

INVESTIGATION OF COLON SPECIFICITY OF NOVEL POLYSACCHARIDE-OKRA MUCILAGE-FILM COATED WITH ENTERIC MATERIALS

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ABSTRACT

- Okra polysaccharide as a microbially triggered material and also as the carrier to colon was previously developed and reported. The release profiles of the reported method revealed that okra mucilage, when used as a matrix tablet, does not protect the drug from being released in the upper parts of the gastro intestinal tract. In the present work the tablets were prepared with okra mucilage and coated with enteric materials to retard the drug release in the upper GI tract. Okra polysaccharide was isolated from *Abelmoschus esculentus* and used for tablet formulation with ibuprofen as model drug. The matrix tablets of okra polysaccharide were prepared by wet granulation method using ibuprofen as a model drug. These prepared matrix tablets were coated with enteric materials by pan coating technique. The *in vitro* release profile was performed with and without rat cecal content, revealed that the enteric coated formulations completely protected the drug from being released in the upper parts of the gastro intestinal tract. Among the enteric coated tablets, the 3% double layer coated tablets exhibited zero *in vitro* release profiles at 2nd and 5th hours. Hence, this tablet was subjected for further pharmacokinetic estimations and was compared with control tablet. At 5.5 h the ibuprofen appeared first in the blood; this showed the tablet was intact throughout the upper GI. The T_{max} and AUC estimates showed that the drug was released from the tablet to colon by bacterial degradation of mucilage.

KEYWORDS

Enteric polymers, okra polysaccharide, double layered film coating, HPTLC, Pharmacokinetic.

INTRODUCTION

Colon specific drug delivery systems have gained increased importance for systemic delivery of drugs^{1, 2}, as well as for local delivery for the diseases of the colon, like ulcerative colitis, Crohn's disease and colon cancer. Colon targeting not only reduces the dose to be administered, and also eliminates the incidence of possible adverse effects associated with these drugs³. Colon-specific delivery systems can be used to improve the bioavailability of protein and peptide drugs^{4, 5}.

An effective colon specific drug delivery system should release minimum amount of drug in the environment of the upper gastrointestinal tract, *i.e.*, in stomach and small intestine. The normal transit time in the stomach is 2 h, while in the small intestine it is around 3 h. The usual colonic transit time varies from 20–30 h. This is for a dosage form to be effective as a colon drug delivery system and the drug release is required to be retarded in the upper GIT conditions. Thereafter, the drug release should be completed within the next 20–30 h⁶.

Well documented approaches to achieve colon-specific delivery include pro-drugs⁷, pH-dependent systems⁸, time-dependent systems⁹, and biodegradable systems¹⁰. Hence, attempts are made to bring out ideal colon-specific delivery systems with improved site specificity and adequate drug release at the appropriate site and developed to accommodate different therapeutic needs. Efficient colon drug delivery system could be formulated with the combination of one or more approaches in a formulation. The use of combination of enteric materials and bacterially degradable polymers for colon-specific drug delivery seems to be a more site-specific approach as compared to other single approaches. These enteric polymers shield the drug from the environments

of the stomach and the small intestine and the drugs are further carried by the natural polysaccharide to the colon. On reaching the colon, they undergo assimilation by micro-organism¹¹ or degradation by enzyme¹² or breakdown of the polymer backbone¹³ leading to a subsequent reduction in their molecular weight and loss of mechanical strength. They are then unable to hold the drug entity any longer¹⁴.

The colon has microflora of 10^{11} - 10^{12} CFU per ml. The main bacterial population present is anaerobic bacteria which proliferate. The main saccharolytic species are Bactericides and Bifidobacterium. The vast microflora in the colon fulfills its energy needs by fermenting the various types of undigested substrates from the small intestine. The undigested portion of the food, *i.e.* truly physiological roughages such as di-, tri-polysaccharides, mucopolysaccharides, etc. reach the colon. To utilize these roughages as a source of carbon, bacteria produce a wide range of reductive and hydrolytic enzymes. Considering the aspect of the anaerobic bacteria of the colon able to react to the constantly changing mixture of complex carbohydrates entering the colon by recognizing a variety of substrates and producing the appropriate digestive enzyme, various systems have been developed for drug delivery to colon^{15, 16}.

In recent years researchers pay much attention to okra mucilage in pharmaceutical formulation. *Abelmoschus esculentus* mucilage had been used as matrix tablets along with ethylcellulose for colon delivery¹⁷, mini matrix for furosemide and diclofenac sodium tablets¹⁸, sulphafuanidine granules and tablets¹⁹ and investigated as well in release of indomethacin from bioadhesive tablets with carbopol²⁰.

Besides, this mucilage had been evaluated as a controlled-release agent in modified release matrices, in comparison with sodium carboxymethyl cellulose (NaCMC) and hydroxypropylmethyl cellulose (HPMC), using paracetamol as a model drug²¹. Our preliminary investigation on this polysaccharide showed that this polysaccharide is safe to use as a pharmaceutical excipients²².

In this investigation, okra in the form of matrix tablets formulated by wet granulation method and further coated with enteric material had been evaluated for its ability to remain intact in the physiological environment of stomach and small intestine. The susceptibility of okra to undergo biodegradation only in colon site is assessed by conducting *in vitro* drug release studies in the presence of rat cecal contents in pH 6.8 phosphate buffered saline (PBS) using ibuprofen as model drug. This research paper also illustrates the *in vivo* performance of the dosage form by ingesting enteric coated okra matrix tablet using rabbit as animal model by estimating the various pharmacokinetic parameters.

MATERIALS AND METHODS

Sodium metabisulfite was purchased from Merck specialties Pvt., Ltd., India, Acetone from Nicechemicals Pvt., Ltd., India, Ibuprofen gifted by Yarrow chem products, Mumbai, Ethylcellulose were from GlaxoSmithKline Pharmaceutical Ltd., Lactose monohydrate, talc, sodium hydroxide and potassium dihydrogen phosphate were purchased from S D-Fine chemicals, Magnesium stearate from Loba chemie Pvt. Ltd., Mumbai, Ethanol from Changshu YangYuan Chemicals(China). Ethyl acetate, n-hexane and anhydrous acetic acid from and Cellulose acetate phthalate from S.D.

Fine Chemicals (India). All other chemical were also of highest grade.

Extraction of the polysaccharide from okra fruits :

The reported method¹⁹ was modified for the extraction of okra polysaccharide. Fresh okra fruits were purchased locally. They were thoroughly washed with water, deseeded, sliced, homogenised with water containing 1% sodium metabisulfite and extrated by filtering through muslin cloth. The crude was centrifuged at 5000 rpm for 30 minutes and the mucilage was precipitated from the supernatant with addition of acetone. It was further dried with the help of microwave oven and pulverised.

Core tablet preparations:

Ibuprofen tablets were prepared by wet granulation, using lactose as the main filler. Okra mucilage was used as a binder. For a batch of 50 tablets, a single tablet ingredient was given in table I. The prepared powder mix was granulated using okra paste (6%). The wet mass formed was passed through a sieve with a nominal aperture size of 2 mm and dried in an oven for 2 hrs. The dried granules were screened through another sieve with a nominal aperture of 1mm. The sieved granules were blended with talc (1.5%) and magnesium stearate (1%). The granules thus obtained were made in to tablets of weighing 130 mg and containing 50 mg of ibuprofen were individually compressed at a maximum force of 4000 kg using 7 mm round and slightly concave punches on 12-station rotary tablet mini press-II MT(Remek, Ahmedabad, India). Tablets were tested for content uniformity, friability, disintegration. Tablets confirmed to IP limits for all the above-mentioned tests. Thickness of tablets was found to be within the permissible limits.

Table-I
Composition of Ibuprofen core tablets

Ingradients (for single tablet)	Quantity(mg) 130
Ibuprofen	50
Lactose	21.6
Magnesium stearate	01
Talcum	02
Okra mucilage	50
Ethylcellulose	0.4
Okra mucilage as paste	05

Tablet coating: The prepared core tablets were equally divided in to three groups. The first group and second group of core tablets were coated with cellulose acetate phthalate and ethyl cellulose respectively. The third group of core tablets was first coated with ethyl cellulose followed by CAP solutions (double layered film coating). Coating solutions were prepared by previously reported method²³.

Coating solutions were prepared using different coating concentrations such as 1%, 2% & 3% of polymers used for coating. The cellulose acetate phthalate, in acetone was used for coating and propylene glycol (1.5%) was used as plasticizer. For ethyl cellulose, in acetone was used and ethyl alcohol was used as plasticizer. The tablets were coated with different polymers, at two or three different concentrations. The desired volume of coating solution was poured on the prewarmed tablet bed in a pan coater. The tablets were coated and dried with the help of inlet air (temperature 35°–45°C). The coating process was repeated till the desired level of coating was achieved. The percent mass increase of the tablets upon coating was taken to be indicative of the coat thickness.

In vitro drug release studies^{24, 25} :

The formulated ibuprofen matrix tablets using okra were evaluated for their integrity in the physiological pH of stomach, the small intestine and colon. These studies were carried out using a USP XXIII dissolution rate test apparatus

(Apparatus 1, 100 rpm, 37 °C). The tablets were tested for drug release for 2 hours in pH 1.2 (900 ml) as the average gastric emptying time is about 2 hours. Then, the dissolution medium was replaced with pH 7.4 phosphate buffer (900ml) and tested for 3 hours as the average small intestine transit time is about 3 hours, the medium was once again replaced with pH 6.8 PBS (900ml) and the study continued for 10 more hours. At the end of each time period, 1ml sample was withdrawn, suitably diluted and analyzed for ibuprofen content at 265 nm using Double beam UV-visible spectrophotometer-2220 (SYSTRONICS, India).

In vitro drug release studies with and without 4% rat cecal contents^{24, 25} :

The tablets were tested for drug release for 2 hours in pH1.2 (100 ml) as the average gastric emptying time is about 2 hours. Then, the dissolution medium was replaced with pH 7.4 phosphate buffer (100 ml) and tested for 3 hours as the average small intestine transit time is about 3 hours, again the medium was replaced with 100 ml of pH 6.8 phosphate buffer with 4% w/v rat cecal contents and also with the same medium (pH 6.8 PBS) but without rat cecal content as control. The release study with rat cecal content was used to assess the susceptibility of the okra mucilage to the enzymatic action of colonic bacteria. At the end of each time period, 1ml sample was withdrawn, suitably diluted and analyzed for ibuprofen content at 265 nm using Double beam UV-

visible spectrophotometer-2220 (SYSTRONICS, India).

The cecal contents were obtained from male albino rats after pretreatment of the animal for 7 days with 1ml of 2% okra dispersion in order to induce enzymes specifically acting on okra gum in the cecum which provides the best condition for the *in vitro* evaluation of okra gum. Thirty minutes before the commencement of drug release studies, the rats were killed by spinal traction, their abdomen opened, the cecal bags isolated and ligated at both ends. The cecal bags were opened, their contents individually weighed, pooled and transferred to pH 6.8 (previously bubbled with CO₂) to give a final dilution of 4% w/v. All the operations were carried out under continuous CO₂ supply. The studies of drug release under the simulated environment in colon were carried out in USP XXIII dissolution rate test apparatus with slight modification. A beaker (capacity 150 ml internal diameter 55mm) containing 100ml of dissolution medium was immersed in water-filled 1000 ml vessel, which in turn placed in the water bath of dissolution apparatus. The matrix tablets were placed in the beaker containing pH 6.8 phosphate buffers containing the rat cecal matter. The experiments were carried out with the continuous CO₂ supply into the beaker to simulate anaerobic environments of cecum. The above study was carried out on optimized okra matrix tablet without rat cecal content also in pH 6.8 phosphate buffer (control).

Pharmacokinetic Investigation of matrix and film coated okra matrix tablets in rabbits:

The method described was applied to quantify the plasma concentration of ibuprofen in a single-dose pharmacokinetic study conducted on three rabbits of New Zealand strains. The protocol was approved by the Institutional ethical committee. The experiments were conducted as per CPCSEA (Committee for Prevention, Control and Supervision of Experimental Animals) guidelines. The rabbits weighing about 1.9 to 2.0 kg were fasted over night before tablet administration and until the

last blood sample was taken, but with free access to water. The interval between administrations was at least 1 week. Matrix and the enteric-coated core tablets were administered orally with 10 ml of water, and 1-mL blood samples were collected from a marginal ear vein using a heparinized syringe before dosing and at 2, 5, 8, 10, 12 and 14 h after administration. Plasma samples were immediately collected by centrifuging the blood sample at 3000 rpm for 15 min and the supernatant plasma layer was separated and stored at -20° C until analyzed. The plasma concentration of ibuprofen was determined by Camag HPTLC with Wincats software and Densitometric analysis of the separated components was carried out using a Camag TLC scanner 3 in the absorbance mode at 222 nm. Integration of the chromatograms was performed using the Camag TLC scanner/integrator system LCI- 100 with UV detection at 254 nm based on a previously described method²⁶ and was standardized with marked formulation. The solvent system consisting of n-hexane-ethyl acetate- anhydrous acetic acid (75:25:2), which gave dense and compact spots with sufficient separation in R_f values of ibuprofen and its metabolites, was selected. The maximum observed plasma concentration and corresponding time were defined as C_{max} and T_{max}, respectively, and the time of first appearance of acetaminophen in the systemic circulation was determined from the individual subject plasma concentration time profile. The finite area under the plasma concentration–time curves (AUC) from beginning to the last sampling time point was calculated with the linear trapezoidal method. The total area under the observed plasma concentration–time curve (AUC) was calculated by using the trapezoidal rule. The maximum observed ibuprofen concentration (C_{max}) and the time at which C_{max} was observed (T_{max}) were reported directly from the profile.

Data analysis:

The calibration curve and the raw dissolution data were analyzed. ANOVA was applied for statistical comparisons.

Ethical committee approval:

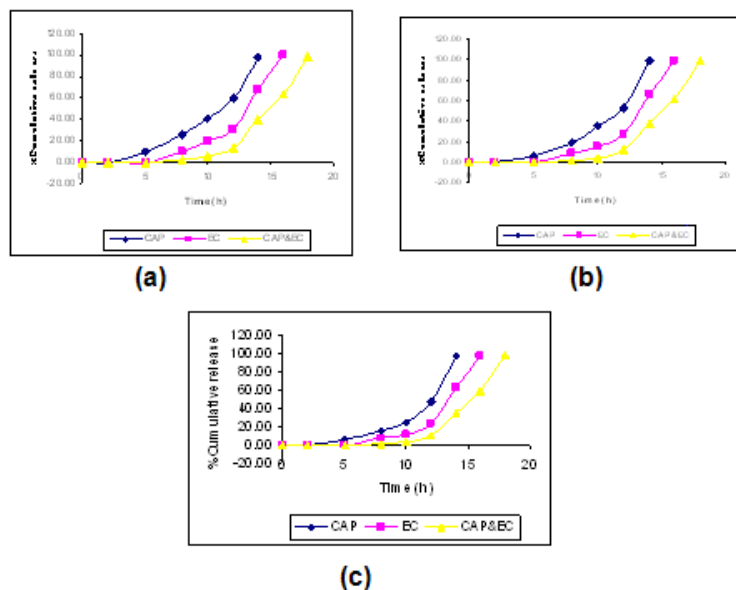
Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA).The Institutional Animal Ethical Committee (IAEC) of Karpagam College of Pharmacy, Coimbatore, Tamilnadu, India has approved the experimental protocols for this work.

RESULTS AND DISCUSSION

The present investigation is an attempt made to utilize the presence of polysaccharide in okra mucilage, as a carrier for microbially triggered colon-site-specific delivery system by film coating with enteric material using ibuprofen as a model drug. It was earlier reported that okra polysaccharide with ethylcellulose could be used as a carrier for colon-specific drug delivery in the form of a matrix tablet.

The sequential dissolution profile of tablets in buffers of pH 1.2, 7.4, and 6.8 is presented in Fig.1. Dissolution media of pH 1.2 and 7.4 were used to simulate the pH conditions in the stomach and small intestine, while pH 6.8 buffers was used to represent colon pH. By varying the concentration of CAP, ethylcellulose and double layer coat (CAP & ethyl cellulose) varied the percent drug release from the tablet significantly at the end of 5 h is evident from Fig.1 that the release of ibuprofen in pH 1.2 and 7.8 buffers was negligible. The formulation coated only with 1%, 2% & 3% of CAP showed 97.54%, 98.75 %, 97.22% drug release respectively at 14thh. In case of ethyl cellulose coated with 1%, 2% & 3% formulation showed about 99.67%, 98.99%, 98.18% drug release respectively at 16th h. At the same time the formulation coated with 1%, 2%, & 3% mixture of ethyl cellulose and CAP showed 99.24%, 99.02%, 98.89% drug release respectively at 18th h. The results indicated that the enteric coating applied appeared sufficient to prevent premature drug release in the stomach and small intestine.

Figure1.
Cumulative percent of drug released (mean ± S.D, n = 3) versus time profile for tablets coated with: a) 1%, b) 2% & c) 3%.



***In vitro* dissolution studies with and without rat cecal matter:**

In vitro dissolution studies of 3% double layered film (CAP & ethyl cellulose) coated tablets were carried out using pH 1.2, pH 7.4 and pH 6.8 phosphate buffer with and without rat cecal matter in order to mimic conditions as in stomach to colon environment. The results of cumulative percentage drug release at pH 1.2 and pH 7.4 were 0, at the 2nd h and 5th h. The percentage cumulative release of the drug in pH 6.8 PBS with out rat cecal matter was found to 98.89 at the 18th h. The study was repeated with one more set of double layer coated tablet, the results of percentage drug release at pH 1.2 and pH 7.4 were found to be 0 at 2nd and 5thh, but the %cumulative release with rat cecal content in pH 6.8PBS was found to be 98.52 at the 14thh.

Pharmacokinetic studies:

The ibuprofen concentrations were estimated using HPTLC method and the chromatogram is shown in the figure 2, 3 & 4.

Table-II shows the T_{max} , C_{max} , and $AUC_{(0-18)}$ values obtained from the mean plasma concentration–time profiles of ibuprofen after oral administration of enteric coated okra matrix tablets and the reference dosage form (i.e., uncoated okra matrix tablet). Table I also shows the first appearance time of ibuprofen in the systemic circulation. Irrespective of the absorption onset, the plasma concentration of ibuprofen reached the maximum and then declined at a slower rate from tablet than the reference uncoated matrix tablet. For enteric coated tablets, the C_{max} and $AUC_{(0-18)}$ values were significantly reduced while T_{max} was increased significantly compared with the reference. Thus, both rate and extent of ibuprofen absorption from coated tablets were significantly decreased. This indicates that the absorption of ibuprofen from coated tablet most likely occurred in the lower GI tract of the rabbit

because it has been reported that the colonic absorption rate of ibuprofen is two or three times slower than that in the small intestine. Ibuprofen was first detected in the plasma samples from the matrix tablets at 1.5 (\pm 0.2) h after dosing. The results indicated that the time frame of gastric retention of the tablet was within 1.5 h because the matrix swells before the drug was released and absorbed after the tablets were emptied from the stomach. This time frame of gastric retention is also believed to be applicable to coated tablets because of the essentially identical tablet size and the same group of rabbits used in the study. In contrast, the average time for the first appearance of ibuprofen in the systemic circulation was 5.5 (\pm 1.0) h after dosing of coated tablets. The delayed onset of ibuprofen absorption can be attributed to the presence of the double coating layer in the coated tablets, which prevented the drug release in the small intestine.

The colon arrival time of coated tablets can be expressed as the summation of the gastric retention time and the transit time in the small intestine. As estimated from the reference, the gastric retention time of uncoated tablets was approximately 1.5 h. Together with the fact that the small intestinal transit of the tablets is about 3 h, it can be approximated that coated tablets arrived at the colon about 4.5 h after dosing. The difference between the onset of ibuprofen absorption and gastric emptying of coated tablets is about 5.5 h; this difference would then correspond to the time frame in which the transit of coated tablets in the small intestine as well as the initiation of drug release in the colon occurred. Therefore, it can be concluded that the onset of drug release from coated tablets took place in the proximal colon in rabbits. Additional experimental evidence supporting this conclusion is the reduced drug absorption and the increase in T_{max} value from coated tablet.

Table-II
Pharmacokinetics of Ibuprofen from enteric double film coated okra tablets and uncoated tablets

Tablets	T _l (h)	T _{max} (h)	C _{max} (mcg/mL)	AUC (mcg x h/mL)
Uncoated	1.5 ± 0.7	3.0 ± 0.7	1166.3 ± 260.6	2046.8 ± 250.4
Double layered film coated	5.5 ± 0.5	8.5 ± 0.9	752.6 ± 154.4	1622.2 ± 258.8

Figure 2
Ibuprofen chromatogram with out plasma. Peak 1 is ibuprofen peak.

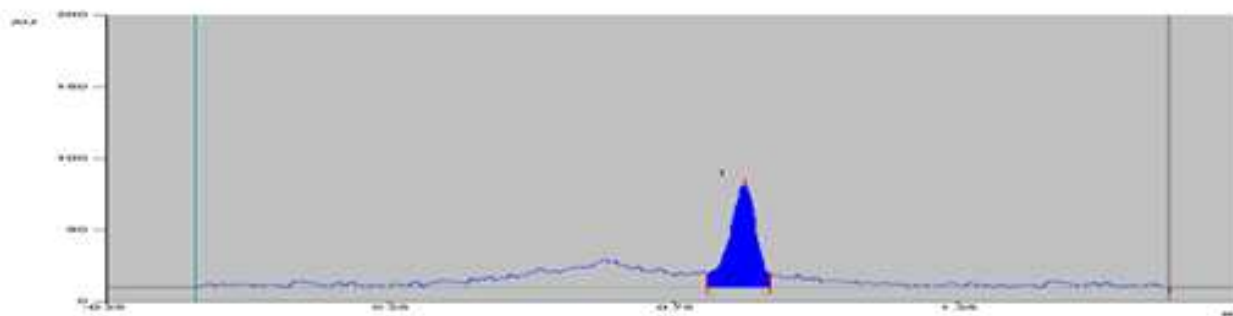


Figure 3
Ibuprofen chromatogram spiked in Plasma. Peak 3 is ibuprofen peak.

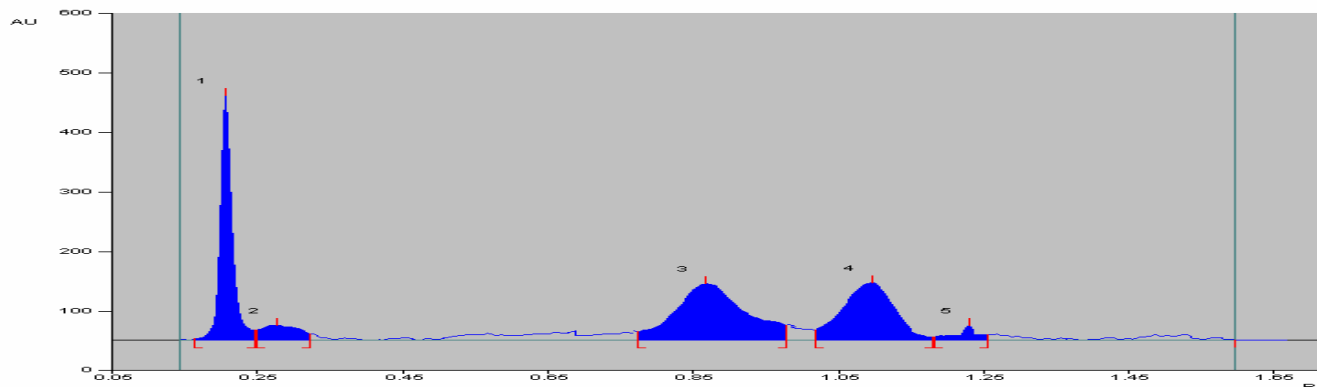
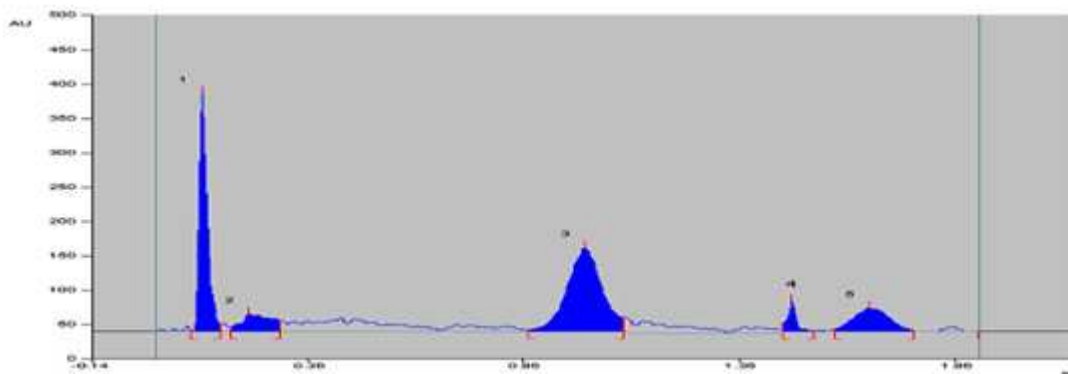


Figure 4

Ibuprofen Chromatogram after ingestion of double layered tablet. Peak 3 is ibuprofen peak.



CONCLUSION

At a coat concentration of 3%, double coat (first with ethylcellulose followed by CAP) provided the most appropriate polymer coat for colon specific drug delivery in the present study, which may be useful for local colonic pathologies and for systemic drug delivery. Okra mucilage is a

natural polymer that is also abundantly available and cost effective. Moreover, the study shows that it provides a site-specific drug delivery. Variation in coat thickness and percentage of polysaccharide can facilitate the drug delivery to terminal ileum, distal or proximal colon.

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