

Isolation, characterization, and evaluation of *Cassia fistula* Linn. seed and pulp polymer for pharmaceutical application

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Abstract

Introduction: Present work, is an effort toward exploring the potential of *Cassia fistula* Linn. seed gum as an extended release polymer and laxative. While, *C. fistula* pulp polymer has evaluated as suspending agent. **Materials and Methods:** For extended release application, total five batches (F1-F5) were prepared by varying the ratio of drug:polymer as 1:1, 1:2, 1:3, 1:4, and 1:5, respectively. The granules were prepared by wet granulation method and further evaluated for micromeritic properties such as angle of repose (θ), Carr's compressibility index (CCI), and Hausner's ratio. Further compacts were evaluated by hardness, thickness, swelling index, *in-vitro* dissolution, and so on. Laxative activity was evaluated by administration of seed polymer (100 mg/kg) alone or in combination with bisacodyl (2.5 mg/kg) in 1% Tween 80. Zinc oxide suspension was prepared by varying the concentration of *C. fistula* pulp polymer and compared with suspension made by use of tragacanth, sodium carboxymethyl cellulose and bentonite. **Results:** Result showed that granules were free flowing, while the compact extended the drug release up to 10 h (72.84 ± 0.98 ; batch F5) and followed Higuchi matrix release kinetics. This extended release might be due to the formation of polyelectrolyte complex because of gluco-mannose in seed gum. Result of *in-vivo* laxative activity showed that seed polymer reduced faeces weight after 24 h compared to control ($P < 0.01$). **Conclusions:** Pulp polymer showed good sedimentation volume, but alone fails to stabilize the suspension for a longer period, so it could be useful in combination with other suspending agents and can be useful as novel excipient.

Key words: Diclofenac sodium, extended release, laxative, suspending agent, swelling index

INTRODUCTION

Since many years, oral route is most preferable amongst all routes for drug administration.^[1] In a recent era near everyone prefers to use sustained release (SR) mode of drug administration as a substitute to conventional dosage form because it has guaranteed features that eventually affects enormity of the pharmacologic response. It reduces ratio between peak and trough which properly maintains the therapeutic level of drug concentration, reduces the dosing frequency and enhance patient's compliance.^[2,3]

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Diclofenac sodium (2-(2,6-dichloranilino)phenylacetic acid) is a nonsteroidal anti-inflammatory drug usually administered to manage a variety of anti-inflammatory disorders such as rheumatoid arthritis, polymyositis, osteoarthritis, and spondylarthritis and so on.^[4,5] SR tablets of diclofenac sodium using natural gums such as Xanthan gum, Guar gum, and synthetic polymers have previously been reported.^[6-8]

Although the synthetic polymers such as ethyl cellulose^[9] and hydroxy propyl methyl cellulose (HPMC)^[10] are widely used to formulate SR preparations, at the present researchers are aiming to implement the use of natural polymers to design and develop the sustain release drug delivery system. Literature reveals that previous work has been conducted on sustainable formulations using natural polymers like Guar gum,^[11] Karaya gum,^[12] Chitosan,^[13] and Xanthan gum.^[14,15] Natural polymers are widely used because they are biocompatible, physiologically inert, inexpensive, widely available and compatible with many drugs.^[16]

Cassia fistula Linn., a plant belongs to family Fabaceae (Caesalpinaceae). It is an ornamental plant, grows wildly in mixed monsoon forests throughout greater parts of India,

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ascending to 1300 m in outer Himalaya. In Maharashtra, it occurs as a scattered tree throughout the Deccan and Konkan.^[17] It is 6-9 m in height with a straight trunk, and bark is smooth, pale grey when young, while gets rough, dark brown when old.^[18] *C. fistula* seed mucilage has been evaluated in tablet formulations as binder.^[19] Carboxymethylated *C. fistula* gum has utilized in aqueous tablet-coating process.^[20]

In the present study, various hydrophilic matrix systems were designed by taking different concentrations of *C. fistula* gum, with a fixed drug concentration. In addition to this, *C. fistula* seed and pulp polymer also screened for its pharmaceutical application.

MATERIALS AND METHODS

Materials

Diclofenac sodium was kindly provided as gift sample by Okasa Pharma, Satara (India). Microcrystalline cellulose (Signet Mumbai, India), magnesium stearate (Loba Chem. Pvt. Ltd. Mumbai), zinc oxide (Molychem, Mumbai, India), n-hexane (High Purity Laboratory Chemicals, Mumbai, India), glycerine (Merck Specialities Pvt. Ltd. Ambarnath), ethanol (E. Merck (India) Ltd., Mumbai, India), methanol (Molychem Mumbai, India), chloroform (Finar Chemical Ltd., Ahmadabad, India), and acetone (E. Merck (India) Ltd., Mumbai, India) were procured from the local market.

Methods

Extraction, purification, and characterization of polymers from seed and pulp

Collection of plant material

The *C. fistula* Linn. was first located in the local area; plant was collected in the flowering stage. Collection was done in the month of April-May from the different locations of Kolhapur. Different parts of the plant such as flowers, leaves, twig, legume, and seed are separated and pressed in blotting paper for preparation of herbarium. Dried plant material was properly stuck on the white paper sheet for herbarium. All necessary details regarding-collected by, collection period, etc. were properly filled.

Identification and authentication

Identification and authentication of the herbarium of *C. fistula* Linn. was done by Dr. M. Y. Cholekar-Bachulkar, Taxonomist and Principal, Shri Vijaysinha Yadav Arts and Science College, Peth-Vadgaon Kolhapur, Maharashtra. Herbarium (Nale A. B No. 1) specimen was deposited in Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur for future referencing.

Preparation of seed powder

Dried seeds (1.5 kg) were separated from pods and powdered in the mixer grinder. Further powdered material was passed through the sieve no. 60, weighed and used for extraction.

Selection and optimization of extraction process

For seed protein

Extraction of protein was carried out by an assortment of methods like salt precipitation, using organic solvents, heating of the solution also precipitate out the proteins. After optimization of isolation method we tried for different salts such as sodium chloride, potassium chloride, calcium chloride, and ammonium sulfate.

For pulp polymers

Extraction of polysaccharide was carried out by assorted methods like extraction with boiling ethanol, chloroform water IP, 1% NaCl solution or boiling water, and polysaccharides were precipitated out by placing this solution in several volume of alcohol. After process optimization chloroform water IP was used for extraction.

Extraction of polymers

From seeds

Initially dried powder of *C. fistula* seeds was extracted with n-hexane for removal of oil. Further, this de-oiled powder was challenged with 3% sodium chloride solution and continuously agitated for 12 h in an orbital shaker at 37°C and 60 rpm, for leaching out of proteins from seed powder. Extract was filtered by Whatman filter paper and removed out the brown colored sodium chloride extract. This extract was further heated till white precipitate should not form at the bottom of solution; it was a separated crude protein which was further purified by dialysis. To avoid the step of removal of oil every time, we have used directly de-oiled seed powder.^[21]

From pulp

Extraction was carried out in chloroform water IP solution. Pulp was placed in chloroform water IP and it was continuously agitated for 24 h in the orbital shaker at 37°C and 60 rpm, for leaching out of carbohydrate polymers from pulp. Extract was filtered by Whatman filter paper, and this filtrate was placed in several volume of acetone to precipitate polysaccharides. These precipitated polymers were dried at 40°C and kept in desiccators for further studies.^[22]

Purification of seed polymers

Heated crude protein extract was cooled, poured into the dialysis tube (Himedia. Mumbai) and kept for 12 h into the beaker containing cold water that was kept in the ice bath. After completion of dialysis, salts were removed out into the surrounding water solution and white residue remains inside the tube. Residue was further removed out from the tube and rinsed with de-ionized water. These separated proteins were homogenized with cold acetone for delipidization in a homogenizer and dried at room temperature.

Determination of total proteins by Lowry method

Total protein content was determined by previously reported Lowry method, which involves the reactivity of the peptide nitrogen(s) with the copper [II] ions under alkaline conditions and the

subsequent reduction of the Folin Ciocalteay phosphomolybdic phosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids.^[23]

Preliminary chemical test

Separated polymeric extract was screened for different biochemical constituents by performing different chemical test.^[24,25]

Solubility and pH study

Solubility of separated seed and pulp polymers were carried out by gravimetric method in which 1 g of polymers were dispersed in different solvents such as water, ethanol, chloroform, and methanol. While pH was determined using 1% solution of seed and pulp polymers in water at room temperature.

Isoelectric pH

Isoelectric pH of *C. fistula* polymers were determined using 1% solution of *C. fistula* seed proteins in water, to this slowly added ethanol till precipitate was formed in solution, now report the pH of the solution that was the isoelectric pH.

Moisture content

Moisture content of the *C. fistula* polymers was an important parameter deciding the stability of the material. Methods like infrared (IR) moisture balance was recommended for the moisture determination, which gives direct display of the weight loss related with the percentage of moisture.

Viscosity and density determination

Viscosity study was carried out by Ostwald's viscometer method. In this method, pulp polymers were dissolved in water, and same solutions were used for density determination at room temperature.^[26]

Melting point

Melting point of polymers was determined by capillary tube method. Polymers filled capillary was placed in the melting point apparatus containing liquid paraffin as a heating medium and continued heating till the entire sample was melted or degraded.

Particle size determination

Particle size determination was carried out by optical microscopy method using motic microscope. Powder was sprinkled on slide and observed under microscope. Diameters of 50 particles were measured.

Determination of microbial contamination

Separated seed and pulp polymers were dispersed in to water and prepared 1% solution of polymers. This aqueous dispersion was inoculated in nutrient agar at 37°C for 24 h. The culture media plates were observed for growth of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* genus. Similar to growth of bacteria it was necessary to check the growth of fungi for which aqueous dispersion of polymers were inoculated on Sabourad's dextrose agar media at 25°C for 48 h.^[27]

Fourier transform infrared spectrophotometer study

Fourier transform infrared (FTIR) spectra of both seed and pulp polymers were recorded using IR spectrophotometer (Jasco-V-530 model). About 2 mg of sample was ground thoroughly with KBr; uniformly mixed sample kept in the sample holder, and spectra was recorded over the wave number 400-4000 cm⁻¹.

Powder X-ray diffraction study

Crystallinity of seed polymers were checked by powder X-ray diffraction (PXRD) study. Dry powder of separated seed polymers was used. Sample was irradiated with monochromatic Cu K α radiation (1.542 Å) between 50 and 550 (using 2 θ) on an X-ray diffractometer (Philips analytical XRD, PW 3710). The voltage and current applied were 40 kV and 30 mA respectively.

Differential scanning calorimeter study

Thermal behavior of separated seed polymers were analyzed by differential scanning calorimeter (DSC) on a Shimadzu differential scanning calorimeter (TA Instruments, Model SDT 2960, USA) equipped with intra cooler, and refrigerated cooling system was used to analyze the sample.

Formulation development

Sustained release diclofenac sodium tablet

For the development of SR formulation, we are using polymers (*C. fistula* seed protein) and Diclofenac sodium was selected as a model drug for formulation of SR tablet.

Preparation of tablets

Different batches F1, F2, F3, F4, and F5 of diclofenac sodium SR tablets were prepared by varying drug:polymer ratios like 1:1, 1:2, 1:3, 1:4, and 1:5 respectively [Table 1]. Seed polymers were used as SR polymer, microcrystalline cellulose, talc and magnesium stearate was used as filler to maintain tablet weight, glidant, and lubricant respectively. The granules were prepared by wet granulation method. All the ingredients were mixed and add sufficient water to form wet mass and this wet mass passed through # 16 sieve and granules were dried for 1 h at 40-45°C. Further granules were passed through # 20 sieve and added talc and magnesium stearate. Then granules were compressed by using KBr press, with the help of 10 mm punches.^[28] Further tablets were prepared by using HPMC for comparison with optimized batch.

Table 1: Composition of tablet formulation

Ingredients (mg)	F 1	F 2	F 3	F 4	F 5
Drug	50	50	50	50	50
Seed polymers	50	100	150	200	250
MCC	196	146	96	46	—
Talc	3	3	3	3	3
Magnesium stearate	1	1	1	1	1
Total weight	300	300	300	300	300

MCC: Microcrystalline cellulose

Evaluation of granules

Bulk density and tapped density

Accurately weighed granules of all batches were taken separately in 100 ml graduated cylinder. Bulk density and tap density were determined in triplicate by reporting the volume occupied by each sample, before (V_b) and after tapping (V_t) using bulk density apparatus (Laboratory Hospital, Mumbai, Maharashtra, India). The bulk density and tap density was calculated using the formulas:^[29]

$$\rho_b = \frac{M}{V_b} \quad (1)$$

$$\rho_t = \frac{M}{V_t} \quad (2)$$

Angle of repose

Angle of repose was determined in triplicate using fixed funnel method and calculated using the formula:^[30]

$$\theta = \tan^{-1} \frac{H}{R} \quad (3)$$

Where “ θ ” is the angle of repose, “ H ” is the height between the lower tip of the funnel and the base of the heap of powder, and “ R ” is the radius of the base of the heap formed.

Carr's compressibility index and Hausner's ratio

Carr's compressibility index (CCI) and Hausner's ratio (HR) for granules of all the batches were calculated in triplicate using the formula:^[30,31]

$$CCI = \frac{TD - BD}{TD} \quad (4)$$

$$HR = \frac{TD}{BD} \quad (5)$$

Evaluation of tablets

Tablet thickness, hardness, and diameter

Thickness and diameter were measured using Vernier caliper and Monsanto hardness tester respectively. The hardness was measured in terms of kg/cm^2 .^[31]

Weight variation

Weighed twenty tablets individually and calculate the average weight of tablets. The tablets meet the United States Pharmacopeia (USP) tests if no more than two tablets were outside the percentage limit and if no tablets were outside the percentage limit.^[31]

Friability

A total of 20 tablets was weighed accurately and placed in the drum of Roche friabilator that revolves at 25 rpm dropping the tablets through a distance of 6 in. with each revolution. The tablets were then dusted and reweighed.^[31] Friability of tablets can be calculated using the formula:

$$\% \text{ Weight loss} = \frac{W_o - W_t}{W_o} \times 100 \quad (6)$$

Swelling index

The extent of swelling was measured in terms of percentage weight gain by the tablet. One tablet from each formulation was kept in a petri dish having pH 7.4 phosphate buffers. At the end of 0.5 h and 1 h, the tablet was withdrawn, dried with tissue paper and weighed. Then for every 1 h, weight of the tablet was noted and the process was continued till the end of 8 h percentage gain by the tablet was calculated by formula:^[32]

$$\text{Swelling index} = \frac{M_t - M_o}{M_o} \times 100 \quad (7)$$

Where,

M_t = weight of the tablet at time t (h) and

M_o = weight of the tablet at 0 time.

Drug content

For the determination of drug content, tablets were triturated into powder and 50 mg equivalent weight of diclofenac sodium in tablet powder was accurately weighed and transferred into a 100 ml volumetric flask. Initially, 10 ml of phosphate buffer (pH 7.4) was added and shaken for 10 min. Then, the volume was made up to 100 ml with buffer. Subsequently, the solution in volumetric flask was filtered, and 1 ml of filtrate was diluted and analyzed at 276 nm using UV-visible spectrophotometer. The drug content of the sample was estimated from their standard curve.

Fourier transform infrared spectroscopy

The pure drug (diclofenac sodium) and physical mixture of pure drug diclofenac sodium and seed polymers (1:1) were studied by FTIR spectra to ascertain any structural or chemical changes in drug after the preparation of tables by using Jasco-V-530 FTIR.

Powder X-ray diffraction

Powder X-ray diffraction patterns of pure drug (diclofenac sodium) and physical mixture of pure drug diclofenac sodium and seed polymers (1:1) were obtained (Philips X-ray diffractometer, PW-3710, Holland), using $\text{Cu K}\alpha$ radiation ($\lambda = 1.5405 \text{ \AA}$) at voltage of 40 kV, and 30 mA current. The data were recorded over a range of $5\text{-}100^\circ$ at a scanning rate of $5 \text{ cps} \times 103 \text{ cps}$ using a chart speed of $5 \text{ mm}/2^\circ$.

Differential scanning calorimetry

Differential scanning calorimetry thermograms of pure drug (diclofenac sodium) and physical mixture of pure drug diclofenac sodium and seed polymers (1:1) were obtained by using a DSC-TGA (TA instruments, model SDT 2960, USA). Zinc metal or calcium oxalate was used as a standard to calibrate the DSC temperature and enthalpy scale. Samples were hermetically sealed in an aluminum crucible. The system was purged with nitrogen gas at a flow rate of 60 ml/min. Heating was done from 10°C to 300°C at rate of $10^\circ\text{C}/\text{min}$.

In-vitro dissolution studies

In-vitro dissolution kinetics of the tablet was carried out in triplicate for every batch in USP Type-II dissolution test apparatus in 900 ml of 0.05 M phosphate buffer, pH 7.5 for 10 h

(37.5 ± 0.5°C and 50 rpm). Each time, 5 ml of aliquots were withdrawn at the interval of 1 h up to 2 h then at the interval of 2 h up to 6 h and then at the interval of 4 h up to 10 h until the end of dissolution study and replaced by equivalent amount of blank. The withdrawn samples were filtered through a Whatman filter paper and further analyzed at respective wavelength by double beam UV-visible spectrophotometer (Jasco V-530 UV). Analysis of data was performed using "PCP Disso V-3" software, Poona College of Pharmacy, Poona, India.

Stability study

Accelerated stability study was carried out for 3 months period at temperature 40 ± 2°C and relative humidity 75 ± 5%. Sampling was done at the end of 1, 2, and 3 months.

Use of pulp polymer as a suspending agent

Preparation of zinc oxide suspension

Zinc oxide sieved through 100 # was used to yield 20% w/v suspension in distilled water. Different suspending agent like tragacanth, sodium carboxymethyl cellulose (CMC) and pulp polymers were used in concentration of 1.0, 1.5, 2.0, and 2.5% w/v to prepare a suspension. For making the suspensions, zinc oxide first levigated with glycerin (1:1) separately. Then weighed amount of these aforesaid suspending agents was added and triturated. Finally volume was made up with distilled water. Benzoic acid (0.1% w/v) was added as a preservative. Composition of suspension has been reported in Table 2. All the suspensions were deflocculated. To determine the degree of flocculation, flocculated suspensions were also prepared for same suspending agents using a flocculating agent, potassium dihydrogen phosphate (0.004 moles).^[33]

Evaluation of suspension

Sedimentation volume

The rate of separation of suspension was studied by keeping 50 ml portion of each suspension in stopped measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted at intervals of 5d up to 45d. The separation ratio was calculated using the formula:^[33]

$$\text{Sedimentation volume} = \frac{H_s}{H_o} \quad (8)$$

where H_s is the height of clear liquid, and H_o is the original height of the sample.

Degree of flocculation

The degree of flocculation was determined from the following equation:

Table 2: Composition for zinc oxide suspension

Ingredients	1%	1.5%	2%	2.5%
Zinc oxide	20	20	20	20
Pulp polymer	1	1.5	2	2.5
Glycerin	20	20	20	20
Benzoic acid	0.1	0.1	0.1	0.1
Water	q.s. 100	q.s. 100	q.s. 100	q.s. 100

$$\beta = \frac{F}{F\alpha} \quad (9)$$

Where F is the ultimate sedimentation volume in the flocculated suspension and $F\alpha$ is the ultimate sedimentation volume in deflocculated suspension.^[28]

Laxative activity and biological effect of seed polymers

Experimental

Animal species

A total of 24 Swiss Albino rats, 6-8 weeks old, weighing in the range of 150-200 g were randomly divided into four groups ($n = 6$), control, standard, and two tests groups.

Housing and feeding

All animals were kept in well-ventilated cages under standard conditions of light and temperature (22 ± 0.5°C). They were housed with access to commercial rodent stock diet with a constant supply of drinking water.

Preparation of animal and doses

Selected healthy rats were divided into five groups ($n = 6$), each group was kept individually in a separate cage. Cages were kept separately at least 5 days prior to start of the study. Doses selected were 100 mg/kg and (1:1) seed polymers and standard drug. Test substance was dissolved in 1% Tween 80 solution and administered by oral gavages.

Procedure for laxative activity

The rats of either sex, fasted for 12 h before the experiment but with water provided *ad libitum*. The animals were divided into four groups of six animals each. The first group of animals, serving as a control, received 1% Tween 80 p.o.; the second group received bisacodyl (2.5 mg/kg, p.o.) in 1% Tween 80 as standard; the other groups received test compounds (seed polymers) at dose of 100 mg/kg, in 1% Tween 80 solution and (1:1) seed polymers and positive control in 1% Tween 80. Immediately after dosing, the animals were placed in metabolic cages suitable for collection of faeces. After 24 h of drug administration, the faeces were collected and weighed.^[34]

Statistical analysis

Data were analyzed by one-way ANOVA followed by Dunnett multiple comparisons test (*t*-test) using Instat® (Graph Pad software, USA). About 95% confidence interval $P < 0.05$ was considered statistically significant. The standard group and test groups shows $P < 0.01$, so it was significant.

RESULT AND DISCUSSION

Extraction and characterization of polymers from seed and pulp

Extraction of seed polymer

About 3% sodium chloride in distilled water had given higher yield (20.15 g) of seed polymer compare to 1% potassium chloride (4.20 g), 1.5% calcium chloride (7.10 g), and saturated solution of

ammonium sulfate in distilled water (3.12 g). As the extraction efficiency for sodium chloride was more and, it is cheaper as compare to other solvents, 3% sodium chloride was selected as a solvent for extraction.

Extraction of pulp polymer

Chloroform water IP had given 7.24 and 17.26 g yield of pulp polymer after 12 h and 24 h respectively. While, in case of water yield was found to be, 2.51 and 6.42 g after 12 h and 24 h respectively. Extraction efficiency of chloroform water IP for 24 h was more and in case of water as the extraction solvent the growth of fungus was observed, and percentage yield was also less as compared to chloroform water IP. Hence, chloroform water IP was selected as a solvent for extraction.

Determination of total protein by Lowry method

From the graph and by calculation total protein content of seed proteins were found to be 23.44% by Lowry method. It was found matched to the figure reported in the literature (19.94%). Calibration curve for bovine serum albumin (BSA) standard has given in Figure 1.

Preliminary chemical investigation

Purified *C. fistula* seed and pulp polymers were subjected for preliminary chemical investigation, seed polymers show the presence of proteins and pulp polymers showed carbohydrates and mainly hexose sugars [Table 3].

Solubility and pH

Cassia fistula seed polymers were sparingly soluble in water, and pulp polymers were completely soluble in water. Both seed and pulp polymers were insoluble in ethanol (95%), methanol and chloroform. pH of 1% solution of *C. fistula* seed and pulp polymer in water was found to be 6.55 and 6 respectively.

Isoelectric pH

Cassia fistula seed polymer was separated by salt precipitation method, and its isoelectric pH was found to be 7.31.

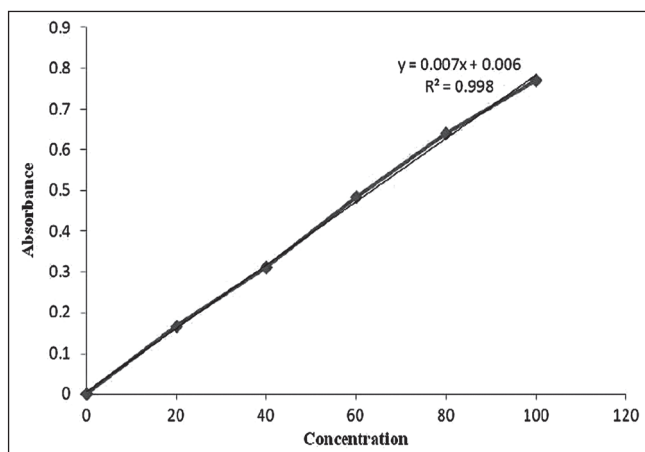


Figure 1: Calibration curve of standard bovine serum albumin

Moisture content

Moisture content of purified *C. fistula* seed and pulp polymer was determined by using IR moisture balance method which was found to be 2.56% and 4.32% respectively.

Viscosity and density determination

The viscosity and density of *C. fistula* pulp polymer were found to be 0.6135 cp and 1.11 g/ml respectively.

Melting point

Melting point of *C. fistula* seed and pulp polymer were determined by capillary method that was found to be in the range of 120-130°C and 160-170°C respectively.

Particle size determination

Particle size of seed polymer was determined by optical microscopy using Motic microscope that was found to be in the range of 105-725 μm. So the nature of particles was coarse fine.

Microbial load

As the separated *C. fistula* seed polymers itself act as an antimicrobial agent, so the 1% solution of *C. fistula* seed polymers not showed any specific type of growth for pathogenic bacteria and fungi. Solution was prepared in distilled water, and the polymers separated by heating up to boiling temperature and passed through semi-permeable dialysis membrane having pore size was low, due to these possible reasons growth of pathogenic bacteria may not occur. Furthermore in case of 1% solution of pulp polymers not showed any specific type of growth for pathogenic bacteria and fungi.

Table 3: Preliminary chemical test for seed and pulp polymer

Name of test	Result
Seed polymer	
Test for proteins	
Biuret test	+
Test for amino acids	
Ninhydrin test	+
Millons test	+
Xanthoproteic test	+
Molisch test	-
Coagulation test	+
Precipitation test	
With absolute alcohol	+
With 5% copper sulfate	+
With 5% lead acetate	+
Pulp polymer	
Test for carbohydrate	
Molisch test	+
Test for reducing sugars	
Fehling test	
Benedict's test	
Test for monosaccharide's	
Barfoed's test	+
Test for pentose sugars	-
Tests for hexose sugar	
Selwinoffs test	+
Tollens test	+
Cobalt chlorides	+

Fourier transform infrared spectrophotometer study

Fourier transform infrared spectra of *C. fistula* seed polymers showed the peaks at wave numbers 1541.71 cm^{-1} for N-H deformation, 1011.99 cm^{-1} for C-H in plane deformation of aromatic compounds, 1438.41 cm^{-1} for C=O stretching, 1338.42 cm^{-1} for C-N stretching of aromatic amines, 1669.88 cm^{-1} for C=C stretching. These peaks were corresponding to -CONH linkage in proteins [Figure 2].

Powder X-ray diffraction study

Powder X-ray diffraction spectra of *C. fistula* seed polymers showed 2θ peaks at 27° , 32° , and 45° . Intensity of these peaks was high so crystallinity of compound was more, so it was crystalline in nature [Figure 3].

Differential scanning calorimeter study

The DSC thermogram of *C. fistula* seed polymers showed an endothermic peak at 125.8°C , corresponding to melting points of protein [Figure 4].

Preparation of tablets

Evaluation of granules

Bulk density for all batches (F1-F5) was found to be in the range of 0.2793 ± 0.001 to 0.3378 ± 0.003 . While, tap density was found to be in the range of 0.3158 ± 0.002 to 0.4098 ± 0.005 . This

indicates good packing capacity of granules. Angle of repose was found to be 17.76 ± 0.22 to 19.51 ± 0.23 . Carr's index was found to be between 11.54 ± 0.53 and 18.66 ± 0.20 . The Hausner ratio was found to be in the range of 1.13 ± 0.006 to 1.22 ± 0.003 for all batches. All values are indicative of good flow properties and interparticulate cohesive properties of the granules. Values are reported in Table 4.

Evaluation of tablets

All the formulations were evaluated for various parameters, such as thickness, diameter, and hardness and the values of all parameters from batch F1 to F5 have reported in Table 5. As there was no much variation in thickness of tablets in each formulation, it shows that granules were consistent in particle size and uniform behavior during the compression process. Thickness and diameter of tablets of all batches was measured by Vernier caliper, and there was no any change in thickness and diameter of tablets respectively. Thickness was in the range of 3.93 ± 0.12 to 4.06 ± 0.04 . While, the diameter ranged between 9.9 ± 0.08 and 10.1 ± 0.08 . The hardness was in the range of 4.06 ± 0.24 to 6.16 ± 0.16 . Tablet hardness reflects differences in tablet density and porosity, affects the rate of penetration of the dissolution fluid at the surface of the tablet, which ultimately contribute in difference release patterns of the drug.

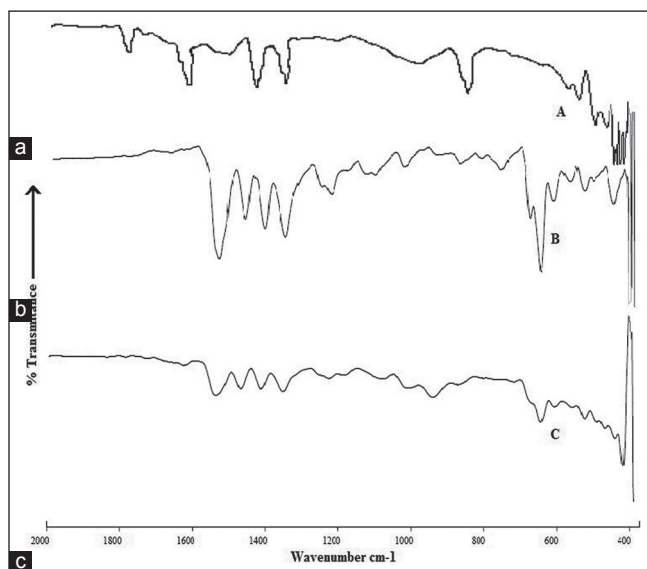


Figure 2: Fourier transform infrared spectra of (a) seed polymer, (b) pure diclofenac sodium and (c) physical mixture of diclofenac sodium and seed polymer

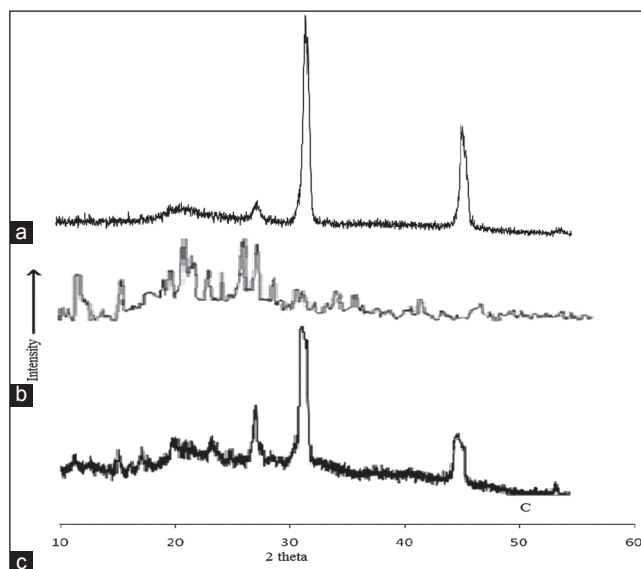


Figure 3: Powder X-ray diffraction spectra of (a) seed polymer, (b) pure diclofenac sodium and (c) physical mixture of diclofenac sodium and seed polymer

Table 4: Micromeritic properties of granules*

Batch	Bulk density (g/cm ³)	Tapped density (g/cm ³)	Angle of repose (θ)	Carr's index (%)	Hausner ratio
F1	0.3378±0.003	0.4098±0.005	18.59±0.23	18.66±0.20	1.22±0.003
F2	0.3138±0.001	0.3623±0.004	19.03±0.22	13.39±0.63	1.15±0.008
F3	0.3254±0.002	0.3694±0.001	19.44±0.21	11.92±0.72	1.13±0.009
F4	0.2941±0.002	0.3334±0.005	19.51±0.23	11.77±0.59	1.13±0.007
F5	0.2793±0.001	0.3158±0.002	17.76±0.22	11.54±0.53	1.13±0.006

*Indicates \pm SD ($n = 3$), SD: Standard deviation

Weight variation

The average weight of tablets ranged from 300.25 to 300.4 and were within the limit. All the batches showed the uniformity of weight [Table 5].

Friability of tablet

The friability was found in the range of 0.0442 ± 0.015 to 0.1222 ± 0.041 and passes the IP test. The friability of tablets depends on the type of filler and moisture contents in it. The values of friability are given in Table 5.

Swelling index

The swelling properties of all formulation batches (F1-F5) were studied, and it was calculated with respect to time. Swelling index increases as the time increases because weight gain by tablet was increased proportionally with rate of hydration up to a certain limit. After some time, it decreases gradually due to dissolution of the outermost gelled layer of the tablet into the dissolution medium. A direct relationship was observed between swelling index and polymer ratio. The swelling index increases as the polymers concentration increases [Table 6].

Drug content

Drug content was in the range of 93.46 ± 0.08 to 99.69 ± 0.1189 indicating good content uniformity in the prepared formulation. The drug content increases from F1 to F5 as polymers concentration increases.

Fourier transform infrared spectrophotometer study

Fourier transform infrared spectrum of diclofenac sodium shows characteristic peaks of C=O stretching of carboxylic group, N-H bending of amine, C-N stretching of amine and C-Cl stretching at 1396.10 cm^{-1} , 1566.43 cm^{-1} , 1279.54 cm^{-1} , and 742.89 cm^{-1} respectively, similar peaks have been reported in FTIR spectra of physical mixture. So this was indicating that no any structural

changes were found in physical mixture. The peaks of diclofenac sodium and seed polymers remained unchanged [Figure 2].

Powder X-ray diffraction study

Diclofenac sodium was found to be crystalline compound, so it was showed string diffraction peaks at 2θ of around 12° , 17° , 23° , 25° , 35° , 41° , and 42° . Hence, the number of intense peaks was found to highest so crystalline nature of the compound was reported. Same as to drug in the physical mixture shows diffraction peaks at 27° , 32° , and 45° . Furthermore, intensity of peaks was slightly reduced which is indicative of somewhat decrease in the crystallinity [Figure 3].

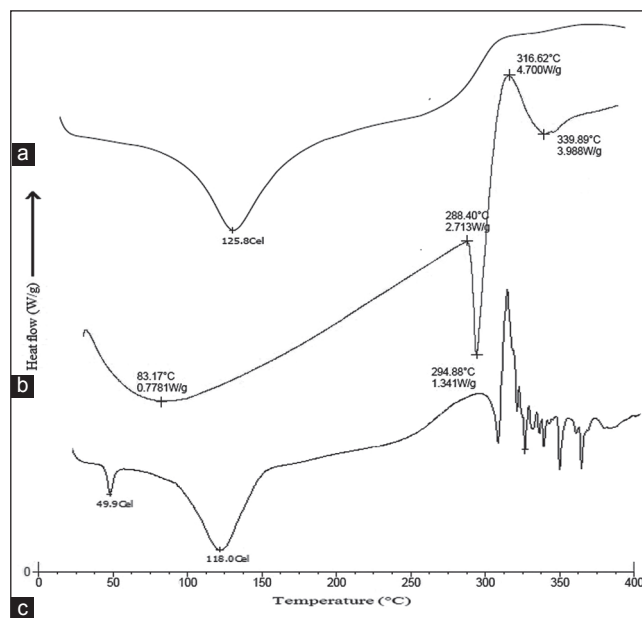


Figure 4: Differential scanning calorimeter thermogram of (a) seed polymer, (b) pure diclofenac sodium, and (c) physical mixture of diclofenac sodium and seed polymers

Table 5: Evaluation parameters of tablet formulation*

Batch	Thickness	Diameter	Hardness	Friability	Weight variation
F1	4.06±0.04	09.9±0.08	4.06±0.24	0.1222±0.041	300.25±0.99
F2	3.93±0.12	10.03±0.04	5.33±0.24	0.0555±0.015	300.25±1.08
F3	4.06±0.12	10.1±0.08	6.16±0.16	0.0688±0.027	300.4±1.01
F4	4.03±0.09	10.06±0.12	4.86±0.04	0.0442±0.015	300.3±0.78
F5	4.00±0.08	10.06±0.09	4.20±0.16	0.0444±0.015	300.3±0.95

*Indicates ±SD (n = 3), SD: Standard deviation

Table 6: Swelling index of F1-F5 batches at different time (h) intervals*

Time (h)	F1	F2	F3	F4	F5
0.5	87.33±0.26	96.99±0.27	96.99±0.27	112.99±0.27	123.99±0.27
1	94.66±0.27	106.99±0.27	106.33±0.26	124.21±0.41	150.99±0.27
2	98.66±0.27	113.99±0.27	112.99±0.27	139.88±0.41	195.33±0.26
3	103.66±0.27	119.66±0.27	124.21±0.41	151.33±0.54	220.88±0.41
4	111.66±0.27	129.33±0.26	139.88±0.41	187.33±0.26	232.33±0.26
5	121.99±0.27	136.22±0.15	151.33±0.54	211.44±0.15	274.99±0.27
6	108.99±0.27	127.33±0.26	123.1±0.41	146.33±0.26	235.99±0.27
7	101.66±0.27	117.99±0.27	118.10±0.41	123.10±0.41	225.66±0.27
8	96.33±0.26	103.23±0.16	105.33±0.26	110.33±0.26	161.33±0.26

*Indicates ±SD (n = 3), SD: Standard deviation

Differential scanning calorimeter study

Thermogram of diclofenac pure drug showed endothermic peak at 58.2°C and 285.8°C which corresponds to glass transition temperature and melting point. While in case of physical mixture minute shifting of peaks are observed at 49.9°C and 281.8°C for diclofenac sodium and at 118°C for seed polymers. So no any incompatible thermal changes were reported in the thermogram [Figure 4].

In-vitro dissolution study

Release of drug from all batches (F1-F5) was due to erosion of the tablet surface because of dissolution medium. Contact of tablet formulations with the dissolution medium leads to the formation of gelling layer due to swelling of the polymer, which contributed to the drug retardation effect of the polymer. Highest polymer content of batch F5 contributed to more retardation of drug release (72.84 ± 0.98 at 10 h) compare to other batches and followed Higuchi matrix release kinetics. Sustain release tablet prepared using HPMC retarded drug release up to 10 h (74.42 ± 0.48). Drug release from batch F5 was significant ($P < 0.05$) as compared to batch containing HPMC. However, conventional tablet of diclofenac sodium (without any polymer) showed $>90\%$ drug release in almost 2 h. This retardation of drug release from compact may be due to the formation of polyelectrolyte complex [Table 7]. So this result supports the use of *C. fistula* gum as sustain release polymer. Plot of percentage cumulative drug release Vs versus time has shown in Figure 5a.

Stability study

There was no change in physical appearance in the dosage form of batch F5 over a period of 3 months in accelerated conditions. Drug content of diclofenac sodium tablet was found to be 99.24%, 98.61%, and 98.08% at the end of 1, 2, and 3 months respectively. While percentage drug release was found to be 73.11%, 74.05%, and 74.74% at the end of 1, 2, and 3 months stability study respectively. Hence, it was observed that with time some amount of drug content was declined, but it was not out of the limit and release retarding principle was found to be active, so these tablets were found to be suitable in 3 months period of stability study without causing any incompatible change in formulation [Figure 5b].

Use of pulp polymers as suspending agent

Evaluation of suspension

Sedimentation volume

Sedimentation volume of sodium CMC at 2.5% concentration gives lower values than tragacanth, bentonite and pulp polymers. From that it was concluded that pulp polymers were not good suspending agent as compared to other suspending agents [Table 8, Figure 6a].

Degree of flocculation

Degree of flocculation of sodium CMC at 2.5% concentration gives higher values than tragacanth, bentonite, and pulp polymers. So it gives better results for degree of flocculation and it was good suspending agents than tragacanth, bentonite, and pulp polymers. From that, it was concluded that pulp polymers were not good suspending agent as compared to other suspending agents [Figure 6b]. Although its degree of flocculation is higher, it fails to stabilize the suspension for a longer period and tends

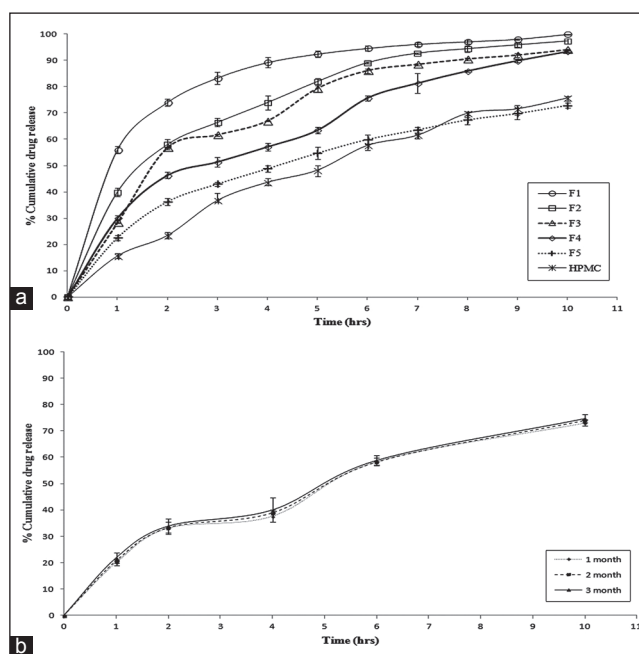


Figure 5: Graph of *in-vitro* percentage cumulative drug release versus time (h) for (a) all batches (F1-F5) and (b) optimize formulation (F5) at 1, 2, and 3 months intervals

Table 7: In-vitro percent cumulative drug release profile for all batches*

Time (h)	F1	F2	F3	F4	F5	HPMC
1	55.91±1.30	39.88±1.68	28.26±0.12	30.03±0.98	22.58±0.97	12.42±1.32
2	73.94±1.40	57.95±1.98	56.77±0.09	46.28±1.21	36.23±1.31	21.36±1.18
3	83.27±2.31	66.36±1.61	61.61±0.45	51.36±1.80	42.96±0.65	36.45±2.68
4	89.14±1.98	73.90±2.68	66.94±0.16	57.10±1.42	48.77±1.24	42.56±1.40
5	92.36±1.23	81.89±1.32	79.32±1.23	63.45±1.21	54.69±2.36	48.15±1.96
6	94.54±0.96	89.05±0.40	86.05±0.86	75.68±0.98	59.89±1.80	54.32±0.69
7	96.12±0.78	92.67±0.23	88.39±0.32	81.32±3.89	63.54±1.20	61.25±0.78
8	97.05±0.69	94.38±1.30	90.46±0.43	85.96±0.43	67.32±1.69	69.58±0.34
9	98.02±0.34	95.87±0.68	91.92±0.68	89.87±0.68	69.76±2.23	72.32±0.32
10	99.85±0.16	97.30±0.97	93.99±0.16	93.34±0.23	72.84±0.98	74.42±0.48

*Indicates \pm SD $n = 3$, SD: Standard deviation, HPMC: Hydroxy propyl methyl cellulose

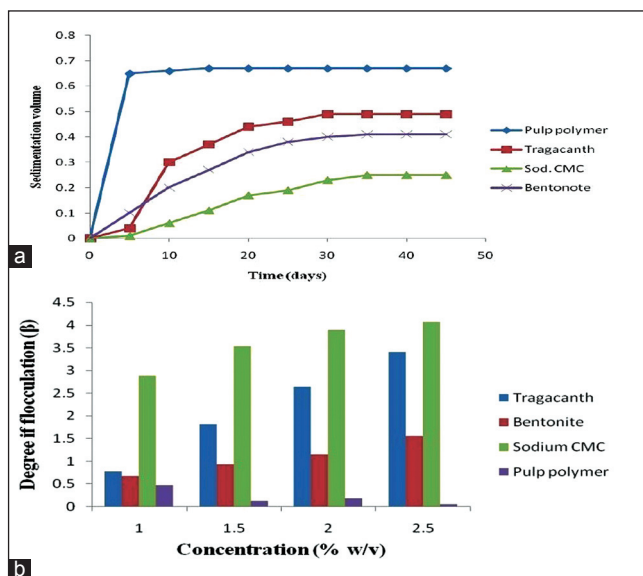


Figure 6: Graph of (a) sedimentation volume of 2.5% suspending agents at different time intervals (days) and (b) degree of flocculation (β) of various suspending agents at different concentrations

to form a cake. So it is better to use it in combination with other suspending agents instead of alone.

Use of pulp polymers as emulsifying agent

Breaking or phase separation of emulsion results during preparation which indicate that the pulp polymers were not very effective emulsifying agent in comparison to acacia gum, so there is no further evaluation tests of emulsion were carried out.

Laxative activity and biological effect of seed polymers

From biological effect of seed polymers, observed that seed polymers do not show laxative activity as it gives results close to the control group but for standard drug (bisacodyl) and seed polymers in combination (1:1) reduced the laxative activity because of SR effect of polymers [Table 9]. So seed polymer can be useful as a laxative agent when used in combination with another laxative agent.

CONCLUSION

Polymer from *C. fistula* Linn. seed and pulp was successfully isolated and screened as excipient to fit into different pharmaceutical purpose. Polymer isolated from the seed extended the release up to 10 h; this retardation is may be dye to polyelectrolyte complex formation between drug and polymer. The release of diclofenac sodium from matrices was influenced by concentration of seed polymer. Increase in concentration directly affected the release rate. Pulp polymer was not found to be satisfactory to use as suspending agent because formulated suspension tends to settle down and could not get easily redispersed. So it can be useful in combination with other suspending agents like tragacanth. So, further work is needed to prove its suspendability. If properly executed it can

Table 8: Sedimentation volume (Hs/Ho) of pulp polymers at different concentrations

Time (days)	1%	1.5%	2%	2.5%
5	0.05	0.62	0.64	0.65
10	0.61	0.62	0.65	0.66
15	0.61	0.63	0.65	0.67
20	0.61	0.63	0.65	0.67
25	0.62	0.63	0.65	0.67
30	0.62	0.63	0.65	0.67
35	0.62	0.63	0.65	0.67
40	0.62	0.63	0.65	0.67
45	0.62	0.63	0.65	0.67

Table 9: Laxative activity and biological effect of seed polymers

Group	Dose (mg/kg)	Weight of faeces (g) after 24 h
Control	1% Tween 80	5.1666±0.03
Standard	2.5 mg/kg bisacodyl in 1% Tween 80	7.3306±0.04**
Test (for laxative activity)	100 mg/kg seed polymer in 1% Tween 80	8.2666±0.038**
Test (for biological effect of polymers)	(100 mg/kg) seed polymer and bisacodyl (2.5 mg/kg) in 1% Tween 80	4.8855±0.04**

Values are expressed as mean ± SD (n = 6), **P < 0.01, when compared to the control, SD: Standard deviation

be a value addition to previously available natural polymers, as it can be used as sustain release polymer, suspending agent and so on.

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