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Colon specific delivery of Eudragit E-100 and Eudragit RL-100 coated tablets of Rifaximin Using chitosan-chondroitin sulphate Interpolymer complex

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ABSTRACT

The present investigation was carried out to develop colon targeted drug delivery system which consists of chitosan-chondroitin sulphate interpolymer complex as a binder containing rifaximin in the core coated with Eudragit E-100 and Eudragit RL-100.The chitosan-chondroitin sulphate interpolymer complex was characterized by Fourier Transform Infrared Spectroscopy. *In vitro* release studies of coated tablets were carried out for 2 h in pH1.2 HCl buffer, 3h in pH 7.4 phosphate buffer and 19 h in pH6.8 phosphate buffer in the presence and absence of rat caecal content. A drug release of 27.13% was observed with uncoated tablets in HCl buffer pH 1.2, 80.56% of drug release in phosphate buffer pH 7.4 and 99.23% was observed in phosphate buffer pH6.8. Also tablets coated with Eudragit E-100 and Eudradgit RL-100 with different coat weight showed less than 10% of drug release in the stomach whereas same tablets showed 23.22%, 15%, 13.39%, 12.83% release of drug in pH 7.4 phosphate buffer and 73.26%, 75.91%, 72.23%, 71.93% release of drug in pH 6.8 phosphate buffer. Histopathology of rat colon after administration of Eudragit E-100 and Eudradgit RL-100 coated tablets containing chitosan-chondroitin sulphate interpolymer complex revealed marked reduction in acetic acid induced colitis in test group.

Keywords: chitosan, chondroitin sulphate, Eudragit E-100, Eudradgit RL-100, rifaximin

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INTRODUCTION

The target for robust therapy is to match the needs of the patient while improving the efficiency and safety of the administered drugs. Oral drug delivery is the most patient complaint and preferred route of delivery. In the area of targeted drug delivery, the colonic region of the gastrointestinal tract (GIT) is the one that has been embraced by scientist and is extensively investigated over the past two decades.[1]

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 large intestine. Targeted drug delivery to the colon has been recognized to have several advantages. A smaller dose is required; therefore reduced incidence of undesirable systemic adverse reactions can be expected. A number of colonic diseases could be treated more efficiently by delivering drug locally in the colon such as Crohn's disease, ulcerative colitis, colorectal cancer and irritable bowel syndrome (IBS).[2]

The present study was to design a delivery system using rifaximin, a non systemic rifamycin-derived antibiotic as a model drug and has been investigated to target it to the colon for treatment of inflammatory bowel diseases (IBD) with core containing chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC complex) coated with Eudragit E-100 and Eudragit RL-100 as release retardant and Eudragit FS30D as a reference material for coating.

MATERIALS AND METHODS

Rifaximin was received as a gift sample from Lupin Pharma (Jammu, India). Chitosan and chondroitin sulphate were purchased from Himedia (Mumbai, India). Eudragit E-100 was procured as a gift sample from Panacea Biotech Lalru, Eudragit RL-100 was procured as a gift sample from Kwality Pharmaceuticals, Amritsar and Eudragit FS30D was obtained from Evonic Degussa, Mumbai. All other chemicals and solvents used were of analytical grade.

Preparation of binder solution of chitosan-chondroitin sulphate interpolymer complex

The interpolymer complex of the polysaccharides was prepared by mixing 7.5 mg of chitosan (CH) in a 1 ml of 3% w/v solution of glacial acetic acid in distilled water with magnetic stirring for 30 minutes so as to enable the chitosan to swell. 7.5 mg of chondroitin sulphate (ChS) was separately dissolved in 1ml of water. To both the mixtures, 2ml of 5 M ammonium acetate was distributed equally.[3] The homogenous solution was used as a binder for the powder mixture during wet granulation

PREPARATION OF COLON TARGETED DRUG DELIVERY SYSTEM

Formulation and evaluation of rifaximin granules and tablets

Tablets (average weight 315mg) containing 100 mg of rifaximin were prepared by wet granulation technique. Rifaximin granules were prepared by passing the wet mass through #22 sieves and retaining on # 44 sieves. The rifaximin granules and magnesium stearate were mixed by tumbling method and compressed using convex three punches, single station Rotary Compression Machine (Popular Cant Laboratory Limited). The blend for the preparation of tablets was evaluated before the compression for parameters like Angle of Repose, Carr's Consolidation Index and Hausner's Ratio. Tablets were evaluated for the parameters like hardness, surface appearance, size measurement, friability, content uniformity, weight variation and disintegration.[4] The formulation of rifaximin tablets is summarized in Table 1.

Table 1: Formulation ingredients for preparing rifaximin tablets

S. No	Ingredients	Quantity (mg)
1	Rifaximin	100
2	Lactose Monohydrate	180
3	Chitosan	7.5
4	Chondroitin Sulphate	7.5
5	PVK-30	5
6	Magnesium stearate	5

COATED TABLETS OF RIFAXIMIN

Preparation of coating solution and coating of core tablets of riafximin

A coating solution of Eudragit E-100 was prepared by dissolving 10% (w/v) Eudragit E-100 in isopropyl alcohol. A coating solution of 5% (w/v) Eudragit RL-100 was prepared in acetone. The tablets were coated to 5% total weight gain for F1, F2, F3, F4 and F5 respectively. A 30% (w/w) aqueous Eudragit FS30D dispersion was used.[5] The percentage coating weight was 5%, 7.5%, 10%, 12.5% and 15% total weight gain for formulation F1, F2, F3, F4 and F5 respectively. The coating was performed with a conventional pan coating machine (A K Industries M1107, Nakodar, India) with a tablet bed temperature of 42°C and rotating speed of pan at 20 rpm.

RELEASE OF RIFAXIMIN FROM FORMULATED TABLETS

***In vitro* dissolution studies of rifaximin uncoated and coated tablets**

Dissolution studies were carried using dissolution apparatus i.e.Apparatus1-Rotating Basket type (LABINDIA DISSO 2000, Digital tablet dissolution test) utilizing temperature of 37 ± 0.5°C with constant stirring rate of 50 rpm. The uncoated as well as coated tablets prepared using chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) as binder were evaluated for drug release by sequential exposure to HCl buffer pH 1.2 (700 ml) for 2 h, Phosphate buffer pH 7.4 (780 ml) for 3 h and finally in Phosphate buffer pH 6.8 (790 ml) for 19 h. A sample of 5 ml

was withdrawn from each dissolution vessel at regular intervals and replaced with equal volume of respective fresh dissolution medium. Amount of drug released was determined by UV-Visible spectrophotometer (Blue Star AU-2701) employing wavelength of 296 nm for both acidic and basic conditions.[6]

Dissolution studies in the presence of rat caecal content

Preparation of rat caecal content medium

The procedure was approved by Institutional Animal Ethics Committee. Wister rats weighing 200-300 g were used. For these studies, thirty minutes before the commencement of drug release studies, two rats were sacrificed by spinal traction. The abdomen was cut open; caecum was ligated at both ends and cut loose[7]. The formed caecal bag was immediately transferred into phosphate buffer pH 6.8. The caecal bags were opened; their contents were weighed, pooled and suspended in phosphate buffer pH6.8. *In vitro* dissolution studies were carried out using dissolution apparatus i.e. Apparatus1-Rotating Basket type (LABINDIA DISSO 2000, Digital tablet dissolution test) utilizing temperature of 37 ± 0.5°C with constant stirring rate of 50 rpm in the presence of rat caecal contents to confirm the susceptibility of chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) to the colonic bacteria.

PHARMACODYNAMIC STUDIES

In order to study the ameliorating effect of rifaximin on the inflamed tissue of colon in inflammatory bowel disease, Acetic acid model was selected which is simple and reproducible.[8]

Induction of colitis in rat

Wistar rats (average weight 140-160g, n=3/group) were used. They were housed in a room with controlled temperature condition (23ºC). The animals were food fasted 23 h before experimentation and allowed food and water after the administration of acetic acid (2 mL of 4% acetic acid in saline). To induce an inflammation, all groups were treated with acetic acid except for the healthy control.

The rats were catheterized 8 cm intrarectal, after anaesthised with ketamine and further, 2ml of 4% acetic acid in saline was injected into colon via butterfly canulla. Animals were then maintained in a vertical position for 45 s followed by washing with 0.9% saline and returned to their cages. The rats were housed without treatment to maintain the development of a full inflammatory bowel disease model for 24 h. The animals of both standard and test groups received uncoated and coated rifaximin tablets by oral route. The animals of all groups were examined for 24 h for rectal bleeding.[8]

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectrographs of rifaximin, chitosan (CH), chondroitin sulphate (ChS) and chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) formed by interacting chitosan (CH) and chondroitin sulphate (ChS) are depicted in Figures 1, 2, 3, 4 and 5 respectively.

Further, in the spectra of rifaximin and chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC), the peaks at 3435.90 cm^{-1}, 1716.82 cm^{-1} and 1646.66 cm^{-1} confirmed that OH stretch and C=O stretch was intact as such which confirmed that there was no interaction between the rifaximin and the chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) as indicated in Figure 5.

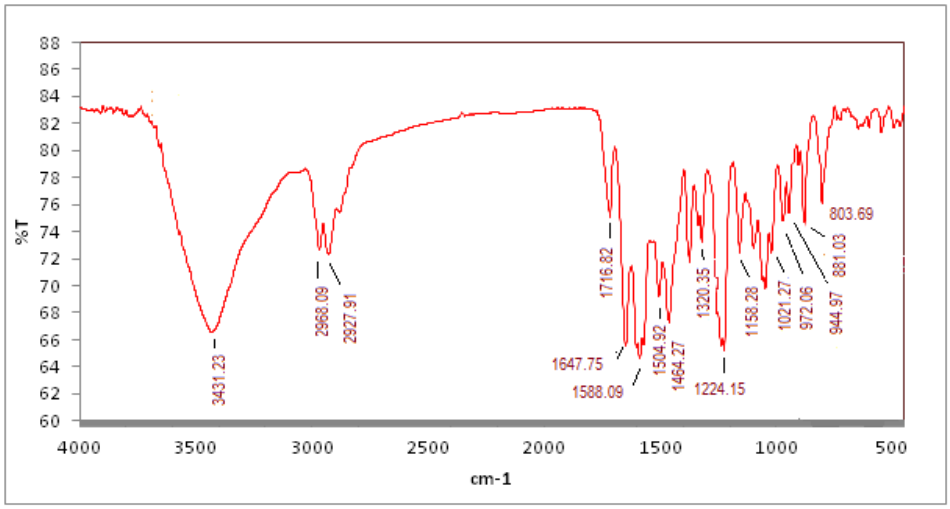


Figure 1: FTIR spectra of rifaximin

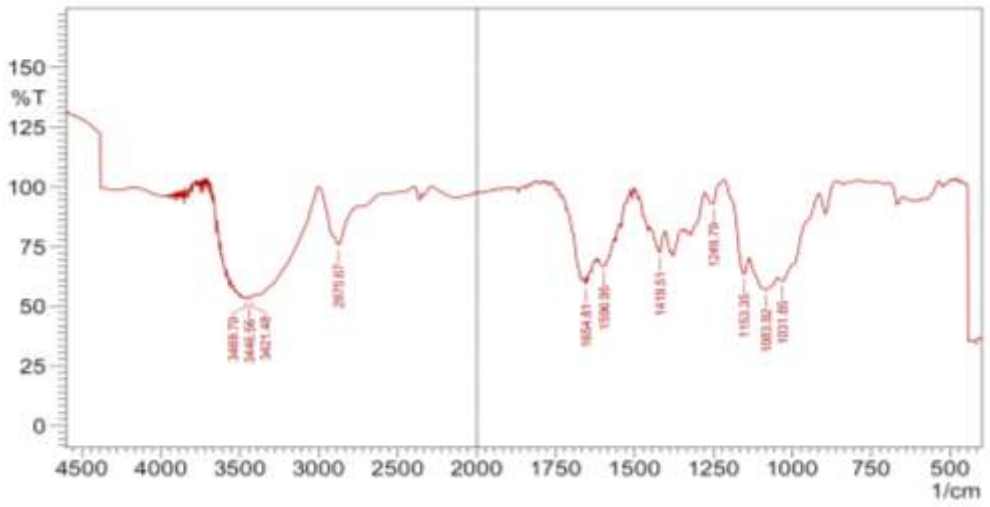


Figure 2: FTIR spectra of chitosan

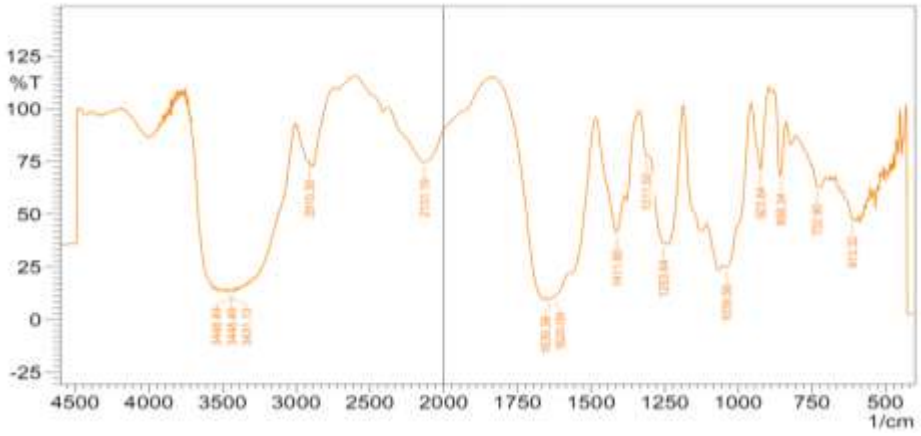


Figure 3: FTIR spectra of chondroitin sulphate

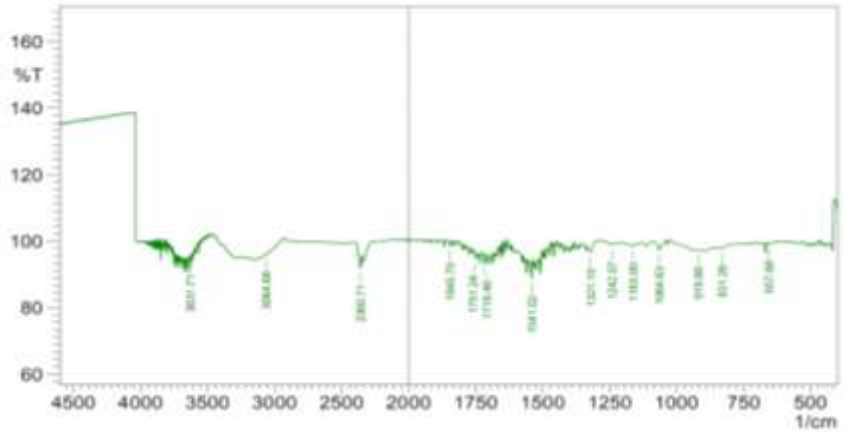


Figure 4: FTIR spectra of chitosan-chondroitin sulphate

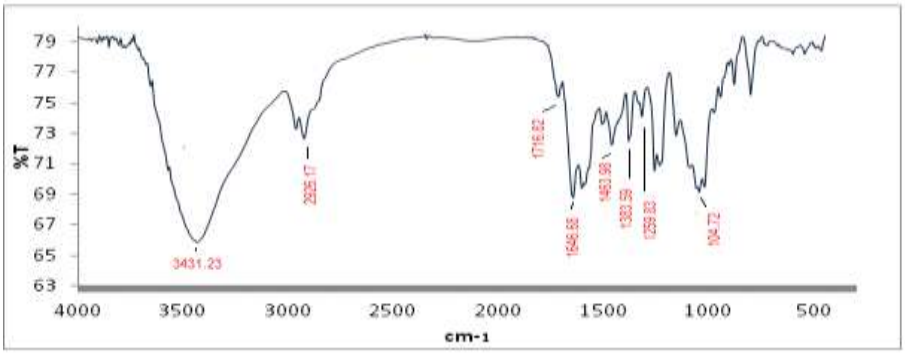


Figure 5: FTIR spectra of rifaximin : chondroitin interpolymer complex

Evaluation

The granules of rifaximin for matrix tablets were prepared using lactose monohydrate as diluents. Angle of Repose, Carr's Consolidation Index and Hausner's Ratio are indicative of relative flow rate, cohesiveness and particle size of granules. It was inferred from Table 2 that there was no significant difference in the flow properties of rifaximin granules containing lactose monohydrate.

The tablets were evaluated as per the Pharmacopoeial tests and all the prepared batches were found to have acceptance value as indicated in Table 3 which is below the maximum 15% USP tolerance limits prescribed in Pharmacopoeia for friability and content uniformity.[9] Hence, all the batches passed the Pharmacopoeial tests.

Table 2: Evaluation of rifaximin granules

Batch Code	Angle of Repose (deg) Mean ± S.D	%Carr's Consolidation Index Mean ± S.D	Hausner's Ratio Mean ± S.D
A	28 ± 1.1	12 ±0.5	0.93 ±0.24
B	25 ± 0.8	12.3 ± 0.5	1.00 ±0.01
C	27 ± 0.9	13.2± 1.1	1.16±0.06
D	22 ± 0.6	13.1 ± 0.6	1.37± 0.54
E	28 ±1.2	14 ± 1.2	1.00± 0.40

Table 3: Evaluation of rifaximin uncoated tablets formulation

Batch Code	Weight (mg) Mean±S.D	Axial diameter (mm) Mean ± S.D	Radial diameter (mm) Mean ±S.D	Hardnes (kg/cm^2) Mean±S.D	Weight variation (%) Mean ± S.D	Friability (%) Mean ±S.D	Content uniformity (%) Mean±S.D
A	315 ± 0.34	4.56 ± 0.04	10.63± 0.22	5.5 ± 0.1	7.5±0.45	0.24±0.01	5.35±0.56
B	317 ± 1.1	4.88 ± 0.14	10.74± 0.22	5.7 ± 0.2	6.23 ± 0.12	0.26±0.07	5.31±0.75
C	311 ± 1.52	4.02 ± 0.007	10.24± 0.15	6.1 ± 0.4	6.46±0.14	0.29±0.032	5.66±0.57
D	314 ± 0.47	4.27 ± 0.04	10.37± 0.22	5.6 ± 0.4	5.68± 0.41	0.23±0.041	5.58±0.36
E	313 ± 1	4.27 ± 0.04	10.34± 0.11	6.1 ±0.3	6.23± 0.12	0.22±0.049	4.58±0.76

Release of Rifaximin From Formulated Tablets

***In vitro* dissolution studies of rifaximin uncoated tablets**

Colon release dosage forms should ideally be tested by sequentially subjecting the tablets to HCl buffer pH 1.2, phosphate buffer pH 7.4 and finally to phosphate buffer pH 6.8.[10] It is evident from the percentage cumulative drug release-time profile in Figure 6 that the uncoated tablets released approximately 27.13% of rifaximin in HCl buffer pH 1.2 and drug release of 80.56% was observed in phosphate buffer pH 7.4 and 99.23% in phosphate buffer pH 6.8. It indicates that uncoated tablets using chitosan and chondroitin sulphate interpolymer complex (CH-ChS IPC) at the concentration of 1.5% w/v as binder when used alone were not capable of preventing rifaximin from being released in physiological environment of stomach and small intestine. Therefore, tablets were further coated with Eudragit E-100 and Eudragit RL-100 using different coated weight solution.

***In vitro* dissolution studies of rifaximin coated tablets**

In gastrointestinal tract (GIT), luminal pH in the proximal small bowel ranges from 5.5 to 7.0 and gradually rises to 6.5–7.5 in the distal ileum. There has been a fall in luminal pH from the terminal ileum to the caecum (range 5.5–7.5) and thereafter pH rises in the left colon and rectum

to 6.1–7.5. During an acute attack of inflammatory bowel disease, the normal pH of the colon lumen often decreases significantly. This pathological drop in the luminal pH of the colon favors the development of a coated dosage form with the acid-soluble coating film of Eudragit E-100 to achieve drug delivery to colon using chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) as a binder in the tablet core.[11] Therefore, tablets were coated further with Eudragit RL-100 as Eudragit RL-100 is a pH independent polymer and expected to ensure sustained and time controlled drug release in the intestine. Thus, it favors the development of dosage form coated with Eudragit E-100 and Eudragit RL-100.[11]

The dissolution profiles given in Figure 7 indicate that all the batches, except F1 were found to release less than 10% of the rifaximin in the stomach, thus complying with USP standards for enteric coated tablets. Hence, the observed release kinetics of rifaximin indicated that the tablets coated with Eudragit E-100 and Eudragit RL-100 in Batches F2, F3, F4 and F5 complied with enteric release requirements while those coated in F1 did not exhibit enteric release compliance.

Dissolution studies in the presence of rat caecal contents

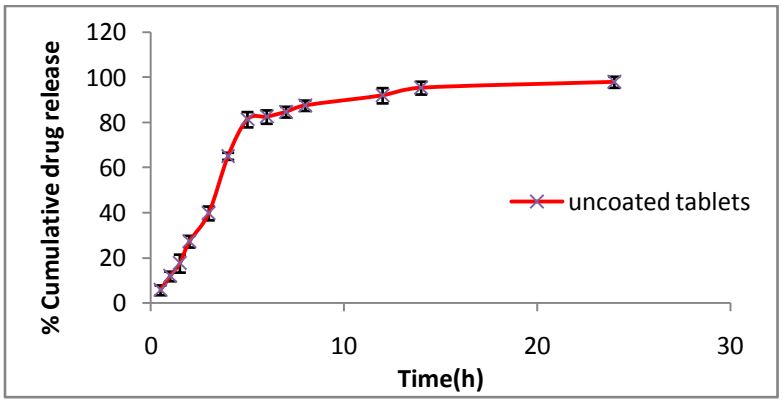


Figure 6: Rifaximin release from uncoated tablets

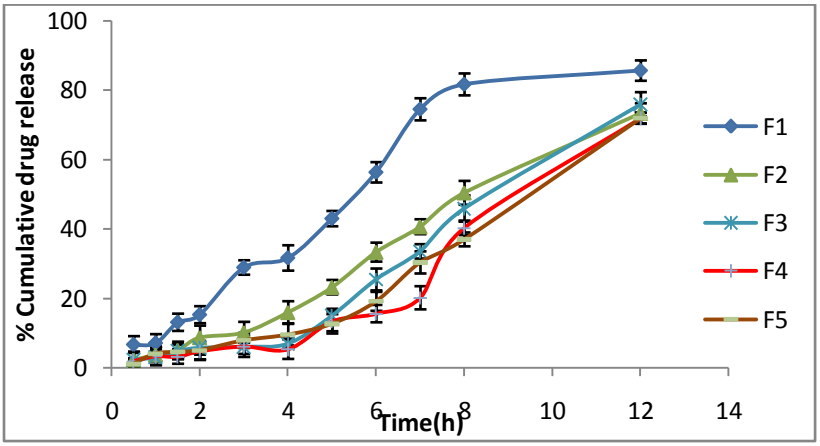


Figure 7: Comparison of rifaximin release from Eudragit E-100 and Eudragit RL-100 coated tablets

Eudragit FS30D is an aqueous dispersion of an anionic copolymer based on methyl acrylate, methyl methacrylate and methacrylic acid, which is insoluble in acidic media, but dissolves by salt formation above pH 7.[12] The tablets were coated with Eudragit FS30D with the weight gain of 10% w/w in formulation F6 and was selected for further comparison with F3 by adding rat caecal contents.

Drug release studies carried out in phosphate buffer pH 6.8 with rat caecal contents indicate higher drug release in presence of rat caecal contents. This may be due to the presence of high concentration of enzymes caused by the multiplication of bacteria present in caecal content which might have further enhanced the rate of biodegradation of coated and matrix polysaccharides.

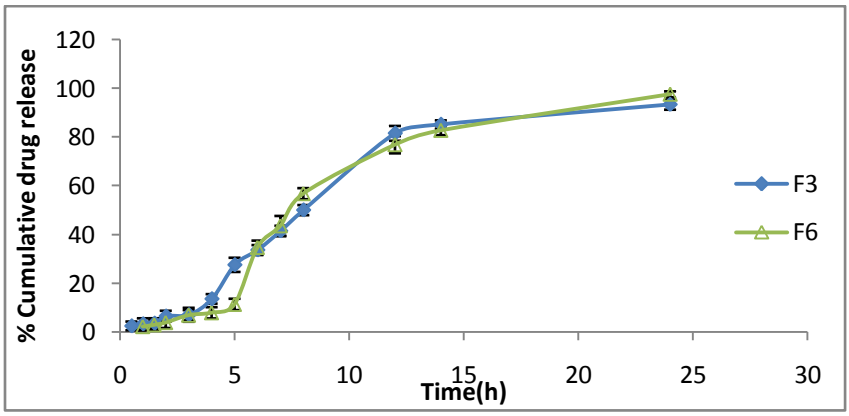


Figure 8: Comparison of rifaximin release from Eudragit E-100 and Eudragit FS30D coated tablets with 10% w/w gain in rat caecal contents

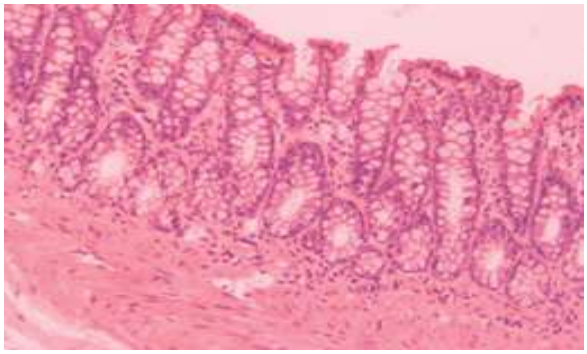
Histopathological Studies

The ameliorating effect of uncoated, Eudragit E-100/Eudragit RL-100 and Eudragit FS30D coated rifaximin tablets were investigated on acetic acid induced colitis in rats.

The results of the histopathological studies revealed no sign of colitis or epidermal damage in healthy group (Group I). Colitis control (Group II) resulted in shedding of mucosal epithelium. The intestinal crypt show mild distortion, otherwise were regularly placed. The lamina propria and submucosa showed intense infiltration Group III receiving uncoated tablets showed slight recovery from mucosal erosions and inflammatory infiltrate. The lamina propria showed mild to moderate inflammatory infiltrate. Group 1V receiving Eudragit E-100 and Eudragit RL-100 coated tablets resulted in slight recovery from inflammatory infiltration. It showed intact mucosal epithelium. The test group V receiving Eudragit FS30D coated tablets also indicating full recovery from acetic acid induced colitis after 24 h. Table 4 depicts the evaluation of colonic damage.

Table 4: Evaluation of colonic damage

Groups	Acetic acid treatment	Dosage form (rifaximin tablet)	Histopathological evaluation (Score)
I	-	-	0
II	+	-	4
III	+	Uncoated	2
IV	+	Eudragit E-100 and Eudragit RL-100coated	1
V	+	Eudragit FS30Dcoated	1



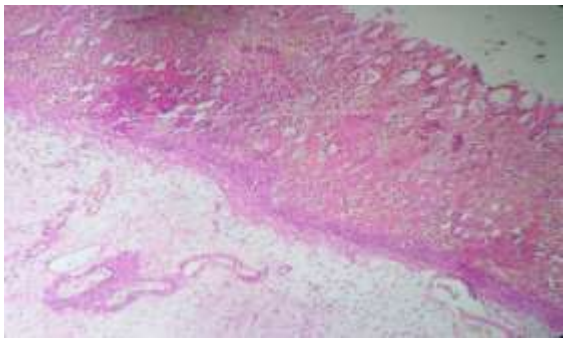


Figure 9 & 10: Histopathology of the healthy rat colon and acetic acid induced colitis

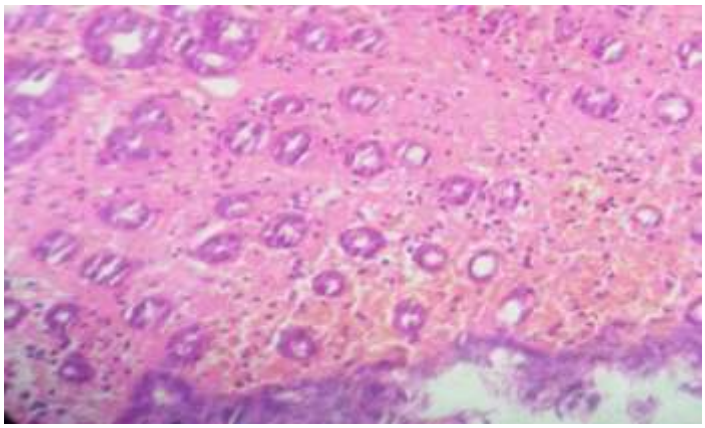


Figure 11: Histopathology of acetic acid induced colitis treated with uncoated tablets

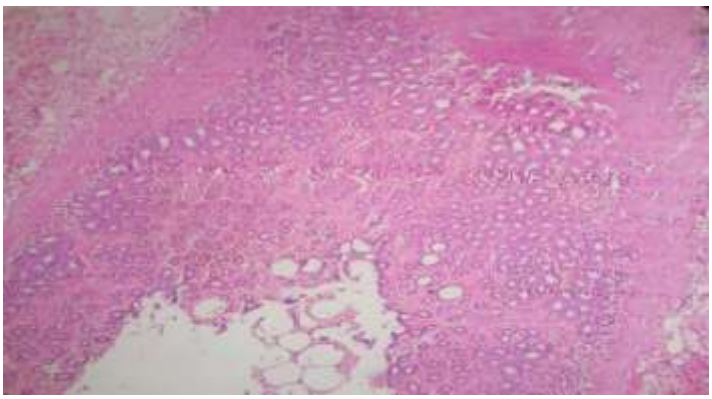


Figure 12: Histopathology of acetic acid induced colitis treated with Eudragit-100 and Eudragit RL-100 coated tablets

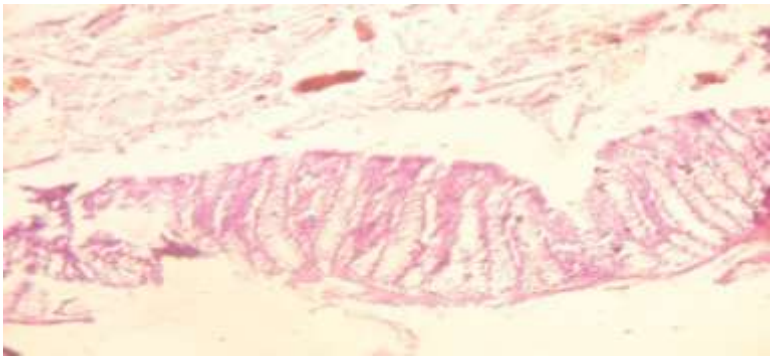


Figure 13: Histopathology of acetic acid induced colitis treated with Eudragit FS30D coated tablets

CONCLUSION

The results of the present investigation revealed distinct advantages of coating tablets containing rifaximin with chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC) in the tablet core as compared to uncoated tablets. FTIR studies revealed that there was no interaction between rifaximin and chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC). The investigation indicated that tablets coated with Eudragit E-100 and Eudragit RL-100 were able to ensure sustained and time controlled drug release in intestine and prevented the dissolution of Eudragit E-100 in the stomach. Further, these formulations were subjected to dissolution studies in HCl buffer pH 1.2 for 2 h, phosphate buffer pH 7.4 for 3 h and then in phosphate buffer pH 6.8 for 19 h in the presence of rat caecal contents. A 93.38% release of rifaximin in 24 h was observed after incorporating the rat caecal contents indicating the specific action of anaerobic enzymes on chitosan-chondroitin sulphate interpolymer complex (CH-ChS IPC). Histopathological studies were carried out using acetic acid induced colitis in rats. Hence, coating with Eudragit E-100 and Eudragit RL-100 delivers the rifaximin to colon in highest efficacy for the treatment of inflammatory bowel diseases.

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