IN VITRO AND IN VIVO EVALUATION OF OKRA POLYSACCHARIDE-BASED COLON-TARGETED DRUG DELIVERY SYSTEMS

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ABSTRACT

Colon targeted tablet formulation was developed using okra polysaccharide (Abelmoschus esculentus) as a microbiologically triggered material and also as the carrier. Okra polysaccharide was isolated from Abelmoschus esculentus and used for tablet formulation with ibuprofen as the model drug. The matrix tablets with four different proportions of the okra (20%, 30%, 40%, and 50%) with 1% ethyl cellulose in all the four formulations and the formulations were coded as WO1, WO2, WO3, & WO4. In all the formulations constant 100 mg ibuprofen were incorporated. The formulations were evaluated for their hardness, weight variation, friability, and drug content and were characterized by FTIR. Matrix tablets were subjected to in vitro drug release studies. The release studies were carried out for 2 hours in pH 1.2, 3 hours in pH 7.4 phosphate buffer and for 10 hours in pH 6.8 PBS. The % Release of these formulations i.e. WO1, WO2, WO3 & WO4 were found to be 20.75, 18.48, 13.37 & 11.99 respectively at 5th hour. The fifth matrix tablet [WOS] with 10% ethyl cellulose, 40% okra polysaccharide and 100 mg ibuprofen was formulated. The % cumulative release of this formulation (WOS) was found to be 4.59 at 5th hour. Among the above, WO3 was chosen as the optimized formulation for further studies. The in vitro dissolution studies were carried out with pH 1.2, pH 7.4 and the study continued in pH 6.8 PBS with rat cecal matter at 6th hour in simulated colonic fluid in order to mimic conditions from mouth to colon. The post five hour studies were carried out without rat cecal matter also as a control. The observation made was that the maximum release was 98.09% at 10th hour with rat cecal matter and a mere 32.70 % and 46.98% without rat cecal matter at 8th and 10th hour respectively. These findings were confirmed by in vivo investigation using X-ray images of rabbits ingested with okra matrix tablets (WOS) containing barium sulphate as contrast medium instead of ibuprofen. The tablet began to disintegrate at 8th hour of tablet ingestion. These observations drive us to conclude that the okra polysaccharide under investigation has the potential to deliver the drug almost intact to the intended site i.e. Colon where it undergoes degradation due to the presence of anaerobic microbes there. Thereby both the aims contemplated are achieved.

Keywords: Okra, Ethylcellulose, wet granulation, microbial triggered, X-ray imaging, Rabbit.

INTRODUCTION

Colon specific drug delivery systems have gained increased importance for systemic delivery of drugs1-3, 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 drugs to the other organs en route. Colon-specific delivery systems can be used to improve the bioavailability of protein and peptide drugs4,5.

Well documented approaches to achieve colon-specific delivery include pro-drugs6, pH-dependent systems7, time-dependent systems8, and biodegradable systems9. Efficient colon drug delivery system is vital since it responds only to the physiological conditions particular to the colon. Hence, attempts are made to bring out an ideal colon-specific delivery systems with improved site specificity and adequate drug release at the appropriate site and developed to accommodate different therapeutic needs. The use of bacterially degradable polymers for colon-specific drug delivery seems to be a more site-specific approach as compared to other approaches.

These polymers shield the drug from the environments of the stomach and the small intestine and are able to deliver the drug to the colon intact. On reaching the colon, they undergo assimilation by micro-organism10 or degradation by enzyme11 or breakdown of the polymer backbone12 leading to a subsequent reduction in their molecular weight and loss of mechanical strength. They are then unable to hold the drug entity any longer13.

The colon has microflora of 1011 -1012 CFU per ml. The main bacterial population present is anaerobic bacteria which proliferate. The predominant species isolated are Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria, etc.. The main saccharolytic species are Bacteroides 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\textsuperscript{14,15}. Recent trends towards the use of natural polysaccharides such as vegetables, animal, and of microbial origin have increased. There are several reports about the successful use of hydrophilic polymers derived from plants, like guar, carrageenan, karaya, locust bean etc. in pharmaceutical preparations\textsuperscript{16,17}.

Guar gum has been investigated for its application in colon specific dosage forms\textsuperscript{16}. Abelmuschus esculentus gum had been used as mini matrix for furosemide and diclofenac sodium tablets\textsuperscript{17}, sulphafuanidine granules and tablets\textsuperscript{18} and investigated as well in release of indomethacin from bioadhesive tablets with carbopol\textsuperscript{19}. Besides, this gum had been evaluated as a controlled-release agent in modified release matrices, in comparison with sodium carboxymethyl cellulose (NaCMC) and hydroxypropylmethylcellulose (HPMC), using Paracetamol as a model drug\textsuperscript{20}.

The Okra (Abelmsuchus esculentus) is a bulky annual plant cultivated throughout the tropical and subtropical areas of the world, particularly in India, gives fruits which are green pods of various shapes. The okra polysaccharide contains the major polysaccharide component differing widely in the molar ratios of galactose, galacturonic acid, and rhamnose\textsuperscript{21} and with some fractions of glucose, mannose, arabinose and xylose\textsuperscript{22}. In recent years researchers pay much attention to okra polysaccharide in pharmaceutical formulation. The present investigation is an attempt made to utilize the presence of polysaccharide in okra gum, as a carrier for microbially triggered colon-site-specific delivery system using ibuprofen as model drug.

In this investigation, okra in the form of matrix tablets formulated by wet granulation method 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 okra matrix tablet containing barium sulphate instead of ibuprofen to rabbit. Images were taken by X-ray in definite time intervals.

**MATERIALS AND METHODS**

Sodium metabisulfite was purchased from Merck specialties Pvt., Ltd, India, Acetone from Nice chemicals Pvt., Ltd., India, Ibuprofen gifted by Yarrowchem products, Mumbai, Ethyl cellulose, Lactose monohydrate, talc, sodium hydroxide and potassium hydrogen phosphate were purchased from S D-Fine chemicals, Mumbai. Magnesium stearate and barium sulfate from Loba chemie Pvt., Ltd., Mumbai, ethanol from Changshu YangYuan Chemicals, China. All other chemical were also of highest grade.

**Extraction of the polysaccharide from okra fruits**

The reported method\textsuperscript{18} 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 extracted by filtering through muslin cloth. The crude was centrifuged at 5000 rpm for 30minutes and the mucilage was precipitated from the supernatant with addition of acetone. It was further dried with the help of microwave oven and pulverised.

**Preparation of matrix tablets**

Different matrix formulations of ibuprofen were prepared by wet granulation technique using varying proportions of okra polysaccharide. Each formulation contains 100mg of Ibuprofen. The formulations were coded as WO1, WO2, WO3, & WO4 by varying the okra polysaccharide percentage 20%, 30%, 40% and 50% respectively and 1%ethylcellulose in all the four formulations. The fifth matrix tablet (WO5) contains 100mg of ibuprofen, 10% ethylcellulose and 40% of okra. 1 % w/v of okra mucilage in each formulation was used as binding agent and the rest as excipients. The lactose was included to adjust the tablet weight to 300mg. Accurately weighed quantities of pre-sieved drug and polymer were mixed thoroughly, granulated and lubricated with a mixture of talc and magnesium stearate (1:0.5). The granules thus obtained were compressed at a maximum force of 4000kg using 8mm round and slightly concave punches on 12-station rotary tablet mini press - II MT (Remek, Ahmedabad, India). Three batches in each formulation containing 50 tablets each were formulated. The matrix tablets were evaluated for hardness (Tablet tester C-W WTDH 500N Thermonik, Campbell Electronics), friability (Thermonik, Campbell Electronics C – FT 10/20) and weight uniformity as per the standard procedures.

**In Vitro Drug Release Studies**\textsuperscript{23,24}

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 pH1.2 (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.
In vitro drug release studies with and without 4% rat cecal content

The tablets were tested for drug release for 2 hours in pH 1.2 (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 polysaccharide 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 265nm using Double beam UV-visible spectrophotometer-2220 (SYSTRONICS, India). The cecal contents were obtained from male albino rats (obtained from Kmcch College of pharmacy, coimbatore, India) after pretreatment of the animal for 7 days with 1ml of 2% okra dispersion in order to induce enzymes specifically acting on okra polysaccharide in the cecum which provides the best condition for the in vitro evaluation of okra polysaccharide. 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 study of drug release under the simulated environment in colon was 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 buffer 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 caecal content also in pH 6.8 phosphate buffer (control).

In vivo X-ray studies

New Zealand rabbit weighing 2.7 Kg was used for in vivo studies. The rabbit was housed singly in restraining cages during the experiment and allowed food and water libitum. The rabbit was pretreated for 7 days with 1ml of 2% okra dispersion. The tablet was ingested by the trachea of the rabbit using gastric intubation. X-ray imaging was adopted to visualize in vivo functioning of a colon-specific drug delivery system. The in vivo transit of the optimized tablet formulation containing barium sulphate was observed visually. X-raying of the tablet ingested rabbit was taken at 2 hrs, 5hrs, 8hrs and 10hrs to visualize the passage of the tablet in the GIT of rabbit and to observe the location at which the tablet begins to degrade.

Data analysis

The calibration curve and the raw dissolution data were analyzed. The statistical parameter for each tablet unit and their mean values were computed.

Ethical committee approval

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

RESULTS AND DISCUSSION

The evaluation of the okra polysaccharide for its ability to retard the release of drugs from tablets and the effect of excipient on the release of the drug were reported by previous researchers. The present work is to find out the ability of the okra polysaccharide to deliver the model drug ibuprofen intact to colon and to undergo microbial degradation there.

The intention is the formulated matrix tablet shall be protected from degradation in stomach or intestine until it reaches the colon. The polysaccharide obtained from Okra is composed of galactose, rhamnose and glucuronic acid. It is contemplated to exploit the presence of above saccharides for the microbiologically triggered drug delivery system of okra matrix tablet to the colon. The in vivo X-ray imaging study on rabbit proved that the okra matrix tablet was almost intact until it reached colon. Ibuprofen has a low water solubility. If a highly water-soluble drug was used, diffusion may have started even before the bacterial degradation of the polymer coating started. Ibuprofen is also a candidate for an intentionally delayed absorption from a dosage form ingested.

Evaluation of okra polysaccharide matrix tablet

The hardness of the tablet ranged between 5.97 and 6.5 kg/cm². The percentage friability of the prepared tablets was well within the acceptable limit. There was no significant weight variation observed between average weight and individual weight of tablets. The percentage drug content in all the batches were within the range of 98.29 - 99.19%, ensuring uniformity of drug content in the formulations.

FTIR spectra analysis

FTIR spectra of pure Ibuprofen, pure Okra polysaccharide ethylcellose and optimized formulation i.e. WO3 is shown in the fig 1, fig 2, and fig 3, fig 4 & fig 5. It is very clear from the spectra that there is no interaction among ibuprofen, okra polysaccharide, ethylcellose and excipients.
Figure 1: FTIR spectra of ibuprofen

Figure 2: Ethylcellulose

Figure 3: FTIR Spectra of okra polysaccharide
Figure 4: FTIR spectra of okra polysaccharide and ethylcellulose

Figure 5: FTIR Spectra of WO₃

Figure 6. In vitro release profile of okra polysaccharide based matrix tablets (Mean ± S.D., n = 3)
**In vitro studies**

The results of drug release profile of okra polysaccharide matrix tablet formulations WO1, WO2, WO3, WO4 and WO5 in pH 1.2 (2 h), pH 7.4 phosphate buffer (3 h) and pH 6.8 PBS are shown in figure 6. The % cumulative release of drug at second hour is observed as 5.62, 3.59, 2.99, 2.49 and 0.94, at fifth hour 20.75, 18.48, 13.37, 11.99 & 4.59 respectively and at 14th hour 98.18, 92.13, 88.44, 82.34, 26.82 respectively.

The result show that the dissolution rate decreased as the okra polysaccharide amount increased and that the dissolution rate increased as excipient lactose increased.

This is evident from figure 6. Thus, okra polysaccharide in the form of matrix tablet is capable of releasing minimal quantity of the drug in the physiological environment of stomach and small intestine. This may be due to the contact of okra polysaccharide matrix with the dissolution medium, followed by absorption of the fluid, swelling and formation of protective layer of hydrated gel that slowed down further seeping-in. Lactose being freely water soluble, dissolve and provide a pathway for erosion of the drug from the matrix.
In vitro dissolution studies with and without rat cecal matter.

In vitro dissolution studies of okra polysaccharide matrix tablet were carried out using pH 1.2, pH 7.4 and pH 6.8 with and without rat cecal matter in order to mimic conditions from mouth to colon environment. The results of % drug release at pH 1.2, pH 7.4, pH 6.8 PBS without rat cecal matter were 2.87, 2nd hour, 4.72 at 5th hour and 88.60 at 14th hour without rat cecal matter. The study was repeated with one more set of tablets (WO3) and the results of % drug release at pH 1.2 and pH 7.4 were found to be 2.83, 14.95 at 2nd and 5th hours respectively, but the%release with rat cecal content in pH 6.8 PBS was found to be 98.09 at 10th hour itself. The figure 7 illustrates the % release of drug without and with rat cecal contents.

In vivo studies on rabbit model

The main saccharolytic species in the colon are Bacteroides and Bifidobacterium. The human large intestine is composed of 8.0 and 7.0 viable counts of Bacteroides and Bifidobacteria respectively. The guinea-pig’s is composed of 7.1 and 8.4 viable counts; rabbit’s is composed of 8.0 and 4.5 viable counts of Bacteroides and Bifidobacteria respectively. This in vivo study had been carried out using rabbit as animal model instead of guinea-pig due to the difficulty to ingest a 200mg tablet to a guinea-pig. The formulation (WO5) was selected for in vivo study. This formulation contains 10% of ethylcellulose instead of 1% in other formulations. It showed much less release at fourteenth hour than other formulations.

X-ray imaging was adopted to pinpoint visually the various stages of in vivo transit of the tablet, from mouth to colon. The X-ray images also reveal the location, the swelling, intactness or otherwise of the matrix tablet, the duration of transit from mouth to colon and thereby substantiate this design rationale. The inclusion of a contrast material into a solid dosage form enables the visualization of the dosage form after oral administration by placing the rabbit under an X-ray machine and the images taken at fixed time points.

It is observed from figure-8 that at 2nd hour the matrix tablet has entered into stomach intact, and at 5th hour it has entered in to the intestine swelled a bit but intact. At 8th hour, the okra polysaccharide matrix tablet has found its way to colon and begins to degrade. There were no remains of matrix tablet at end of 10th hour. It indicates that the tablet was susceptible to colon bacteria.

CONCLUSION

In vitro release studies of the formulations prepared from okra polysaccharide with and without rat cecal contents indicated that rate of drug delivery enhanced in the presence of rat cecal contents, which enhance the rate of biodegradation of the polysaccharide used. This is due to the presence of enzymes secreted by the bacteria present in the cecal contents. Comparison of the release profiles of the formulation indicated that, drug release depends on the nature of the matrix and amount of polysaccharides. Okra polysaccharide at a concentration of 40% w/w with 1%w/w of ethylcellulose tablet showed controlled drug release. When 10% w/w ethylcellulose was used as matrix material, it had shown a maximum effect in controlling the drug release. These are the optimized composition for effective drug delivery. It was confirmed by in vivo x-ray images. With the present experimental work, it can be concluded that, okra polysaccharide proved to be the most suitable polysaccharide. The study revealed that natural polysaccharide can be used for selective delivery to colon for the treatment of local as well as systemic disorders. However, further investigations have to be realized in order to improve the system, and to study other variables.

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