Original Article

Carboxymethyl Tamarind Seed Kernel Polysaccharide Formulated into Pellets to Target at Colon

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ABSTRACT

Objective: Tamarind seed polysaccharide (TSP) modifying to carboxymethyl tamarind seed kernel polysaccharide (CMTSP) could be potential polymer over synthetic one to target colon diseases. Ibuprofen was loaded in CMTSP to achieve colon targeted sustained action of pellets due to viscous nature of CMTSP. **Methods:** Ibuprofen loaded CMTSP pellets prepared by extrusion spheronization technique were optimized using three-level two-factor full factorial design. **Results:** Higher was the amount of CMTSP; better was the crushing strength of pellets, needed to target at the colon. *in vitro* dissolution test of the pellets at pH 1.2, 6.8 and 7.4 phosphate buffer showed drug release within 10 h. Further, the effect of enzyme induced in the colon portion of rat, in response to fed CMTSP and TSP, was carried out by performing dissolution study of pellets at 2 and 4% w/v of rat caecal matter in an anaerobic environment. The optimal calculated parameters were CMTSP and MCC, each at 300 mg, with highest crushing strength (23.6 N) and drug release (98%) within 9 h in presence of rat caecal content (4% w/v). Scanning electron microscopy revealed the spherical nature of pellets with rough surface and small pore openings for the drug release. **Conclusion:** Ibuprofen loaded pellets of CMTSP were successfully targeted at colon, due to increase in viscosity and decrease in swelling index of CMTSP than TSP.

Key words: Carboxymethyl tamarind seed polysaccharide, Extrusion, Spheronization, Pellets, Colon drug delivery, Ibuprofen, Rat caecal content.

INTRODUCTION

The specific delivery of drugs to the colon is highly desirable for treatment of colorectal cancer and inflammatory bowel diseases such as ulcerative colitis and Crohn's disease. The colon offers reduced digestive enzyme activity, neutral pH, a long transit time and enhanced bioavailability. Colon is an ideal site for the delivery of agents to cure the local diseases of the colon.[1] However, due to its location at the distal part of the alimentary tract, the colon is particularly difficult to access. The intestinal microflora is composed of about 500 different strains. Many of them (about 95%) are anaerobic and fastidious in their growth requirements.[2] The bacterial floras present in the colon like Bacteroides and Bifid bacterium are predominantly anaerobic and carry out a variety of metabolic reactions like hydrolysis, decarboxylation, dealkylation and reduction.[3] Based upon this natural phenomenon, the colon specific drug delivery using chemically modified polysaccharide can be a promising technique to target colon. It mainly involves the enzymatic action of colonic bacteria on polysaccharides.[4]

Tamarind seed kernel polysaccharide (TSP), a glucosaminoglycan derivative, is extracted from the kernel of seeds of *Tamarindus indica* Family Fabaceae. It consists of galactoxyloglucan polysaccharide (55-65%), a monomer of glucose, galactose and xylose (molar ratio of 3:2:1).[5,6] It is insoluble in organic solvents, but disperses in hot water

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to form a highly viscous mucilaginous solution with a broad pH tolerance and good adhesivity.[7] It exhibits higher viscosity than most starches at equivalent concentration of polysaccharide. TSP has been widely used as stabilizer, thickener, gelling agent and binder in food and pharmaceutical industries.[8] *in vitro* studies carried on TSP, suggests its degradation in the presence of rat caecal contents under conditions mimicking colon.[9] However, it has several drawbacks, such as low solubility in cold water, fast biodegradability, unpleasant odor and dull color.[10] Hence, need arises to modify TSP polysaccharide to improve and alter its physicochemical and pharmaceutical characteristics in terms of aqueous solubility, stability, swell ability and release kinetics.[11] Carboxymethylation of TSP disrupts the polysaccharide structure, thus exposing the network of hydration and enabling an anionic nature to the polymer.[12] This results in higher viscosity and lower biodegradability than TSP, ultimately enhancing the shelf life.[13] The presence of carboxymethyl groups makes the molecule resistant toward enzymatic attack.[11] Carboxymethyl xyloglucan possess improved properties which are required for the retardation of release of drug, thus helping to achieve sustained release action.[14]

The colonic region of gastrointestinal tract can be targeted using modified release rate of pellets for local treatment of variety of bowel diseases such as ulcerative colitis, amoebiasis and colonic cancer.[15] Single unit dosage forms for colonic delivery may suffer from the disadvantage of unwarranted disintegration of the formulation due to high inter- and intra-subject variability and poor reproducibility, which may lead to loss of local therapeutic action in the colon. Little emphasis has been laid on the formulation of single unit dosage forms[16] in comparison to multi-particulate delivery system. Due to possible benefits, like better bioavailability and decreased risk of local irritation multiparticulate system is able to reach the colon and retain there for a long period of time.[17]

Pellets are spherical, discrete units and free-flowing granules. Pellets offer less friable dosage form, narrow particle size distribution and low risk of side effects associated with dose dumping of drug. Pellets can be either filled into the capsules or compressed into the tablets. Extrusion spheronization method is commonly used in the pharmaceutical industry to make uniform size pellets at minimum amount of excipients.[18] This method is advantageous to load high dose drugs to achieve controlled release oral solid dosage form in the form of pellets.[19]

Ibuprofen was a drug of choice due to its poor water solubility, shorter half-life (1.3-3h) and absorption window throughout the gastrointestinal tract. The efficacy of ibuprofen in the treatment of colorectal cancer has been studied earlier.[20] Literature study revealed that regular long-term use of ibuprofen might be an effective way to reduce colorectal cancer risk.[21] Ibuprofen inhibits proliferation and induces apoptosis in human colorectal cancer cells. The cyclo-oxygenase (COX) is the rate limiting enzyme for synthesis of eicosanoids such as prostaglandin and arachidonic acid. Ibuprofen has COX inhibitory activity on cell proliferation and apoptosis in colorectal cancer.[22]

Literature study revealed that tropicamide-loaded carboxymethyl tamarind seed kernel polysaccharide (CMTSP) has been explored for formulation of nanoparticles for ophthalmic drug delivery.[23] Carboxymethyl xyloglucan was used as matrix forming polymer for sustained release matrix tablets of tramadol HCl[24] as well as for formulation of metronidazole loaded cryogels.[25] A novel hybrid nanocomposite has been prepared using *in situ* incorporation of nano-sized filler (silica) onto CMTSP.[26] Researchers have used earlier chitosan and CMTSP, in the form of interpolymer complexed film, for colon drug delivery of budesonide.[27] To target colon various natural polymers were used such as pectin-surelease coated colon-specific pellets of budesonide;[28] coated tablet of pectin, inulin and shellac;[29] derivatives of chitosan succinate and chitosan phthalate for matrix tablets;[30] calcium pectinate[31] and amidated pectin in matrix tablet,[32,33] gaur gum matrix tablet.[34,35,36] We hypothesized that more viscous nature of CMTSP than TSP and increased resistance at acidic pH could be potential candidate to delay the release and target the drug in the colonic region. Being abundant in nature, TSP by modifying to CMTSP could be economical and suitable polymer, over synthetic polymer, to treat colon diseases. However, to the best of our knowledge, there is no scientifically reported study available on the use of CMTSP pellets to target the colorectal cancer. In light of this, an attempt was made to derivatize the TSP to CMTSP and target at colon in the form of ibuprofen pellets prepared by extrusion spheronization technique to treat colorectal cancer.

MATERIALS AND METHODS

Ibuprofen was kindly supplied as gift sample by Wockhardt, Aurangabad, Maharashtra, India. Tamarind seeds were purchased from local market. All other chemicals were of analytical grade and were used as received.

Isolation of TSP

The seeds of *Tamarindus indica* (500 g) were washed with water to remove the dirt and adhering material. The

seeds were heated in sand (1:1) and crushed to remove outer brownish testa. These seeds were soaked in water for 24 h. Soaked seeds were boiled for 1 h and kept aside for 2 h to liberate sufficient mucilage into water. Mucilage was removed from the marc by squeezing the soaked seeds through the muslin cloth. Mucilage was then isolated with equal quantity of acetone and dried at 50°C in the hot air oven (Remi, India) powdered and passed through sieve number 80 to get uniform size fine powder of TSP. Powder was then stored in airtight container at room temperature till further use.[37]

Derivatization of TSP to CMTSP

Isolated TSP powder (10 g) was dispersed in alkaline (0.090-0.180mol, NaOH aqueous methanol solution), 100ml. Followed by addition of solid mono-chloro acetic acid, placed in a thermostatic water bath (70°C), with occasionally shaking for 60 min.[12] The optimum ratio of sodium hydroxide: mono-chloro acetic acid: TSP was found to be 3.16:1.8:1, as reported by the Goyal and workers.[12] After the completion of reaction, obtained mass was filtered through the Whatman filter paper no1. The product was then dissolved in water, neutralized with dilute hydrochloric acid, precipitated in ethanol (AR grade 90%) and washed thrice with aqueous methanol solution (80:20). Further, pure methanol washing was given to the product. The obtained product (CMTSP) was dried initially at room temperature, then in vacuum oven at 40°C for 4 h and weighed to get the product yield. The Fourier Transform Infra-red Spectroscopy (FTIR) was performed to confirm the conversion of TSP to CMTSP.[13] The IR spectrum of TSP and CMTSP was obtained on over a wave range of 4000-400 cm $^{-1}$ in FTIR instrument (Alpha Bruker, Germany).

Characterization of isolated and modified TSP

Isolated TSP and CMTSP were characterized for angle of repose, density and compressibility index. Viscosity of both, TSP and CMTSP was determined by using Brookfield viscometer (LVDVE-Brookfield Engineering Ltd. Inc., USA). Accurately weighed (1 g), dried and fine powder was suspended in 75 mL of distilled water and kept aside for 5 h. Volume was made up to 100 mL to get 1% w/v of the solution. The mixture was then homogenized by mechanical stirrer for 2 h and viscosity was determined at spindle number 61, 500 rpm and 25°C using a Brookfield viscometer (LVDVE-Brookfield Engineering Ltd. Inc., USA). The swelling index of powder was measured to know the water retention capacity. Accurately weighed powder (1 g) was transferred to 100 mL measuring cylinder. Initial volume occupied in the cylinder was noted. Distilled water was added to it and shaken gently. Measuring cylinders were kept aside for 24 h at room temperature. The change in volume occupied by swelled polymer was noted. Swelling capacity of polymer was expressed in terms of swelling index (SI) and calculated according to the following equation (1).[38]

$$SI = \frac{S_1 - S_0}{S_0} \times 100 \quad (1)$$

Where, S_0 is initial volume of the powder in graduated cylindrical and S_1 is volume occupied by swollen gum after 24 h.

Formulation of drug loaded pellets

Drug loaded pellets were prepared by extrusion-spheronization technique.[39] Accurately weighed quantities of ibuprofen (100 mg), microcrystalline cellulose quantity (MCC 100-300 mg) and CMTSP (300 to 500 mg) were mixed in planetary mixer (Avon, India) in increased geometric proportion. Granulating liquid, 10% w/v aqueous dispersion of PVP K30 was added slowly to the powder blend, which was then mixed until a homogeneous, cohesive and plastic mass was obtained. The resulting wet mass was extruded through an extruder plate 1 (Shakti Pharma, India) at a speed of 25 rpm. Extruded mass so obtained was subjected to spheronize in the spheronizer (Shakti Pharma, India) using the rotating plate of cross-hatch geometry at 1000 rpm for 4 min to get pellets of desired size. Pellets were cured in hot air oven at 30°C for 24 h.[40]

Selection of formulation parameters and process variables

Preliminary trials of formulation were undertaken to establish physical parameters of pellets by studying concentration of CMTSP (50 to 500 mg), concentration of MCC (200 to 600 mg) and stirring speed of both extruder (20 to 50 rpm) and spheroniser (800 to 100 rpm). Effects of these various parameters were studied on spherical nature, crushing strength, drug content and drug release from pellets. Based on these preliminary results, independent variables were selected for the optimization process.

Experimental design

A 3^2 full factorial design was applied to optimize the pellets. Using the software Design Expert® (version 9.0.3), two factors were evaluated each at three levels; low, medium and high. The factors; concentrations of

both carboxymethyl tamarind seed polysaccharide (X_1) at 100, 200 and 300 mg and microcrystalline cellulose (X_2) at 300, 400 and 500 mg, were selected as independent variables. The response variables considered, were crushing strength (Y_1) and drug release (Y_2), selected as the dependent variables. The formulation batches of F1 to F9 suggested by factorial design are shown in Table 1. To compare the effect of TSP and CMTSP on the characteristics of pellets, TSP pellets (F10) were also prepared with composition of optimized batch (300 mg each of CMTSP and MCC).

Evaluation of pellets

The pellets were evaluated for bulk density, tapped density, Carr's index and angle of repose.[41,42] To determine the drug content, accurately weighed pellets (900 mg) were powdered in mortar and pestle. Powder equivalent to single dose of ibuprofen (100 mg) was weighed and transferred to 100 mL volumetric flask containing 50 mL of methanol. The flask was sonicated for 1 h and the volume was made up to 100 mL with the methanol. The resulted solution was centrifuged at 5000 rpm for 20 min and filtered.[43] After suitable dilution, drug content was estimated at 221 nm using UV spectrophotometer (1800, Shimadzu, Japan) using methanol as blank solution.

Crushing strength of pellets

Crushing strength of pellets was determined using Texture Analyser (CT3 Texture Analyser, Brookfield Engineering Lab. Inc., USA) operating at 3 kg load cell. Pellets were placed in lower flat plate, centered under the 25.4 mm diameter and 35 mm length of clear acrylic upper punch. The punch was moved downwards at a constant rate of 0.3 mm/s.[44] Force (N) vs time (s) plot was recorded using the software (TexturePro CT).

In vitro release study of ibuprofen

In vitro drug release study of pellets (F1 to F10) was carried out in 900 mL of 0.1N HCl (pH 1.2) for 2 h using USP dissolution test apparatus I (TDT-08L, Electrolab, India) at100 rpm maintained at temperature 37±0.5°C. The dissolution study was continued further in phosphate buffer pH 6.8 for 3 h and then in pH 7.4 phosphate buffer solution. Samples were withdrawn at specific time intervals of 1h and replaced with the same volume of preheated fresh dissolution medium, to maintain sink condition. The samples were filtered using a Whatman filter paper and analyzed using double beam UV visible spectrophotometer at λmax 221 nm. All experiments were performed in triplicate.

In vitro drug release study in presence of rat caecal content

Drug release study was performed in presence of rat caecal content to study the effect of intestinal microflora in response to polysaccharides (both TSP and CMTSP) in the colonic portion. This study was carried out as per the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India. The protocol was approved and performed in accordance with the guidelines of Institutional Animal Ethics Committee, India and approval number is IAEC-14-004. Eight healthy male wistar rats of uniform body weight (200 – 250 g) with no prior drug treatment were selected for the study.

Two sets (A and B) of rats each containing three rats were taken for the study. Animals of set A were fed with normal diet. Then the Rats were humanly killed by spinal traction thirty min before the start of the *in vitro* drug release study. Abdomen of each rat was carefully opened. The caecal contents were isolated, ligated at both ends and cut loose. The contents of all the rats were pooled together after opening the caecal bags and immediately transferred to pH 6.8 phosphate buffer solution to get required caecal dilution of 2% w/v, previously bubbled with nitrogen.[45] The caecal solution was immediately transferred into the dissolution medium of pH 6.8 phosphate buffer solution to study the *in vitro* release for F7RN (F7 indicates pellets made of CMTSP; R indicates in presence of rat ceacal matter; N indicates rats fed with normal diet) and F10RN (F10 indicates pellets made of TSP).

In vitro drug release study in presence of CMTSP induced enzyme rat caecal content

Animals of set B were fed with CMTSP, to induce the enzymes specifically acting on it in the caecum. Teflon tubes were inserted in the food pipe of rats. One mL of 2% w/v dispersion of CMTSP in water was administered directly into the stomach. The tubing of each rat was removed. The same treatment was continued for 7 days. Rats were then humanly killed by spinal traction thirty min before the start of the drug release study. The same procedure was followed as mentioned for set A to obtain the ceacal content. The obtained caecal content, equivalent to 4 and 8 g, was added, individually, to 200 mL of pH 6.8 phosphate buffer solution to give a final caecal dilution of 2 % and 4 %, respectively. As the caecum is naturally anaerobic, all these operations were carried out under supply of nitrogen. *in vitro* release study for F7RE1 (CMTSP pellets in presence of

enzyme induced rat ceacal content 2%w/v) and F7RE2 (CMTSP pellets in presence of enzyme induced rat ceacal content 4%w/v) were carried out.

Both the sets (set A- F7RN and F10RN; set B -F7RE1 and F7RE2**)** were further exposed to *in vitro* dissolution study. *in vitro* drug release method described earlier was slightly modified with some modification in the dissolution test.[46] The modifications in the dissolution testing were performed due to two main reasons; firstly, less amount of dissolution medium present in the intestinal portion. Secondly, the amount of rat caecal content collected from each rat was very less (about 1g per rat). The test was performed in 250 mL beaker immersed in the jar of dissolution test apparatus. Drug release study was performed in 150 mL of 0.1 N HCl for 2 h. The study was continued for next 3 h in pH 6.8 phosphate buffer solution (0.2 mol/L trisodium phosphate was added to the dissolution media to get 200 mL pH 6.8 buffer solution) in presence of 2% and 4%w/v caecal content. The test was carried out in presence of a continuous supply of nitrogen. At specified time intervals of 1h, one ml of aliquot was withdrawn each time and replaced with same quantity of caecal content (2% or 4%) maintained under anaerobic conditions. The samples were filtered using a Whatman filter paper No 1 diluted and analyzed using double beam UV visible spectrophotometer at λmax 221 nm.

Release kinetics

In order to determine the suitable drug release kinetic model describing the dissolution profile, the model dependent approach was studied. The data obtained from *in vitro* dissolution study (F7) was fitted to different models like zero order, first order, Korsmeyer-Peppas, Higuchi equation and Hixson-Crowell model.

Scanning electron microscopy

Scanning electron microscopy (SEM) of optimized batch F7 was performed to visualize the surface morphology of the pellets.

Stability study

Accelerated stability study was performed, according to ICH guidelines, to ensure a stable product till shelf life. Optimized formulation was placed in vials and stored in stability chamber (Thermolab, India) at 30ºC±2ºC/ 65% RH±5% RH. The samples were evaluated for the drug content, crushing strength and *in vitro* drug release study after 7, 15, 30 days and 3 months.

RESULTS AND DISCUSSION

Characteristics of TSP and CMTSP

The percentage yield of isolated TSP and modified TSP (CMTSP) was found to be 70.5 and 65% w/w, respectively, sufficiently high enough to use polymer as an excipient in the formulation. Both the powders were brownish in color. Swelling index and viscosity (aqueous dispersion of 1% w/v) of TSP and CMTSP were found to be 200% and 8.80 cps; and 133% and 15.45 cps, respectively. Greater viscosity of CMTSP than TSP indicated the suitable candidate to sustain the drug release in the colonic region. Angle of repose of all batches was found in the range of 25º to 30º, which indicated good flowability of pellets, as reported by Lalla and Bhat.[47] The bulk density, tapped density and compressibility of both revealed good flow properties to formulate in the form of pellets.

The IR spectrum of TSP showed the bands at 2878.24cm^{-1} for C-H stretching at 1230.36cm^{-1} for C-O stretching and one strong bond at 1072.23cm^{-1} was attributed to CH_2-O-CH_2 stretching vibrations (Figure 1a). In case of CMTSP, IR spectrum showed two additional peaks; one at 1746.23 cm^{-1} and other at 1669.09cm^{-1}, for the carboxyl (– COO^-) group, in addition to those peaks observed for TSP, which confirmed the formation of CMTSP (Figure 1b). The obtained peaks for TSP and CMTSP were found in the same range as reported in the literature.[13] The IR spectrum of CMTSP confirmed the presence of carboxyl group, thus assured the conversion of TSP to CMTSP.

Experimental design

Experimental trials were performed for nine possible formulations suggested by 3^2 factorial design. Mathematical treatment of the possible combinations of batches F1-F9 is shown in Table 1.

Evaluation of pellets

Pellets prepared by extrusion spheronization technique showed good flow property and compressibility index. Formulated ten batches (F1 to F10) were spherical in appearance, hence suitable for further formulation process. Drug content was found to be uniform for all batches, which confirmed batch to batch uniformity in the formulation of pellets.

Crushing strength of pellets

The crushing strength value obtained for F1 to F10 formulations is shown in Figure 2a. Comparison of the three batches F1, F4 and F7 which contained 300 mg MCC and increased concentration of CMTSP (100, 200 and 300 mg, respectively) showed 8.89, 19.58 and

Table 1: Composition of pellets.						
Run	Batch	Ibuprofen (mg)	TSP (mg)	CMTSP (mg) (X1)	MCC (mg) (X2)	PVPK30 10% w/v
1	F1	100	-	100	300	q.s
2	F2	100	-	100	400	q.s.
3	F3	100	-	100	500	q.s
4	F4	100	-	200	300	q.s.
5	F5	100	-	200	400	q.s
6	F6	100	-	200	500	q.s.
7	F7	100	-	300	300	q.s
8	F8	100	-	300	400	q.s.
9	F9	100	-	300	500	q.s
10	F10	100	300		300	q.s.

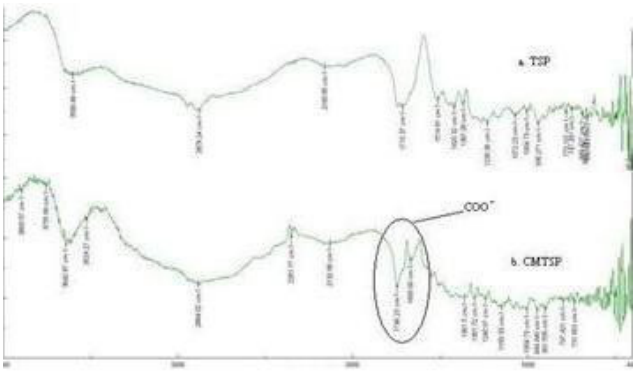

Figure 1: FTIR of a) TSP and b) CMTSP

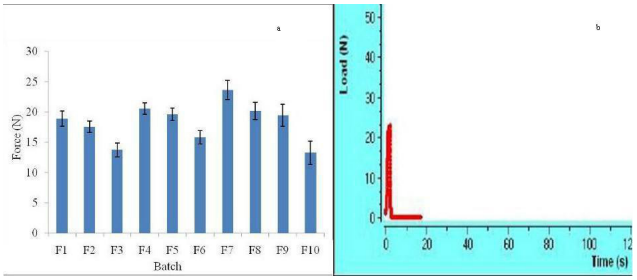

Figure 2: Crushing strength of pellets a) formulations F1 to F10 b) Formulation F7

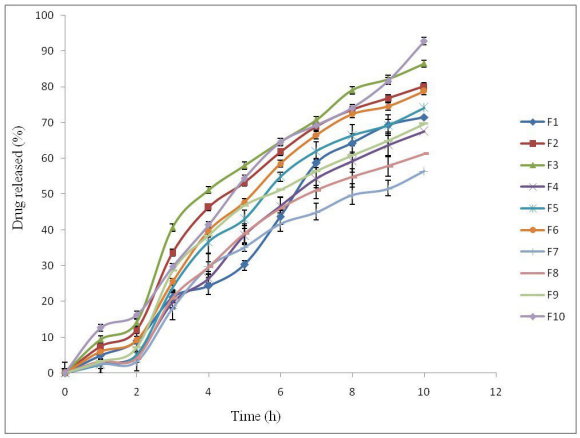

Figure 3: Drug release profile of pellets

23.63 N Figure 2b force required to break the pellets. Thus, from the data obtained, it was concluded that higher the concentration of CMTSP, higher was the crushing strength of pellets. The obtained results deviate from the result of Jadhav and co-worker.[48] The reason for this could be the use of talc along with MCC in previous work. It was observed that addition of CMTSP to MCC provided a moist environment to the pellets, thus imparted a suitable binding strength to the pellets due to its viscous nature.[39] As the concentration of CMTSP was increased, the binding strength was imparted to pellets which in turn increased its crushing strength.

In vitro drug release study

Formulations F1, F2 and F3 released 9.01%, 11.99%, 14.13%,respectively,of druginpH1.2within 2h(Figure3). Further, the release was increased to 30%, 53% and 57% respectively, in pH 6.8 and continued to approximately 71%, 80% and 86%, respectively, in pH 7.4 within 10 h. The pellets of these batches exhibited limited swelling and further disintegrated into smaller granules after 10 h. Formulations F4, F5 and F6 released 4.86%, 5.04% and 9.17%, respectively, of ibuprofen in pH 1.2 within 2 h. Further, the release was increased to 38%, 42% and 47%, respectively, at pH 6.8 and continued to 67%, 74% and 78%, respectively, in pH 7.4 within 10 h. The pellets of batches F4, F5 and F6 exhibited limited swelling. Pellets of formulation F6 disintegrated during dissolution study within 10 h, while F4 and F5 remain intact throughout the dissolution study (10 h).

Formulations F7, F8 and F9 showed 3.14%, 4.20% and 7.09%, respectively, of drug release in pH 1.2 within 2 h. Further, the release was increased to 35%, 39% and 46% respectively, in pH 6.8 and continued to 56%, 61% and 69% respectively, in pH 7.4 within 10 h. *in vitro* dissolution test of F10 (TSP pellets) released 92.75% of

ibuprofen within 10h in absence of rat caecal content. The pellets of batches F7, F8 and F9 exhibited the swelling and remained intact during dissolution study (10 h). The effect of increased amount of MCC and unchanged amount of CMTSP on drug release was studied in three groups; F1, F2, F3; F4, F5, F6 and F7, F8, F9. *in vitro* dissolution profile revealed that more the amount of MCC, more was the amount of drug released. Whereas, increased amount of CMTSP (100-300 mg) delayed the drug release of pellets. As discussed earlier, more crushing strength of pellets at higher amount of CMTSP was found to be responsible to delay the drug release. The viscous nature of CMTSP exhibited the delayed release. Not more than 10% drug was released in the acidic dissolution medium in first 2 h, hence used as potential polymer for colonic release.

Based on formulation batches (F1 to F9) suggested by software, F7 (CMTSP) showed slowest drug release (3.14%) within 2h in acidic media. Furthermore, at pH 6.8, F7 showed 35% drug release within 5 h. Transport of pellets in gastrointestinal tract require 5-6 h to enter the colon portion. Thus, approximately 65% drug was still available for further release into the colonic region. At the end of 8 h, 49% ibuprofen was released in the colon. F7 showed most retardant drug activity in colon. Slow release of ibuprofen was expected to target colon region to treat colorectal cancer.[20] TSP pellets F10 (at same concentration of variables of F7), however, released the drug at faster rate compared to F7. Thus, modified TSP was found to be considered a good choice to delay drug release at colon.

Effect of formulation variables on crushing strength and drug release

Analysis of variance (ANOVA) was applied to determine the significance and the magnitude of the effects of the independent variables and their interactions on crushing strength (Y_1) and drug release (Y_2). Responses of different batches obtained by using factorial design are expressed individually in equations 2 and 3. The regression coefficient for Y_1 (crushing strength) showed in equation (2), in terms of coded factors, explained the predictions about the crushing strength response for given levels of each factor.

$$Y_1 = +17.89 + 3.85\ X_1 - 0.52X_2 \qquad (2)$$

A linear regression equation (2) was obtained for crushing strength (Y_1) with significant with F value 4.86 ($p < 0.0475$). Positive sign before the factor X_1 shows synergistic effect of concentration of CMTSP on crushing strength. However, negative sign before the X_2 shows reciprocal effect of concentration of MCC on crushing strength. Higher numerical value of X_1 shows more influence of factor X_1 than X_2.

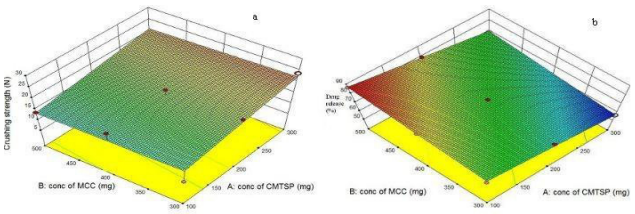

Figure 4: Three dimensional surface plots of (a) crushing strength (b) drug release

The regression coefficient (Y_2), shown in equation (3), in terms of coded factors, explained the predictions about the response drug release for given levels of each factor as follows:

$$Y_2 = +72.00 - 8.45\ X_1 + 6.58X_2 \qquad (3)$$

CMTSP shows more reciprocated effect than direct proportionality effect of MCC on drug release, due to its higher numerical value, and follows linear model with significant with F value 96.43 ($p<0.0001$). According to the software of factorial design, percent error obtained by using predicted value and actual value for Y_1 and Y_2 was found to be 2.63 and 2.56, respectively, which was found to be very less. The individual and combined effects of these factors on the dependent variables are further explained with the help of three dimensional response surface plots as shown in Figure 4a and 4b.

In vitro drug release study in presence of rat caecal content

Formulation F7RN released 4.15% of ibuprofen in pH 1.2 within 2 h. Further, the release was increased to 74.69% within 10h and F10RN showed about 92% of drug release within 10 h. The above results revealed that drug release was faster in presence of rat caecal content. When drug released data (in presence of rat caecal content of rats fed with normal diet) of both, TSP pellets (F10RN) and CMTSP pellets (F7RN) were compared, F7RN was found to retard the drug release by 4 h compared to F10RN. The obtained data are depicted graphically in Figure 5.

F7RN showed more drug release as compared to F7 indicating faster drug release. When drug released data (in presence of rat caecal content) of both, TSP pellets (F10RN) and CMTSP pellets (F7RN) were compared, F7RN was found to retard the drug release compared to F10RN. The above results indicated that the polysaccharide was eroded by the action of colonic microbial flora. About 30% of the total drug remained in the pellets of F7RN.

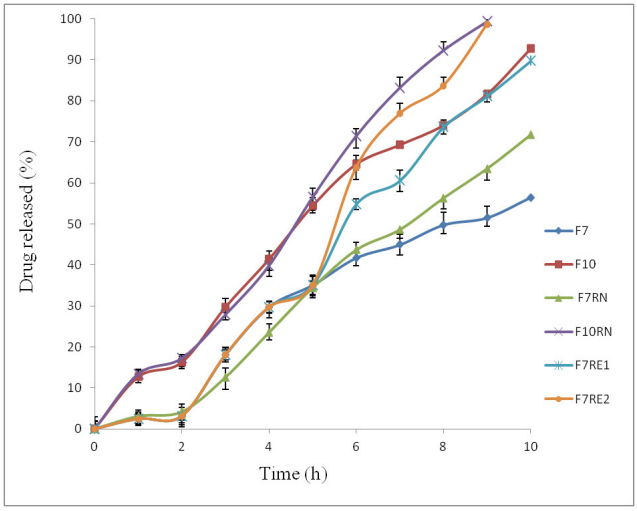

Figure 5: Drug release study from TSP and CMTSP pellets in dissolution media

F7- CMTSP pellets in absence of rat cecal content, F10- TSP pellets in absence of rat cecal content, F7RN (F7 indicates pellets made of CMTSP; R indicates in presence of rat ceacal matter; N indicates rats fed with normal diet) and F10RN (F10 indicates pellets made of TSP; R indicates in presence of rat ceacal matter; N indicates rats fed with normal diet). F7RE1 (CMTSP pellets in presence of enzyme induced rat ceacal content 2% w/v) and F7RE2 (CMTSP pellets in presence of enzyme induced rat ceacal content 4%w/v)

In vitro drug release study in presence of CMTSP induced enzyme rat caecal content

In vitro study of formulations (F7RE1 and F7RE2), containing 2% and 4% w/v solution of CMTSP enzyme induced rat caecal content in dissolution media, showed the drug release of about 89% within 10h and 98% within 9h, respectively. Higher amount of caecal content solution (4% w/v) released the drug completely at faster rate (9h).

The data obtained after dissolution study in presence and absence of rat caecal content showed the effect of caecal matter. Presence of rat caecal content showed enhanced drug release, by causing erosion of CMTSP pellets. However, when study was performed in presence of rat caecal content fed with CMTSP, the enzymes responsible for biodegrading the polysaccharide, were found to be induced in the rat colon and acted on pellets. Ultimately, this resulted in faster drug release. Higher amount of caecal content in the solution (F7RE2) (4% w/v) released the drug completely at faster rate. This indicated that anaerobic microflora present in the colon degraded the carboxymethylated tamarind seed polysaccharide; therefore, responsible for faster drug release from the pellets. However, some of the researchers showed incomplete drug release in presence of 4% w/v rat caecal content in the dissolution medium. Li-Fang *et al* reported 60% theophylline release through the chitosan/Kollicoat SR 30D coated tablets (in the presence of 4% w/v rat caecal content).[49] Kaur *et al* reported incomplete drug release of budesonide from the inter polymer complexed film of chitosan and CMTSP even after 24 h in presence of 4% w/v rat caecal content.[27]

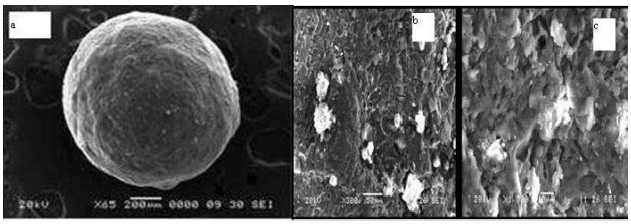

Figure 6: Scanning electron microscopy of pellets

The viscous nature of CMTSP could be beneficial to sustain the drug release at the colon. CMTSP was found to degrade at colon due to induction of enzymes and anaerobic microflora present in the colon.

Release kinetics

The release kinetics of ibuprofen from pellets was explained by Korsmeyer-Peppas, Higuchi equation and Hixson-Crowell model. The regression coefficient (r^2) was found to be 0.9683, 0.9712 and 0.9795, respectively. The value of release exponent (n value) obtained by Korsmeyer-Peppas was 0.956. The pellets were found to follow Higuchi equation as well as Hixson-Crowell model. Thus, drug release was explained by the combination of both diffusion and erosion of polymer. CMTSP pellets swelled in the dissolution media and then further eroded releasing the drug.

Scanning electron microscopy

The morphology of the drug-loaded pellets analyzed by scanning electron microscopy is shown in Figure 6. Micrographs revealed the spherical pellet with rough surface (Figure 6a). Small pore openings appeared on the surface confirmed the sites for the drug release (Figure 6b and 6c). Jadhav *et al* proposed that spherical nature of pellets offered good flow property with satisfactory physical properties for further processing into various dosage forms.[48]

Confirmation of optimized batch

The data and plots generated by computer software were used to get the optimum formulation of pellets. The response variables crushing strength and drug release were considered to optimize the process. Formulation F7 was found to achieve optimum level by showing highest crushing strength of 23.63 N and 56% of drug release in 10 h. Even though, formulations F8 and F9

released 61% and 69% drug which was more than F7, but exhibited less crushing strength, 20.15 and 19.45 N, respectively. Further, the release study of F7RE2 in presence of rat caecal content supported the degradation of carboxymethyl TSP and released the drug within 9 h. Scanning electron micrographs and flow properties of F7 confirmed the formulation F7 as optimized batch which contained 300 mg each of carboxymethyl tamarind seed polysaccharide and microcrystalline cellulose.

Stability study showed drug content of F7 reduced from 98.3% to 97.4%, which was found to be within acceptable range. The crushing strength was found to unchanged (23.23 N), when performed after 90 days. The drug release of F7, after 10 h, was found to be 54.69±1.57. There was no significant change in drug content, crushing strength and drug release after 90 days. Use of modified form of natural polymer, as a polymer for colon release, highlighted the risk of change in the drug release characteristics. However, accelerated stability study performed for the period of three months revealed no change in the release characteristics of the formulation. Thus, modified natural polymer can be a good choice for formulation of colon targeted pellets.

CONCLUSION

The study was aimed to investigate the carboxymethyl tamarind seed polysaccharide for formulation of pellets to target colonic disease. The pellets were successfully prepared by extrusion spheronization technique using microcrystalline cellulose as one of the important excipient of pellets. Pellets offered more surface area to release drug at the colon region. Rats fed with CMTSP confirmed the enzyme induced in their colon in response to polysaccharide and showed entire drug release. The optimal parameters found were CMTSP and MCC, each at 300 mg, having highest crushing strength and extended drug release of 9 h in presence of rat caecal content. Colon targeted drug delivery of pellets has been achieved without incorporation of any coating method. CMTSP showed extended drug release in the colon compared to TSP, due to increase in viscosity and decrease in swelling index of xyloglucan polysaccharide. Thus, carboxymethyl tamarind seed polysaccharide can be considered better candidate for colon specific drug delivery than the tamarind seed polysaccharide.

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CONFLCIT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TSP: Tamarind seed polysaccharide; **CMTSP:** Carboxymethyl tamarind seed kernel polysaccharide; **MCC:** Microcrystalline cellulose; **NaOH:** Sodium Hydroxide; **COX:** Cyclooxygenase; Page no S546; **AR:** Analytical Reagents; **FTIR:** Fourier-transform infrared spectroscopy; **PVP:** Polyvinylpyrrolidone; **HCl:** Hydrochloric acid; **SEM:** Scanning Electron Microscopy; **ANOVA:** Analysis of variance.

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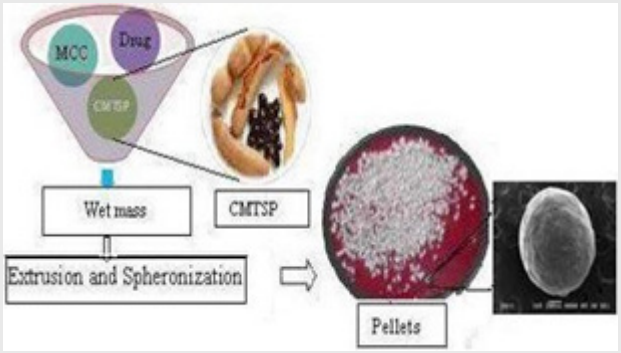
SUMMARY

- Carboxymethyl tamarind seed kernel polysaccharide (CMTSP) obtained by modifying Tamarind seed polysaccharide (TSP) acted as a potential polymer over synthetic one to target ibuprofen in the colonic region. Ibuprofen loaded CMTSP pellets were prepared by extrusion spheronization technique using three-level two-factor full factorial design.The pellets prepared with the higher amount of CMTSP exhibited better crushing strength needed to target the colon.
- *In vitro* dissolution test of the pellets carried out at pH 1.2, 6.8 and 7.4 phosphate buffer showed controlled drug release within 10 h. Not more than 10% drug was released in the acidic dissolution medium in first 2 h, indicating their potential to be used as polymer for colonic release. Also the effect of enzyme induced in the colonic portion of rat, in response to fed CMTSP showed entire drug release in colon. Colon targeted drug delivery of pellets has been achieved without incorporation of any coating method. CMTSP showed extended drug release in the colon compared to TSP, due to increase in viscosity and decrease in swelling index of xyloglucan polysaccharide. Thus, carboxymethyl tamarind seed polysaccharide can be considered better candidate for colon specific drug delivery than the tamarind seed polysaccharide.

PICTORIAL ABSTRACT



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