydrophilic layers. While the release rate was increased in case of F_1 (3:1) CTDC, F_6 (4:2) CTDC, F_7 (3:2) CTDC and F_8 (4:3) CTDC found to be 9.797%, 7.839%, 8.936% and 8.464% due to increased inner hydrophilic layers in comparison to outer enteric coating layers. F_4 (4:4) CTDC released 7.498% of drug as its inner hydrophilic and outer enteric coating layer thickness is same. Minimal release was observed in case of F_2 (3:4) CTDC, F_3 (2:4) CTDC, F_5 (Acasia3:4) CTDC, F_9 (1:3) CTDC and F_{10} (2:3) CTDC indicated its potential to remain intact in simulated gastro-intestinal conditions at 0.1 N Hcl & pH 6.8 respectively. The deviation among formulations may be due to increased thickness of enteric Eudragit S-100 layers as gastro resistant in nature as compared to HPMC layers which in turn promoted hydrophilicity on the coat resulted into fast erosion.

The percent drug release from F_2 (3:4)CTDC, F_3 (2:4) CTDC, F_9 (1:3) CTDC, F_{10} (2:3) CTDC and F_5 (Acasia3:4) CTDC at the end of 24 h study was found to be 44.837%, 43.506%, 40.667%, 37.938%, 26.181% in dissolution media containing plane PBS (pH 7.4). Similarly, in case of formulation F_8 (4:3) CTDC, F_6 (4:2) CTDC, F_1 (3:1) CTDC, F_7 (3:2) CTDC and F_4 (4:4) CTDC the total cumulative percent drug released was observed to be 40.76%, 43.339%, 39.352%, 35.194%, and 40.105% during 24 h dissolution studies. This showed that for formulation F_2 (3:4)CTDC, F_3 (2:4) CTDC, F_9 (1:3) CTDC and F_{10} (2:3) CTDC by increasing the enteric layers in comparison to hydrophilic layer the initial drug release pattern was significantly decreased as gastro resistant in nature. While the total cumulative percent drug release was more during 24 h studies due to fast erosion of coating layers in presence of PBS at pH 7.4. F_5 (Acacia 3:4) CTDC shows only 26.181% drug release at the end of 24 h dissolution study. This is because of the binding capacity of acacia that is used in the formulation.

In F_2, F_3 formulations there is no significant drug release up to 5 h after that significant drug release was observed up to 24 h when compared to other formulations. So, these two are the optimized formulations.

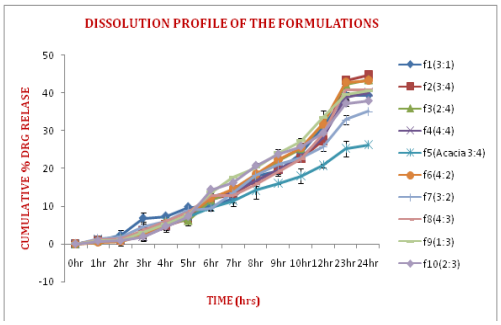


Fig II: Dissolution Profile of the Formulations

Release kinetics:

According to Regression coefficient values all formulations follow Zero order release kinetics by Diffusion mechanism. So, the dug release from the formulation is independent of concentration.

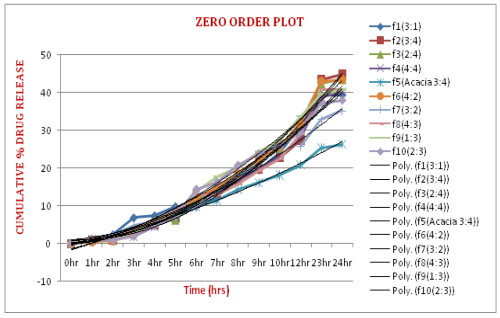


Fig III (a): Zero Order Release

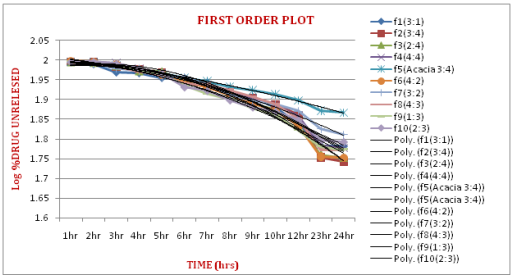


Fig III (b): First Order Release

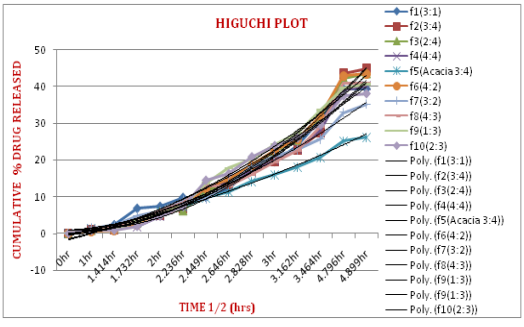


Fig III (c): Higuchi Plot (Diffusion Release)

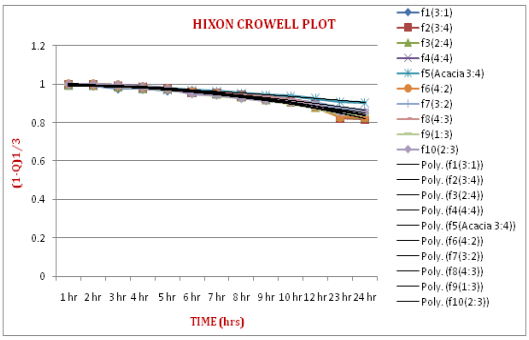


Fig III (d): Hixon Crowell Plot (Erosion Release)

Fig III: Release Kinetics of Different Formulations

Bioadhesion Studies

Mucoadhesion Testing by *In-Vitro* Wash-Off Test:

At the end of 30 min, at the end of 1 h, and at hourly intervals up to 10 h the weight of carbopol decreases. So, as time increases carbopol was washed-off from the tissue.

Table IV: Mucoadhesion testing by *In-Vitro* wash-off test

Time (h)	Total weight (g)	Setup weight(g)	Actual weight (g)
0	102.73	101.34	1.39
After 0.5	102.98	101.34	1.64
After 1	103.24	101.34	1.90
After 1.5	103.56	101.34	2.22
After 2	103.71	101.34	2.37
After 2.5	103.10	101.34	1.76
After 3	102.84	101.34	1.50
After 3.5	102.64	101.34	1.30
After 4	102.58	101.34	1.24
After 4.5	102.41	101.34	1.07
After 5	102.28	101.34	0.94
After 5.5	102.15	101.34	0.81
After 6	102.01	101.34	0.67
After 6.5	101.96	101.34	0.62
After 7	101.91	101.34	0.57
After 7.5	101.84	101.34	0.50
After 8	101.76	101.34	0.42
After 8.5	101.67	101.34	0.33
After 9	101.56	101.34	0.22
After 9.5	101.50	101.34	0.16
After 10	101.46	101.34	0.12

***Ex-Vivo* Residence Time**

Initial time: 10.00 am

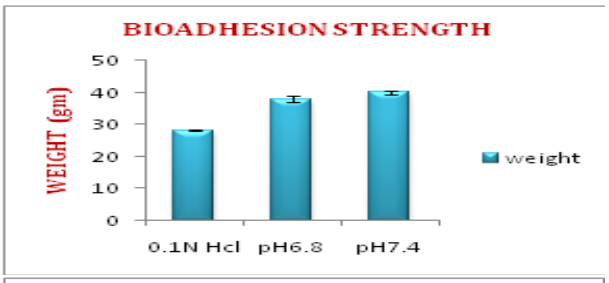
Ending time: 8.00 pm

After 10 h also some amount of carbopol adhere the colon tissue. So, the *Ex-Vivo* Residence Time of carbopol was more than 10 h. It shows that long time the carbopol attaches the colonic mucosa and prolongs the drug release.

***Ex-Vivo* Mucoadhesive Strength (Bioadhesion strength) & Peel adhesion Strength:**

Bioadhesion and Peel adhesion strength of carbopol was more in pH 7.4 when compared to pH 6.8 and 0.1 N Hcl.

Fig IV (a) and V (a): Instruments for determination of ex-vivo Mucoadhesion and peel adhesion strengths



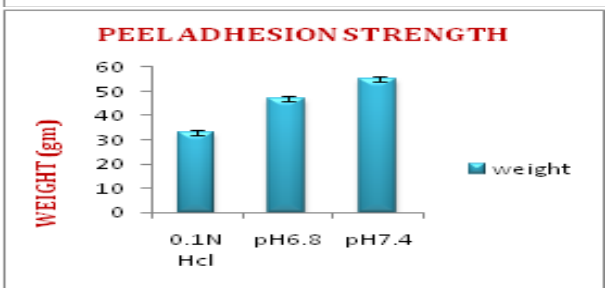


Fig IV (b) and V (b): Bioadhesion & Peel adhesion strengths of carbopol

Degree of Swelling

Wt of the sample before swelling (w_o) = 1.53 g

Wt of the sample after swelling (w_s) = 8.74 g

$\alpha = w_s\text{-}w_o/w_o$

$\alpha = 4.712$

Thumb Test:

Carbopol adheres very firmly to the thumb.

Rheological Studies

Viscosity & Thixotropic Studies

In Viscosity studies up to 5 h the viscosity was more in 0.1N Hcl and pH 6.4 buffers when compared to pH 7.4 buffers. But up to 5 h the capsule was present in small intestine after 6th h only it reaches the colon. Carbopol was released from the capsule in the colonic pH only, because Eudragit S-100 was a pH sensitive polymer (pH 7.0).

Viscosity studies also shows that at 6th h the viscosity of carbopol was more in pH 7.4 when compared to pH 6.8 and 0.1 N Hcl. Because of the increased viscosity carbopol adheres to the colon and releases the drug slowly. At 8th h viscosity was more in pH 6.8 than that of 0.1 N Hcl and pH 7.4 but at that time the formulation will be present in colon that was pH 7.4. So the effect of viscosity in pH 6.8 will be negligible at the end of 8th hr. In most of the experimental conditions viscosities were decreased up to 2.5 rpm, after that as rpm increases, viscosity also increases.

If the formulation present in colonic region it should withstand the peristaltic movement of the colon. If the viscosity of the formulation or system decreases it will be easily wash-off. To retain the dosage form or formulation at that site it is necessary to maintain higher viscosities. But, in colon peristaltic movements are less when compared to stomach and small intestine.

In all formulations rate of shear was progressively increased and the corresponding stress was measured using Viscometer. Along with the shear stress, rate of shear of all formulations were increased and all formulations behaves like either pseudoplastic systems or plastic systems. At first carbopol was in Gel state and it shows high consistency (multiple contacts were present between molecules) so that only it adheres to the colonic mucosa. On shearing, contacts were break down (shows low consistency) and it was in Sol state and it releases the drug in the colon.

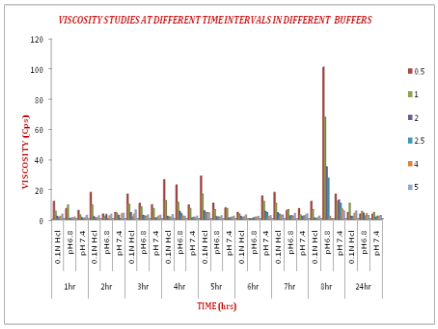


Fig VI: Viscosity Studies at Different Time Intervals in Different Buffers

Drug Penetration though Mucosal Membrane: (Diffusion Studies):

As time increased the % drug release from the formulation was also increased. This showed that the drug was penetrated though the membrane.

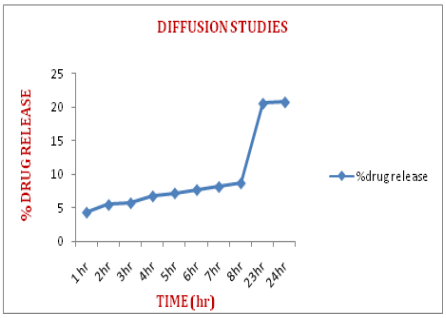
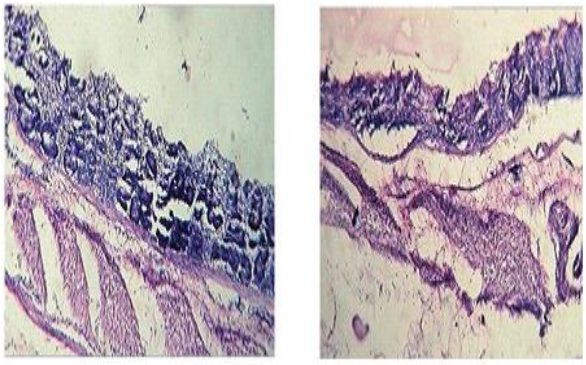


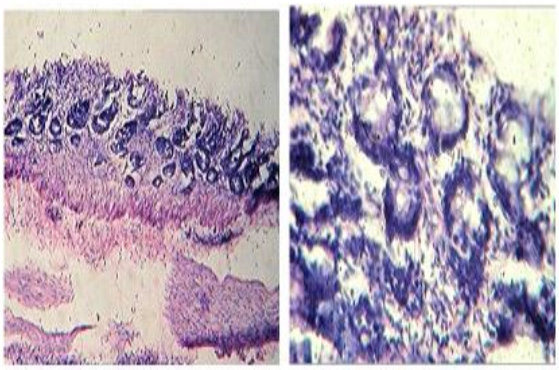
Fig VII: Diffusion Studies

Histopathology

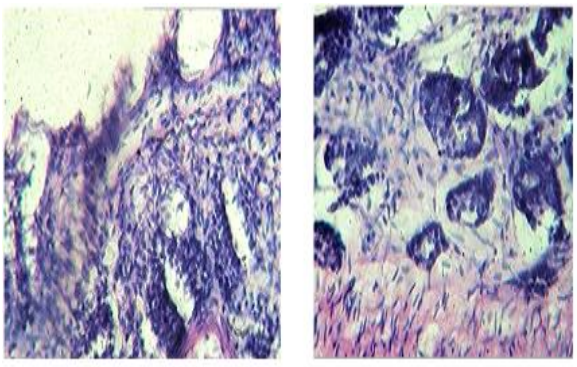
Figure VIII (a), VIII (d) shows the original mucosal layer structure of *Ovis* colon mucosa. Figure VIII (b), VIII (e) shows that the structure of the mucosal layer after treatment with carbopol which indicates destruction of mucosal cells because of the interactions between bioadhesive and mucus polymer chains. (That is adhesion of carbopol to the mucosa). When colonic mucosa was treated with carbopol and drug combination (figure VIII(c), VIII(f)) the cells of mucosa undergone swelling and erosion of some parts of mucosa observed, which indicates the penetration of the drug though the mucosal layer.



(a) *Ovis aries* Colon Mucosa (In 180 X Magnification) (b) *Ovis aries* Colon Mucosa Treated With Carbopol (In 180 X Magnification)



(c) *Ovis aries* Colon Mucosa Treated With Carbopol And Satranidazole (In 180 X Magnification) (d) *Ovis aries* Colon Mucosa(In 400 X Magnification)



(e) *Ovis aries* Colon Mucosa Treated With Carbopol (In 400 X Magnification)(f) *Ovis aries* Colon Mucosa Treated With Carbopol and Satranidazole (In 400 X Magnification)

Fig VIII: Histopathological studies

Compatibility Studies

Differential Scanning Calorimetry: (DSC)

Sharp endotherm was observed for Satanidazole at 187.59^{0}C, corresponding to its melting transition point. DSC scans of the Carbopol showed two endothermic peaks at 67.78^{0}C and 135.82^{0}C.

Physical mixture of Satanidazole and Carbopol exhibited endothermic peaks at 186.42^{0}C, 67.93^{0}C and 135.97^{0}C. In Satanidazole and Carbopol physical mixture smaller Changes was observed in Satanidazole melting transition point and in Carbopol

endothermic peaks when compared to pure Satranidazole and pure Carbopol. Hence it can be concluded that there is no chemical interaction between drug and excipient.

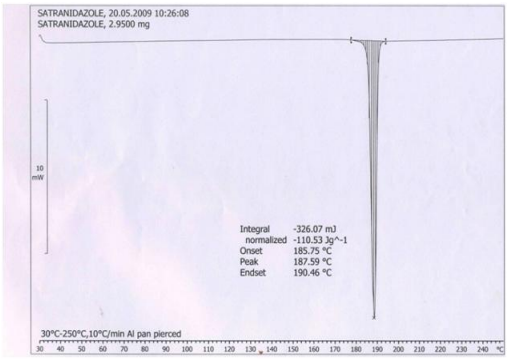


Fig IX (a): DSC endothermic peak of satranidazole

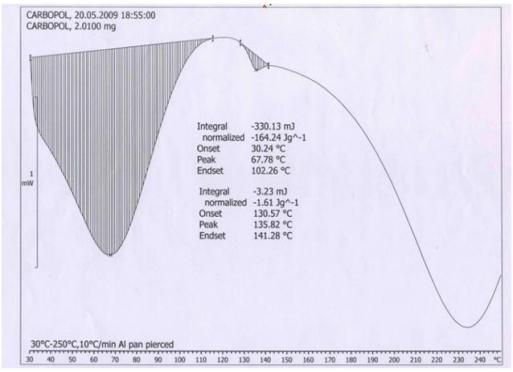


Fig IX (b): DSC endothermic peak of carbopol

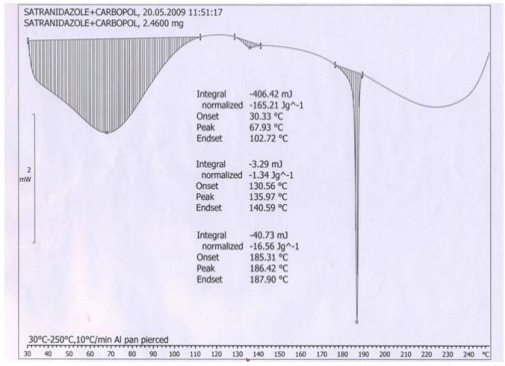


Fig IX(c): DSC endothermic peak of mixture of Satranidazole and Carbopol

Infrared Spectrometry (IR)

By observing the IR Spectrum of Satranidazole the Active functional groups which were present in the structure were observed at different peaks.

By observing the IR Spectrum of Satranidazole and excipient the Active functional groups which were present in the structure were observed at different peaks.

In Drug and excipient IR Spectrum the broad peak at 3433.08 cm^{-1} shows that the OH group present in the carbopol.

There was no appreciable change in the position and intensity of peak of the IR Spectrum of formulation (Drug and excipient) with respect to IR Spectra of Pure Satranidazole. Hence it can be concluded that there is no chemical interaction between drug and excipient.

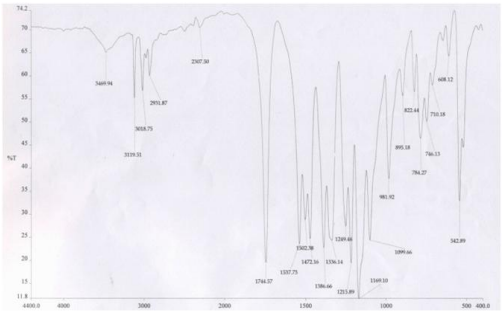


Fig X (a): IR Spectrum of Satranidazole

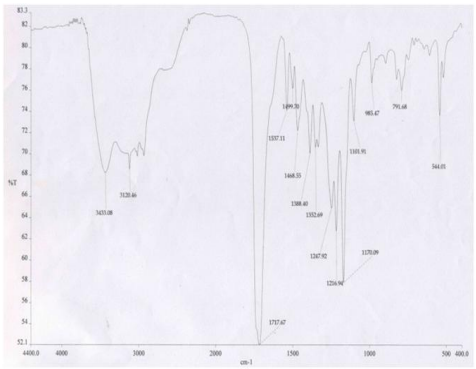


Fig X (b): IR Spectrum of mixture of Satranidazole and Excipient

CONCLUSION

There was a minimal drug release in case of F_2 (3:4) and F_3 (2:4) in 0.1 N Hcl and pH 6.8 buffer respectively at the end of 5 h study. They show more drug release in pH 7.4 (Colon pH) when compared to other formulations at the end of 24th h. So, F_2 and F_3 formulations were the optimized formulations for colonic drug delivery.

Bioadhesion Studies and Viscosity studies shows that carbopol is effective at pH 7.4 than 0.1 N Hcl and pH 6.8 buffers. So, it is useful for the adhesion of the colon mucosa. IR and DSC studies show that there were no interactions between the drug and excipients.

In conclusion Eudragit coated drug matrixed carbopol capsules can deliver and make available the intact drug for local action to the colon.

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REFERENCES

1. V. Ravi, T.M. Pramod kumar and Siddahramaiah. Novel Colon Targeted Drug delivery system using Natural Polymers. Indian. J. Pharm. Sci. Jan-Feb 2008, Vol 70 (1): 111-113.
2. Lanjhiyana Sanjay Kumar and Dangi Jawahar Singh, Development and *In-Vitro* Drug Release Studies of Methotrexate from Modified Pulsatile Release Guar Gum Based Enteric Coated Capsules for Colon Specific Drug Delivery. Indian. J. Pharm. Education& Research. Apr-Jun 2008; Vol 42(2): 154-159.
3. Lanjhiyana Sanjay Kumar and Dangi Jawahar Singh. Colon Specific Drug Delivery of 5-Fluorouracil- An *In Vitro- In Vivo* Testing Utilizing Guar Gum Polysaccharide as Carrier Matrix. J. Pharm. Research. Apr 2008; Vol 7(2): 101-105.
4. S.P. Vyas and Roop K. Khar. Controlled Drug Delivery- Concepts and Advances. 1st edition. Delhi: 2002, p. 218-253.
5. A Florence, Pu Jani. "Novel Oral-Drug Formulations-Their Potential in Modulating Adverse-Effects" Drug Saf. 1994, 410(3): 233-266.

6. Anlar S, Capan Y and Hincal A. "Physico-Chemical and Bioadhesive Properties of Polyacrylic Acid Polymers." Pharmazie. 1993, 48(4): 285-287.
7. Chang H S, Park H, Kelly P, Robinson J R. Bioadhesive Polymers as Platforms for Oral Controlled Release Drug Delivery-II: Synthesis and Evaluation of Some Swelling, Water- insoluble Bioadhesive Polymers. J. Pharmacol. Sci. 1985, 74: 229.
8. Smart J D. Some Formulation Factors influencing the Rate of Drug Release from Bioadhesive Matrices. Drug Devel. Ind. Pharm. 1992, 18(2): 223-232.
9. Smart J D. An *In-Vitro* Assessment of some Mucosa-Adhesive Dosage Forms. Int. J. Pharm. 1991, 73(1): 69-74.
10. Davies, N.M., Farr, S.J.,Hadgraft, J. and Kellaway, I.W. Evaluation of Mucoadhesive Polymers in Ocular Drug Delivery II. Polymer-Coated Vesicles, Pharm. Res. 1992, 9(9): 1137-44.
11. de Leeuw B J, Lueben, H L, Pérard D, Verhoef A C, de Boer A G, Junginger H E. The Effect of Mucoadhesive Poly (Acrylates) Polycarbophil and Carbomer on Zinc and Calcium Dependent Proteases, Proceed. Intern. Symp. Control. Rel. Bioact. Mater. 1995, 22: 2123.
12. Lehr C M, Bouwstra J A, Tukker J J, Verhoef A C, de Boer A G, Junginger, H E, Breimer D D. Oral Bioadhesive Drug Delivery Systems - Effects on G.I. Transit and Peptide Absorption. Pharm. Res. Sep 1990, 7(9): (Suppl.) PDD 7226.
13. Leung S H, Irons B K, Robinson J R. Polyanionic Hydrogel as a Gastric Retentive System. J. Biomater. Sci, Polymer Edition. 1993, 4(5): 483-492.
14. Longer M A, Chang H S, Robinson J R. Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery- III: Oral Delivery of Chlorothiazide Using a Bioadhesive Polymer. J. Pharm. Sci. 74(4), ETIN 1985; 16: 406-411.
15. Lueben H L, Leh C M, Rentel, C O, Noach A B J, de Boer A G, Verhoef J C, Merckle H P, Junginger, H.E., Effect of Poly(Acrylates) on the Enzymatic Degradation of Peptide Drugs by Intestinal Brush Border Membrane Vesicles. J. Control. Release. 1993, 29(1): 329-338.
16. Lueben H L, Bohner V, Pérard, Langguth P, Verhoef A G, de Boer A G, Verhoef J C, Junginger H E. Bioadhesive Polymers for the Peroral Delivery of Peptide Drugs (Polycarbophil, Carbopol® 934P NF, Chitosan). Proceed. Intern. Symp. Control. Rel.Bioact. Mater., 1995, 22: 2124.
17. Stephen W. Hoag, The Compaction Properties of Eudragit Polymers, http://www.pharmacy.umaryland.edu/faculty/shoag/
18. De Silva et al., Hydroxypropyl methylcellulose (HPMC) lubricant facilitates insertion of porous spherical orbital implants. Ophthal Plast Reconstr Surg. 2005 Jul, 21(4):301-302.
19. Williams et al., Method to Recover a Lipophilic Drug from Hydroxypropyl Methylcellulose Matrix Tablets. AAPS. Pharm. Sci. Tech. 2001, 2(2): article 8.
20. Richard A. Kendall and Abdul. Polymers in drug delivery. 2006, Newyork, p. 38-40.
21. Munira Momin and K.Pundarikakshudu. Design, Development and In Vitro Evaluation of Sennosides Taablets Containing Pectin HPMC for Colonic Drug Delivery. Ind. J. Pharm. Sci. May-Jun 2007, 394-401.
22. Munira Momin K.Pundarikakshudu and S.A.Nagori. Design and Development of Mixed Film of Pectin: Ethyl Cellulose for Colon Specific Drug Delivery of Sennosides and Triphala. Ind. J. Pharm. Sci. May-Jun 2008, 338-343.
23. Sanjay K. Jain, Anekant Jain, Yashwant Gupta, and Manisha Ahirwar. Design and Development of Hydrogel Beads for Targeted Drug Delivery to the Colon. AAPS. Pharm. Sci. Tech. 2007; 8(3): Article 56.
24. Leon lachman and Herbert A.Liberman, The Theory and Practice of Industrial Pharmacy. 3rd edition, Lea & Febiger, U.S.A. 1987, p. 392.
25. S.Jayaprakash, S.Mohamed Halith, P.U.Mohamed Firthouse, Jain Abraham, M.Nagarjan, V.Sankar. Colon Targetted Drug Delivery System of Metronidazole. Int. J. Pharm. Research. Jun-Dec 2008, Vol 1:19-24.
26. KPR. chowdary and Y. Srinivasa rao. Design, *In-Vitro* and *In-Vivo* Evaluation of Mucoadhesive Microcapsules of Glipizide for Oral Control Release- A Technical Note. AAPS. Pharm. Sci. Tech. 2003, 4(3): Article 39.
27. Vishnu M. Patel, bhupendra G. Prajapati, and Madhabhai M. Patel. Effect of Hydrophilic Polymers on buccoadhesive Eudragit Patches of Propranolol hydrochloride Using Factorial Design. AAPS. Pharm. Sci. Tech. 2007, 8(2): Article 45.
28. V.M. Patel et al., Design and Characterization of chitosan containing mucoadhesive buccal patches of propranolol hydrochloride. Acta Pharm. 2007; 57: 61-72.
29. Amal E, Kamel, Magda Sokar, Viviane Naggar and safaa A Gamal. Chitosan and Sodium Alginate-Based Bioadhesive Vaginal Tablets. AAPS. Pharm. Sci. Tech. 2002; 4(4): Article 44.
30. N.K.Jain, controlled and Novel drug Delivery. 1 st edition, 1997, Delhi. p. 364.
31. N.A.Nafee et al. Design and characterization of mucoadhesive buccal patches containing cetylpyridinium chloride. Acta Pharm. 2003; 53: 199-212.
32. Robert M. Silverstein, Francis X. Webster. Spectrometric Identification of organic Compounds. Newyork. 7 th edition, 2005.
33. Avinash Nangia. Science and Technology of Bioadhesive-Based Targeted Oral Delivery Systems. Pharm. Tech. Volume 32, Issue 11, p. 100.