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Formulation development using different natural and semi synthetic polymers, *in vitro* evaluation of colon targeted Sulfasalazine tablets for ulcerative colitis

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Key words: Sulfasalazine, Colon, Targeted, Bioavailability.

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Abstract

In this present research work, the aim was to develop colonic targeted matrix tablets of Sulfasalazine for colonitis treatment. Matrix tablets of Sulfasalazine were prepared using microsomal enzyme dependent polymers by wet granulation compression method.The colon targeted tablets were prepared using various polymers in combination. All pharmacopeia tests were performed to ensure its specifications. The colon-targeting tablets prepared via wet granulation method. Different polymers used in controlled release formulation for colon. The compatibility was assessed using FT-IR,XRD and DSC studies for pure drug, polymers and their physical mixtures. The physicochemical properties of all the prepared matrix tablets batches were found to be in limits. The drug content percentage in the optimized formulation H was found to be 99.24 ± 0.10%. Formulations H was containing 2.8% of pectin and 5.7% of HPMC K15M and 11.5% Guar gum and release of drug is about 4% in basic medium (6.8pH) for 3 hrs. This was acceptable range for colon targeting drugs in 3 hrs using medium of 6.8pH. The study concluded that that colon targeted tablets can target the colon and extend the release of sulfasalazine in colon for many hours and also ensure enhanced bioavailability.

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Introduction

Targeted drug delivery towards the colon is highly desirable forthe treatment of different bowel diseases such as ulcerative colitis(Lamprecht *et al.*, 2001) references should be presented in sequence. Please check the whole text, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs. In modern era, much revolutionary exploration has been made across the formulation and development of dosage forms to improve the performance of drugs and to improve patient's compliance (Lamprecht *et al.*, 2001). The colon targeting dosage forms designed to attain a targeted and prolonged therapeutic level by slow and continuous release of drug over an extended period of time after a single administration. The concept of targeted action of drug to the desired area of effect has attained a great appeal from last few decades. The colon targeted formulations reduce the frequency of drugs administration having specially those shorter half-lives. It is a quickly growing technology for developing colon targeted dosage forms. The occurrence of ulcerative colitis ranges from 10 to 70 per 100,000 people, but current studies in Rochester, Manitoba and Canada have shown occurrence is high as 200 per 100,000 people.(Loftus *et al.*, 2000;Loftus Jr and Sandborn, 2002) Such inflammatory conditions are usually treated with conventional oral dosage forms.(Patel *et al.*, 2007, Camma *et al.*, 1997) If the drug substances were directly acted on the site of action in the colon then treatment might be more efficient .Systemic side effects might be reduced and for this, lower doses might be required. A numberof some serious diseases of the colon, like. colorectal cancer, can also be capable of being treated more efficiently by targeting the drugs on the colon.By therapeutic point of view when delay absorption is required in colon, it can be also be acquired by Colon targeted drug delivery system (CTDDS). The present investigation focuses on targeting of drugs to the colon for local treatment of inflammatory bowel disease (IBD), specifically ulcerative colitis. Ulcerative colitis is a chronic inflammatory disease of the intestines in which the wall of the small or large intestine becomes sore, inflamed, and swollen requiring acute as well as chronic therapy. The area most frequently affected by ulcerative colitis is the end of the small intestine and beginning of the large intestine. Sulfasalazine is useful in mild to moderate conditions of ulcerative colitis thus, providing localized effect. Current marketed preparations of sulfasalazine is conventional immediate release tablets, enteric coated and thus do not provide localized effect in colon. Thus, there is a need to develop colon specific drug delivery systems for these drugs. Colon targeting systems are commonly designed using the approaches like: pH-dependent release, time-dependent release, or bacterial degradation in the distal ileum/colon. Colonic delivery systems based solely on time or pH dependency of release have not been reliable because of the inherent variability of pH and transit times through the gastrointestinal tract (GIT) (Kothawade *et al.*, 2015). Polysaccharide which specifically degrade by colonic bacterial enzymes, suffer from the same constraint as pH-dependent carriers do; a premature release of their drug load to a certain extent in the upper segments of the gastrointestinal tract (GIT). This early discharge of drug is associated with the swelling of the carrier, a crucial process, which allows cleavage by colonic enzymes(Pravda, 2014). For that reason, most enzymatically controlled colonic drug carriers cannot function optimally without the aid of a protective coat (primary carrier), whether pH-dependent or depending on the erosion of a physical barrier. The release of drug in various parts of the GIT can be triggered by using intestinal enzymes. In colon gut Micro floraresides in high number intestinal enzymes from where these are derived. More than 500 different types of enzymes are present in colon liberating symbiotic anaerobes. These microbes that are liberated by enzymes are used to degrade matrices/coatings and also to break bonds between an active agent and an inert carrier i.e. drug release from the polymeric pro-drugs. Microflora count of cecum and intestine has a vast difference. As the contents move from ileum to ascending colon in gastrointestinal tract, retardation of movement of contents occur because of the widening of the intestinal lumen(Pravda, 2014).

Cecum is the favorite region for microbial settlement because of its bag shaped and other facts.The aim of the study was to prepare colon targeted tablet by using combination of natural, semisynthetic and synthetic polymers as well as this formulation follow three technique for colon target like pH sensitive, delay release and enzymatic degradation control.

Materials and methods

Sulfasalazine (BASF-SE, Ludwigshafen, Germany) sodium hydrogen phosphate (Merck KGA, Germany), HPMC k15M (Aquacoat, FMC Biopolymers, Belgium), polyvinyl pyrolidine K30 (Kollicoa;BASF, AG,Germany),EudragitS 100 and Eudragit L100 respectively;AG, Darmstadt, Germany), pectin NF (Noveon Inc., Cleveland, OH, USA), croscarmellose sodium (Ac-Di-Sol; FMC Biopolymer, Belgium), guar gum (Morflex, Greensboro,USA), talcum (Luzenac Europe,France), magnesium Stearate and lactose anhydrous(southwest dairy farmer industry USA) and silicon dioxide (Aerosil 255; Evonik Industries, Germany) were used as received. All other reagents were purchased of analytical grade and were used without further purification.

Instruments

Compression machine single punch (AR-400 Erweka Germany), Friabilator (Pharma test PTFE), Hardness Tester (TB-24 Erweka Germany), PH meter (210, Hanna, Romania), USP Dissolution apparatus type II (Pharma Test Germany), UV Spectrophotometer (PG 1600 SHOMADZO), VernierCalliper, Weighing balance (Shimadzu, Japan), FTIR (1345 Brooker), DSC (universal V4.5A TA), X ray defrection.

Method of preparation of tablet

The most effective method of modulating drug release in colon is microbial flora dependent system. The colon-targeting tablets prepared via wet granulation method. Many polymers used in the formulation of based controlled released drug delivery system in colon. Reports found on the use of polymers like Guar Gum and pectin for the preparation of colon targeting formulation of different drugs. Different viscosity grades of polymers are widely used for designing colon controlled drug delivery system like HPMC k15M because their flexibility to provide a desired drug release profile and cost effectiveness and broad regulatory acceptance. However, the use of combination of polymer like Guar gum, pectin for extending drug release for the highly degraded able in microbial flora and drug is restricted due to HPMC k15M , for such drug inclusion of the binder like PVP K30 becomes essential in the matrix systems. Hence, in the present work, an attempt has made to formulate the extended release matrix tablets of sulfasalazine using different ratios of HPMC k15M polymer with and without binder (PVP K30). Nine formulations of Sulfasalazine developed as shown in table1. The granules formulated according to wet granulation method .All the raw drugs and excipients passed through a 40-mesh size sieve separately. Active drugs, polymers and lactose mixed thoroughly. The PVP K30 paste formed using granulating solventisopropyl alcohol (IPA) .The PVP K30 paste thoroughly mixed with the mixture of drugs and polymers. The mixing product passed through the 20-mesh size sieve. The granules were dried at 40 °C in an oven dryer for 30 min .The granules thus formed were also passed through a 18 mesh size sieve. The granules then mixed with lubricating agent talcum and magnesium stearate before final compression. Compression performed by using 9nine punch rotary compression machine.

Apparent Bulk densit (Santomaso et al., 2003)

Apparent bulk density of grains was estimated by placing pre-sieved granules into a graduated cylinder and measuring the volume and weight as it is. It was calculated by using formula.

Bulk density = Mass / volume of granules

Tapped density(Santomaso et al., 2003)

For determined the tapped density weighed sample of granules and transferred to a graduated cylinder and was tapped for a fixed number of taps (100). Tapped density was calculated by following formula which is mentioned in equation.

Tapped Density = Weight of granules/Tapped volume

Hausner's Ratio(Kumar et al., 2015)

The Hausner's ratio is a number that is associated to the flow ability of granules material and powder material. It is consider by formula given in equation Hausner's Ratio = Tapped Density / Bulk Density.

Compressibility Index(Pant et al., 2015)

It is a very simple test to evaluate tapped density and bulk density of granules and the rate at which it is packe down. It is determined by the following formula which is given below.

Carr's Index (%) = (Tapped density-Bulk Density) x100]/Tapped Density).

Angle of Repose(Pant et al., 2015)

The angle of repose of material was evaluated by using funnel method. For determined the angle of repose, accurately weighed granules were taken in the funnel.

The length of the funnel was accustomed in such a way that the tip of the funnel just touched to the apex of the material. The material was allowed to flow throughout the funnel freely onto the surface. The distance of the powder cone was measured and angle of repose was evaluated using the following formula.

$\tan \theta = h/r$.

Compatibility studies(Patil et al., 2015)

DSC Analysis

A differential scanning calorimetry (DSC, Perkin Elmer, USA) was used to study the thermal analysis of drug-excipients compatibility. Firstly, the sulfasalazine was mixed with HPMC, pectin, guar gum and Eudragit S100. The drug-excipients mixture was scanned in the temperature range of 50-300℃ under nitrogen atmosphere. The heating rate willbe 20℃/min and the obtained thermograms were observed for any type of interaction.

Polymer drug interaction by FTIR study(Patil et al., 2015)

The drug with polymer, drug with excipients and polymer-polymer interaction was studied by FTIR spectrometer through KBr pellets.The spectra were recorded from 6000 to 400/cm. The Fourier transform infra-red analysis was conducted for the analysis of drug polymer interaction during granules formation and stability of drug during compression process. Fourier transform infra-red spectrum of pure active drug sulfasalazine along with guar gum, HPMC k15M, pectin, Physical mixture (formulation) were recorded.

Preparation of tablets

Sulfasalazine material compresses with HPMC k15 M, as time-dependent, and Pectin, Guar gum as enzyme-dependent polymers by direct compression method again. The sulfasalazine core tablets were compress at 690 mg by using 12.0 mm concave punches. Different polymer was mixed in different ratio and makes a blend for compression. Then, it was compressed into the tablet. All the ingredients together with drug and excipients were weighed accurately according to the batch formula.

The drug and all the excipients excluding lubricants were taken with the help of stainless steel spatula on a butter paper and the ingredients were mixed in the order of ascending weights and blended for 15 min in mortar- pestle. After homogeneous mixing of all ingredients, lubricants was mixed and again mixed for 5 minute. The prepared blend of formulation was compressed into tablet weighing 690 mg using 12.0 mm concave or flat punches in a rotary tablet press (Rimek, mini-press 9-station rotary machine).

Enteric coating of prepared tablets

Final sulfasalazine tablets were further coated by using enteric coating polymers by dip coating method. Required quantity of Eudragit S100 and CAP as shown in Table 7 was mixed in IPA70% and acetone using a magnetic stirrer. After complete solubilizations of polymer (Eudragit S100) add castor oil (10% w/w of dry polymer) as plasticizer. Talc (0.2% w/v) was added as anti-adherent and the solution was stirred for 20 min. Pre-weighted tablets were dipped for 4-6 times into the coating solution until 10% weight gain.

Drug content uniformity(Commission et al., 2001)
Take the finished tablet of sulfasalazine and was powdered by mortar- pestle and the powder was transferred into a 100 ml volumetric flask. Initially, 50 ml of methanol was added and allowed to stand for 10 minutes in sonicator with intermittent shaky to ensure the complete solubility of the drug in the methanol.

The volume make up to 100 ml using methanol. One ml of the above solution was suitably diluted, filtered and the drug content was estimated using UV Visible spectrophotometer at 359 nm and methanol was taken as blank. The drug content was estimated by using calibration curve.

Disintegration study(Rawas-Qalaji et al., 2015)
The disintegration studies of core tablets of 6mm which was 1st compressed evaluated by using disintegration apparatus according USP specifications. Put one tablet in each of the six tubes of the apparatus basket. Place the plastic disc to each tube and start the machine using 900 ml of acidic medium of pH 1.2 and Phosphate buffer of pH 7.4 as the immersion liquid. The basket assembly should be lowered and raised between 30 cycles per min in distilled water maintained at 37°C in water bath . The time in minutes for complete disintegration of the tablets with no palpable mass lasting in the apparatus was recorded and measured.

In vitro Swelling study(Samanta et al., 2014)
Swelling index for the final compressed tablet was determined in different medium like (HCl buffer pH 1.2, PBS 7.4 and pH 6.8).

The initial weight of the tablet was determined (W1)and then tablet was put in 20 ml HCl buffer of pH 1.2 for 2 h then placed in 20 ml of PBS pH 6.8 for 3 h and finally place the tablet in 20 ml of PBS pH 7.4 up to 24 h in a Petri-dish. The tablet was withdrawn at different time intervals (1, 2, 3, 4, 5... 24 h) blotted with cellulose filter paper and again weighted (W2). The swelling index is calculated by the formula:
Swelling index = 100 (W2 - W1)/ W1

Where; W1 = initial weight of the tablet.
W2 = final weight of the tablet.

In vitro Drug Release Studies(Mallick et al., 2014)
Sulfasalazine release from the final coated tablets was determined by dissolution testing using the USP dissolution test apparatus type II (rotating paddle) at a rotation speed of 50 rpm and temperature maintained at 37.0±0.5°C (pharma-test).

The release study was performed in 300 ml HCl buffer pH 1.2 for 2 h, followed by 300 ml PBS pH 6.8 for another 3 h. Finally 300 ml 3 % microbial flora plus PBS pH 6.8 till the end of the 24 h to simulate the pH pertaining to the stomach, proximal part and middle of small intestine (duodenum and jejunum), and distal small intestine (ileum), respectively. 1 ml of dissolution medium was withdrawn at 1 h interval up to 24h and replaced with an equal volume of media. The collected solution was filtered through 0.45 µm cellulose membrane filter and analyzed at UV-VIS spectrophotometer at 359nm.

Results

Pre-formulation Studies ofSulfasalazine
Different test were performed to assure the specification of material and final Tablet.

Appearance:(Commission et al., 2001)
This is Bright yellow or brownish-yellow crystalline (Pharmacopoeia, 2007), fine powder, we have checked its appearance, it is according to BP specification.

Morphology of Raw material was checked through SEM and was found according to specification, in this figure we can see its particle size and crystalline morphology, powder of sulfasalazine show excellent morphology according to BP specification and that powder was compare with the standard material from validated source.

Physical properties of the powder
Compatibility Study
Determination of drug excipients interaction was

checked by X-ray diffraction, DSC technique and FTIR. DSC is one of the most convenient methods for investigating the compatibility of drug and polymer blends; therefore it was used to investigate thermo dynamic compatibility of Sulfasalazine with Guar Guam, Pectin and HPMC k15Mbased on crystalline melting temperature and the glass transition temperature.

Table 1. Formulation scheme with different material.

Code	Sulfa	Mgst	Pvpk30	Lactose	Guar gum	Talcum	Pectin	Hpmc-k15	Total weight
A	500	0.4	5.00	159.40	10.00	0.2	10.00	5.00	690.00
B	500	0.8	5.00	138.80	20.00	0.4	15.00	10.00	690.00
C	500	1.2	5.00	118.20	30.00	0.6	20.00	15.00	690.00
D	500	1.6	5.00	97.60	40.00	0.8	25.00	20.00	690.00
E	500	2.0	5.00	77.00	50.00	1.0	30.00	25.00	690.00
F	500	2.4	5.00	81.40	60.00	1.2	10.00	30.00	690.00
G	500	2.8	5.00	60.00	70.00	1.4	15.00	35.00	690.00
H	500	3.2	5.00	40.20	80.00	1.6	20.00	40.00	690.00
I	500	3.6	5.00	19.60	90.00	1.8	25.00	45.00	690.00
j	500	4.0	5.00	00.00	100.0	2.0	30.00	50.00	690.00

Table 2.*Physical properties of powder. Standard deviation to include*

Formulation	Bulk density	Tap density	Carr's index	Hausner's ratio	Angle of repose
A	33	30	9.09	0.90	27.38
B	34	32	5.88	0.94	29.27
C	32	31	3.12	0.96	29.52
D	34	31	8.82	0.91	30.63
E	35	32	8.57	0.91	32.91
F	32	30	6.25	0.93	28.3
G	33	29	12.12	0.87	28.81
H	34	30	11.76	0.88	30.72
I	33	30	9.09	0.90	29.52
J	34	29	14.70	0.98	33.1

Table 3. Weight variation test of final tablets.

Formulation	Maximum weight	Minimum weight	Average weight	S.D
A	690.4	689.7	689.4	0.302765
B	690.8	690.1	690.6	0.223358
C	690.2	689.5	689.1	0.566176
D	690.6	690.2	690.5	0.210819
E	690.2	689.3	689.4	0.406749
F	690.4	689.4	689.1	0.379473
G	690.2	689.2	689.5	0.434613
H	690.4	689.9	690.2	0.258414
I	690.6	690.1	690.4	0.223358
J	690.4	689.7	689.9	0.302765

X-raydiffraction

X-ray diffract gram of sulfasalazine exhibited several peaks of different intensities between 2θ = 2°and 80°whileX-raydiffractogramofsulfasalazine blend show edlessintense peaks in the same range (Fig.2).

Differential scanning calorimetry (DSC) studied by a heating rate of 20°C/min and the weight range of 4 – 10 mg over the range of 0 – 325°C). As shown in Fig. 3, sulfasalazine exhibited clear endothermic peak at 175 and blend exhibited peak at 210 which indicate

there is no more shift of peak occur. However polymer melting peaks can be observe in blend. The FTIR spectra of sulfasalazine alone and with all polymers physical mixture are shown in Fig.4. FTIR spectrum of sulfasalazine and blend showed compatibility. Sulfasalazine showed characteristic peaks at 1735.6 cm−1 due to the carboxyl group, at 1175.5 cm-1 due to presence of C–O stretch, the C = C (aromatic stretch) at 1609.9 cm−1, –CH3 bend at 1437.2 cm−1, and due to the presence of O–H (carboxylic acid), the peak was found at 2868.1 cm−1.

Table 4. Friability test of tablets.

Formulations	Initial weight	Final weight	Difference	Friability (%)
A	690.4	689.7	0.7	0.1013
B	690.8	690.2	0.6	0.0865
C	690.2	689.2	0.9	0.1448
D	690.6	690.1	0.5	0.0724
F	690.2	689.3	0.9	0.1303
G	690.4	689.4	1.0	0.1448
H	690.2	689.2	1.0	0.1448
I	690.4	689.9	0.5	0.0724
J	690.6	690.1	0.4	0.0725

Table 5. Hardness of tablets.

FORMULATIONS	HARDNESS(kg/cm2)	RANGE
A	10.2	4-11 (kg/cm2)
B	10.3	-do-
C	10.3	-do-
D	10.7	-do-
E	9.9	-do-
F	10.4	-do-
G	8.9	-do-
H	9.8	-do-
I	10.1	-do-
J	10.7	-do-

Physicochemical properties of the tablets

The tablets were evaluated physically according to internal and pharmacopeia specifications; tablets were of round shape. For physical evaluation tablets of different formulations were used for weight variation, thickness, diameter, friability and hardness during and after compression.

Matrix Index Study

Nine representative tablets from a batch were evaluated for matrix index by using standard formula in simulated gut fluid by using following method. Methods of Preparation of colonic fluid.

Table 6. Thickness of tablets.

Formulations	Thickness	Diameter
A	6.65	12±0.04
B	6.60	12±0.03
C	6.68	12±0.04
D	6.65	12±0.05
E	6.65	12±0.06
F	6.68	12±0.05
G	6.65	12±0.07
H	6.65	12±0.06
I	6.65	12±0.09

Simulated colonic fluid

Human Fecal matter solution of 3% was prepared and it was added in phosphate buffer solution of PH 7.4 in1:10 proportions mixed it well and small amount of pepsin and pancreatic was added in it.

Isolation of microbial flora of human fecal matter

Human fecal matter is representative of *microbial flora of colonic environment tract of human being*.

GIT of human being contains various types of microbes and it was determined by isolating the microbial flora from human fecal matter. Microbial flora from GIT was isolated by following *method.*

Table 7. Drug release (%) profile in simulated microbial colonic fluid 7.4Ph.

Formulation Code	2hour	4hour	6hour	8hour
A	20.4	41.4	58.4	81.3
B	26.3	31.3	52.4	82.4
C	23.3	29.3	63.1	89.4
D	18.3	29.1	59.3	83.4
E	23.2	47.4	68.4	73.4
F	21.2	42.4	72.3	89.4
G	17.4	31.7	68.4	91.4
H	18.9	32.4	72.8	92.8
I	19.5	39.8	65.8	89.7

PHASE 1 - Isolation of microbial flora from human fecal matter was collected.

PHASE 2 - Identification of isolated bacteria was carried out by using morphological characteristics, Gram reaction and by using selective medium.

PHASE 1 - Isolation of microbial flora from feces. The fresh human feces sample was collected in sterile plastic container and used within 1 Hr. after collection. Pour plate technique was done by the following way-The fecal sample was made in peptone water having dilution 1:100.Then Mc-Conkey agar medium (PH-7-7.2) was prepared, and it was sterilized by autoclaving at 121C temperature,15 lbs pressure for 15 minutes.

Then agar medium was allowed to cool, approx. at 40-50C. Then 0.1ml of previously diluted sample was aseptically inoculated in sterile Petri plates, the medium was poured and mixed well and solidify the Petri plates. Then the Petri plates were incubated at 37C for 24-48 Hr. after incubation period the colonies were observed.

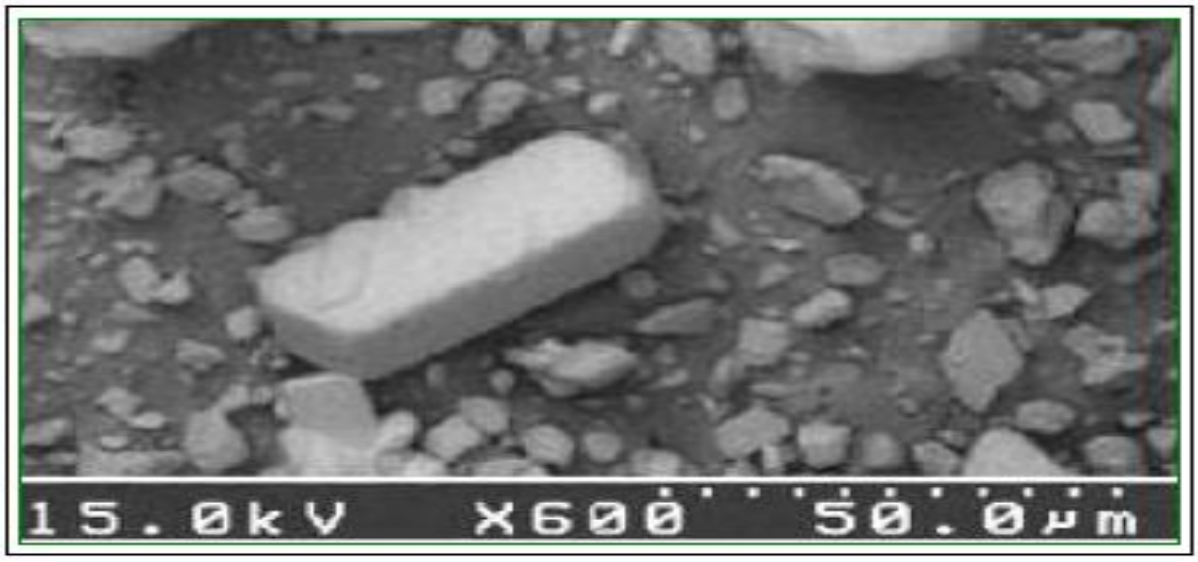

Fig. 1. Powder of Sulfasalazine.

PHASE 2- The isolated bacteria were identified by morphological characteristics. The colonies from Mc-Conkey agar having different morphological characters (shape, size, opacity).

In-vitro drug release study

On the basis of results of matrix index all formulation was tested only by *in-vitro* drug release method.

Test was carried out using USP apparatus II (paddle) and the medium was simulated colonic fluid. This dissolution medium consisted 900 ml.

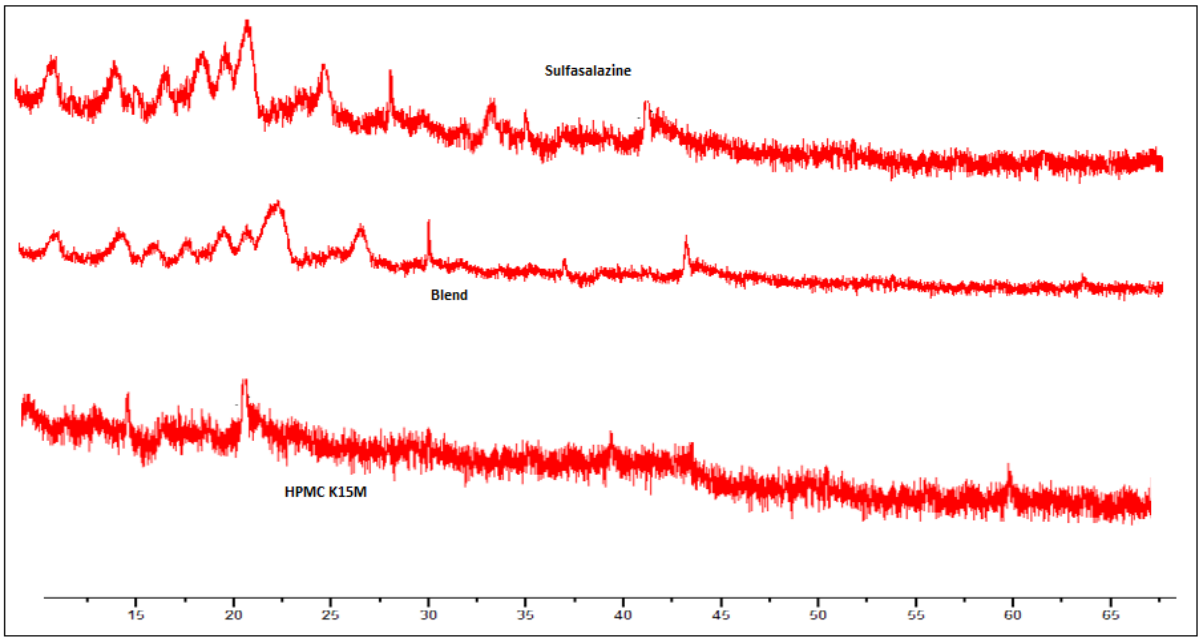

Fig. 2. XRD of HPMC K15M.

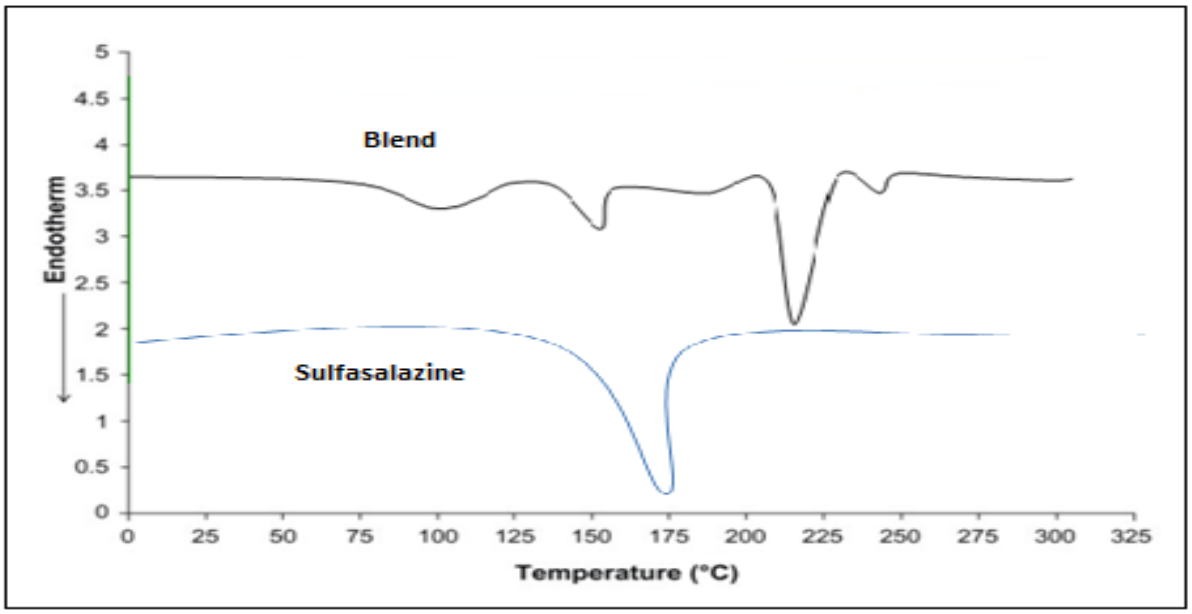

Fig. 3. DSC of Sulfasalazine and blend.

The speed of paddle was 50 rpm and temperature of dissolution medium was 37C. One tablet was placed in the dissolution medium and apparatus was run. At intervals of 2, 4,6nd 8 hours, 5 ml aliquots were withdrawn and replacement was made each time with 5 mL of fresh dissolution medium. Each 5 ml sample was filtered through Whatman filter paper no. 41. The absorbance measured at 359 nm.

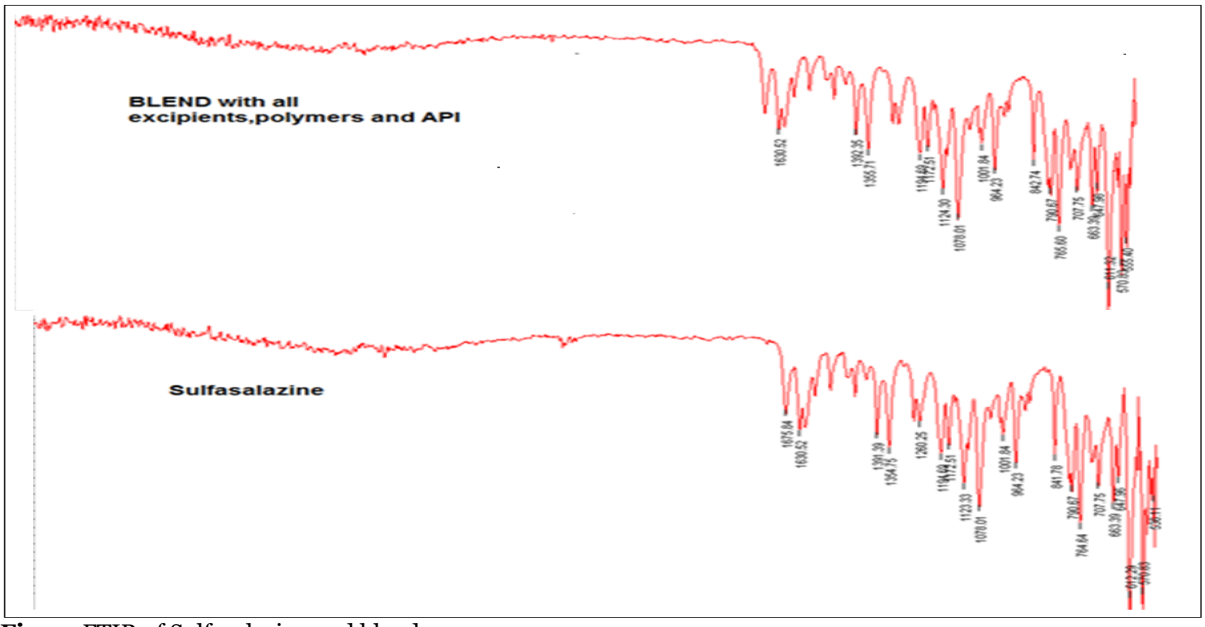

Fig. 4. FTIR of Sulfasalazine and blend.

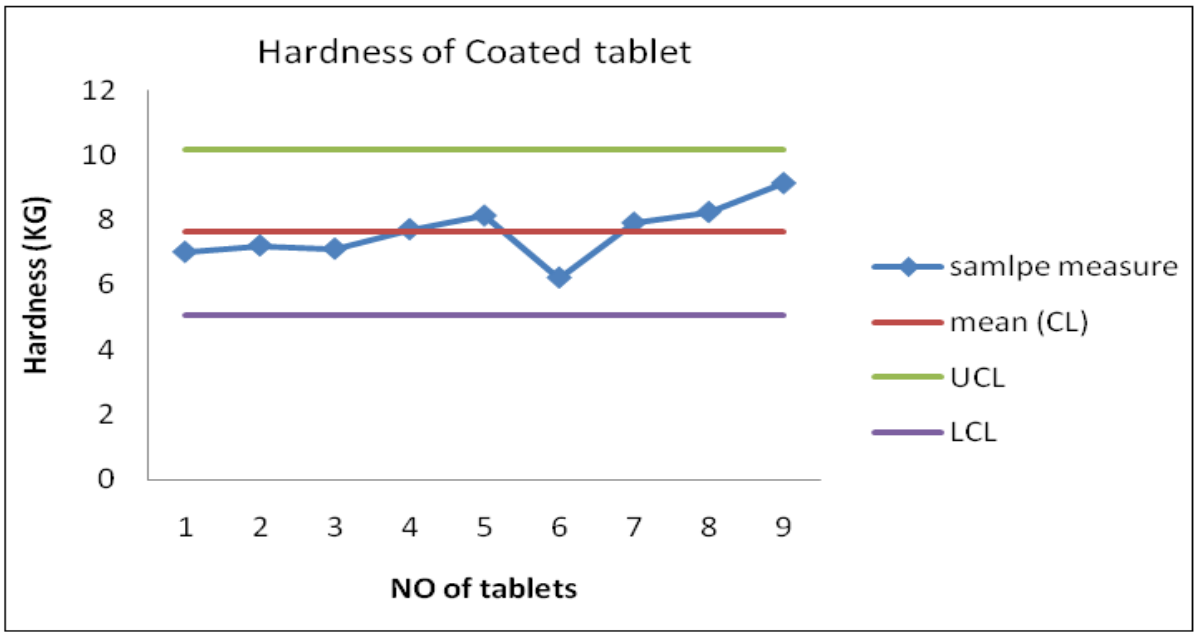

Fig. 5. Graph representation of hardness of tablets.

Discussion

Sulfasalazine mostly used for colon disease(Klotz *et al.*, 1980). The intention of present investigation is to develop the sulfasalazine colon specific delivery to facilitate the maximum drug delivery at the required site to gain the therapeutic benefit. In this study guar gum, HPMC and pectin enteric coated tablets were prepared to achieve the colon specific release of sulfasalazine and evaluated to prove the colon specificity. In a study reported in the literature, Guar Gum in the coat weight of showed similar type of result(Krishnaiah *et al.*, 2002).

Weight variation, thickness, hardness, friability, and drug content of all the tablet formulations were complied with USP pharmacopoeial standards, (Pharmacopoeia, 2003)so all the tablets were with acceptable physical characteristics. The average percentage deviation of all tablet formulations was found to be within the specified limit, and hence all the formulations passed the uniformity of weight as per the official requirements of USP Pharmacopoeia.

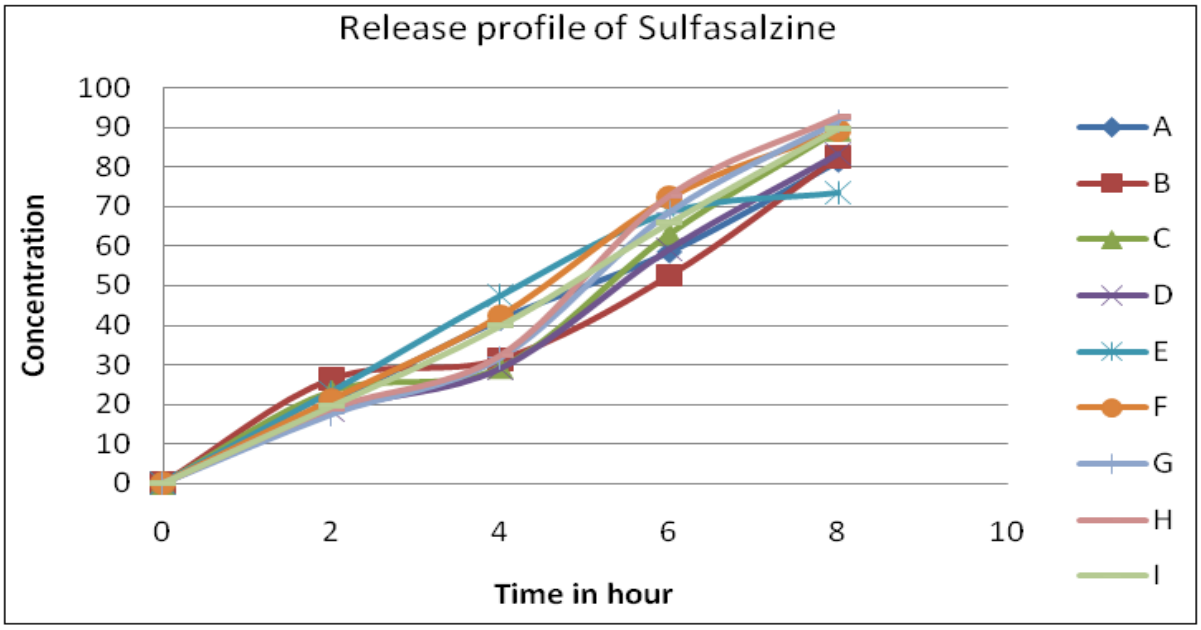

Fig. 6. Sulfasalazine release profile in simulated fluid.

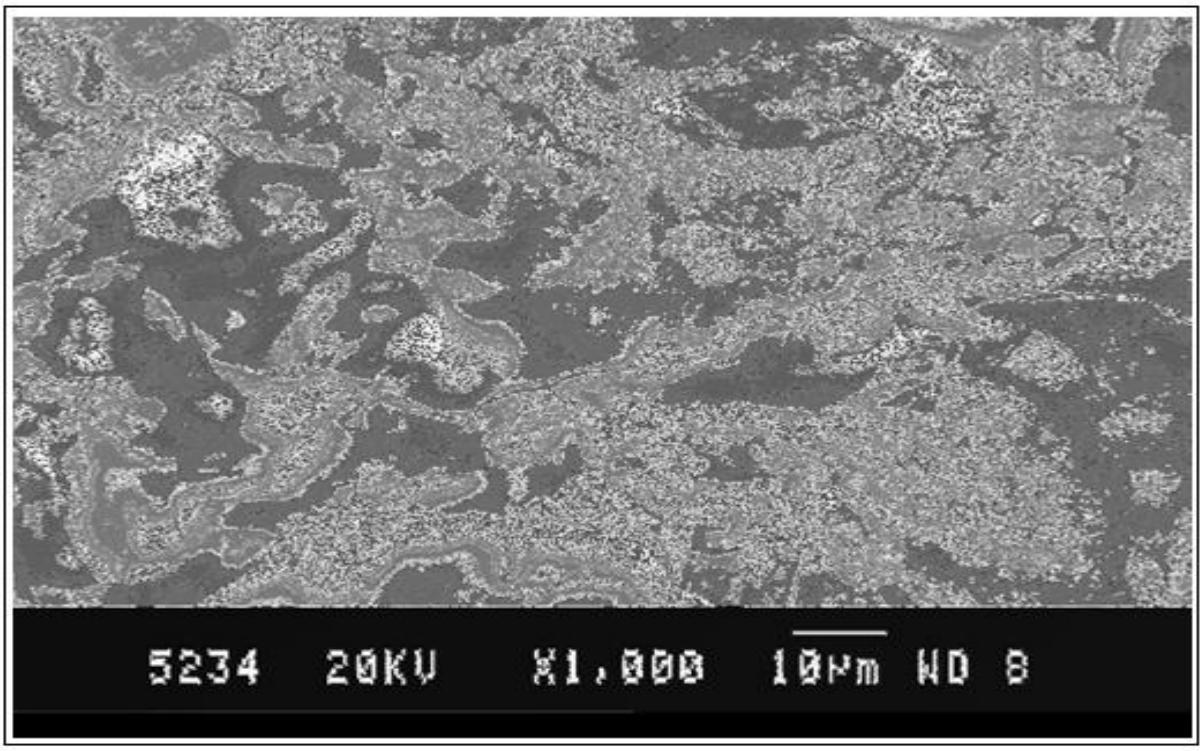

Fig. 7. SEM photographs of formulation before dissolution in simulated colonic fluid.

In weight variation test, the pharmacopoeial limit for tablets is not more than 5% of the average weight(Pharmacopoeia, 2003). The average percentage deviation of all tablet formulations was found to be within the specified limit, and hence all the formulations passed the uniformity of weight as per the official requirements of USP Pharmacopoeia 2015. The drug release kinetics showed high correlation coefficient values for zeroorder than first order indicating that the drug release from compression coated tablets followed zero-order patterns.

Zero-order release was also observed in a study with sulfasalazine using HPMC guar gum and pectin in the compression coat (Krishnaiah *et al.*, 2002).

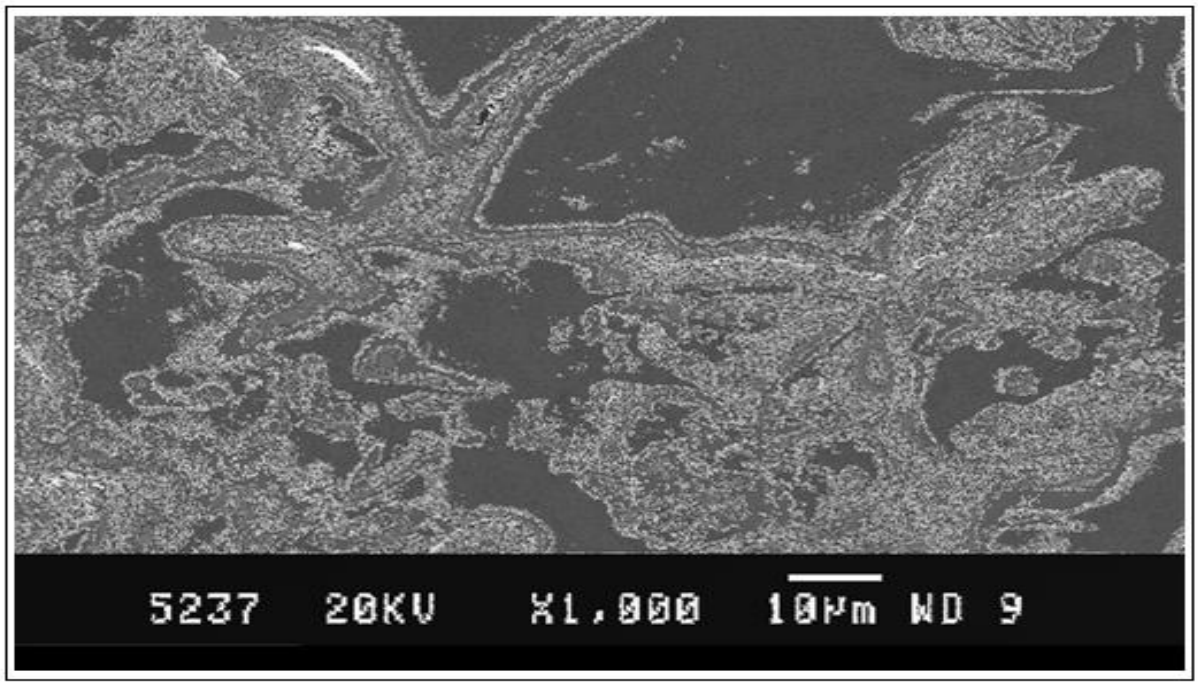

Fig. 8. SEM photographs of formulation after dissolution in simulated colonic fluid.

The optimized enteric coat weight for the better drug release profiles suitable for colon specific release of sulfasalazine was studied by formulating the enteric coated tablets with different coat formulations. From the dissolution study, it was found that 690 mg is the suitable enteric coat weight for colon targeting. Eudragit S100 was used in enteric coated which was describe in literature.(Sinha and Kumria, 2003) The FTIR spectra of sulfasalazine alone and with all polymers physical mixture are shown in Figure 4. FTIR spectrum of sulfasalazine and blend showed no incompatibility. Sulfasalazine showed characteristic peaks at 1735.6 cm^{-1} due to the carboxyl group, at 1175.5 cm^{-1} due to presence of C–O stretch, the C = C (aromatic stretch) at 1609.9 cm^{-1}, $-CH_3$ bend at 1437.2 cm^{-1}, and due to the presence of O–H (carboxylic acid), the peak was found at 2868.1 cm^{-1}. The *in vitro* drug release studies of the of core tablets, i.e., A to I, showed 81.3% ±0.95%, 82.4% ±0.82%, 89.4% ±0.70%, 83.4% ±0.95%, 73.4% ±0.82%, 89.4% ±0.70%, 91.4% ±0.95% and 92.8% ±0.82% drug release within 8 h in pH 7.4 phosphate buffer. Scanning Electron Microscopy (SEM) analyses were performed on the optimized formulation (H) with dissolution media (pH 7.4) which we have compare with literature and found similar results(Khan *et al.*, 1999). SEM photomicrographs of the outer surface of the coated matrix tablet of sulfasalazine showed a homogenous and quite compact structure during dissolution, as depicted in Figure 7 and 8.

Conclusion

From the *in vitro* drug release studies, H formulation showed a significant level of drug release in the colon with negligible release in the first 5 h.

The drug release from these tablets showed zero-order profile, and the drug released mechanism was supercase-II transport. The accelerated stability studies proved that the stability of this formulation was excellent. In conclusion, development of mixture of polymers compression coated tablets based on microbial-dependent method is a good approach for colon targeting of sulfasalazine. The *in vitro* drug release studies of enteric coated colon targeted tablets of sulfasalazine (H) revealed that they give considerable amount of drug release in the colon without loss in the upper gastrointestinal tract. Further the pharmacokinetic evaluation in healthy volunteers is required to prove the capacity of colon specific release of sulfasalazine. These results showed that the colon targeted enteric coated tablets did not release the drug appreciably in upper GIT but released it slowly and progressively in the colon.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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References

Camma C, Giunta M, Rosselli M, Cottone M. 1997. Mesalamine in the maintenance treatment of Crohn's disease: a meta-analysis adjusted for confounding variables. *Gastroenterology* **113,** 1465-1473.
https://doi.org/10.1053/gast.1997.v113.pm9352848

Commission BP, Council GM, Commission G. BM. 2001. British pharmacopoeia, Her Majesty's Stationery Office.

Khan MZI, Prebeg Ž, Kurjaković N. 1999. A pH-dependent colon targeted oral drug delivery system using methacrylic acid copolymers: I. Manipulation of drug release using Eudragit® L100-55 and Eudragit® S100 combinations. Journal of Controlled Release **58,** 215-222.
https://doi.org/10.1016/S0168-3659(98)00151-5

Klotz U, Maier K, Fischer C, Heinkel K. 1980. Therapeutic efficacy of sulfasalazine and its metabolites in patients with ulcerative colitis and Crohn's disease. New England Journal of Medicine **303,** 1499-1502.
https://doi.org/10.1056/NEJM198012253032602.

Kothawade P, Gangurde H, Surawase R, Wagh M, Tamizharasi S. 2015. Conventional and novel approaches for colon specific drug delivery.

Krishnaiah Y, Satyanarayana V, Kumar BD, Karthikeyan R. 2002. In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil. European journal of pharmaceutical sciences **16,** 185-192.
https://doi.org/10.1016/S0928-0987(02)00081-7

Kumar DV, Sitharavamma Y, Nagalakshmi K, Prasanna T, Ravindra K. 2015. Formulation and Optimization of Nitrendipine Sustained Release Tablets Journal of Controlled Release, **1**.

Lamprecht A, Ubrich N, Yamamoto H, Schäfer U, Takeuchi H, Maincent P, Kawashima Y, Lehr CM. 2001. Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. Journal of Pharmacology and Experimental Therapeutics **299,** 775-781.

Loftus E, Silverstein M, Sandborn W, Tremaine W, Harmsen W, Zinsmeister A. 2000. Ulcerative colitis in Olmsted County, Minnesota, 1940–1993: incidence, prevalence, and survival. Gut, **46,** 336-343.
https://doi.org/10.1136/gut.46.3.336

Loftus JR, EV, Sandborn WJ. 2002. Epidemiology of inflammatory bowel disease. Gastroenterology Clinics of North America **31,** 1-20.
https://doi.org/10.1016/S0889-8553(01)00002-4

Mallick S, Sagiri S, Singh V, Pal K, Pradhan D. Bhattacharya M. 2014. Effect of Processed Starches on the Properties of Gelatin-based Physical Hydrogels: Characterization, in vitro Drug Release and Antimicrobial Studies. Polymer-Plastics Technology and Engineering **53,** 700-715.
https://doi.org/10.1080/03602559.2013.877927

PANT S, Sharma PK, Malviya R. 2015. Evaluation of Different Concentration of Binders on the Dissolution Profile of Paracetamol Tablets. Advances in Biological Research **9,** 82-85.

Patel M, Shah T, Amin A. 2007. Therapeutic opportunities in colon-specific drug-delivery systems. *Critical Reviews™ in* Therapeutic Drug Carrier Systems **24,** 147-202.
https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v24.i2.20

Patil H, Tiwari RV, Upadhye SB, Vladyka RS,

Repka MA. 2015. Formulation and development of pH-independent/dependent sustained release matrix tablets of ondansetron HCl by a continuous twin-screw melt granulation process. International journal of pharmaceutics **496,** 33-41 https://doi.org/10.1016/j.ijpharm.2015.04.009

Pharmacopoeia B. 2007. British Pharmacopoeia commission office. London, UK**, 2,** 1078-1080.

Pharmacopoeia U, National Formulary 21. USP Convention, Rockville. 2003.

Pravda J. 2014. Materials and methods for treatment of disorders associated with oxidative stress. Google Patents.

Rawas-Qalaji MM, Werdy S, Rachid O, Simons FER, Simons KJ. 2015. Sublingual Diffusion of Epinephrine Microcrystals from Rapidly Disintegrating Tablets for the Potential First-Aid Treatment of Anaphylaxis: In Vitro and Ex Vivo Study. AAPS PharmSciTech **16,** 1203-1212 https://doi.org/10.1208/s12249-015-0306-0

Samanta A, Jena AK, Das M, De A, Mitra D. 2014. Determination of efficacy of a natural tablet binder: Characterization and in-vitro release study. Asian Journal of Pharmaceutical and Clinical Research **7.**

Santomaso A, Lazzaro P, Canu P. 2003. Powder flowability and density ratios: the impact of granules packing. Chemical Engineering Science **58,** 2857-2874. https://doi.org/10.1016/S0009-2509(03)00137-4

Sinha VR, Kumria R. 2003. Coating polymers for colon specific drug delivery: A comparative in vitro evaluation. *A*CTA Pharmaceutica-Zagreb- **53,** 41-48.