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Original Article

Development of Colon Targeted Extended Drug Delivery System for Mebendazole Using Grafted Natural Polymers

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ABSTRACT

Background: Even though the natural polymers are biodegradable, non-toxic and cost effective, but these suffer with some of drawbacks like uncontrolled drug release, enzymatic degradation, reduction in viscosity on long term storage, thermal instability etc. The aforesaid limitations can be overcome by grafting of natural polymers to obtain high performance biomaterials for drug delivery system. **Materials and Methods:** Purified fenugreek and mesquite gums were grafted with acrylamide using Ceric Ammonium Nitrate (CAN) as free radical initiator. Confirmation of grafting was done by elemental analysis and FT-IR spectroscopy. Grafted copolymers were tested for its biodegradability in microorganisms and toxicity in *Drosophila melanogaster*. Nine batches of mebendazole tablets (F1 to F9) were prepared using mixture of acrylamide grafted fenugreek and mesquite gum polymer by 3^2 factorial designs. Compressed tablets were tested for post-compression compendia parameters. **Results:** FT-IR confirmed the grafting and compatibility between drug and synthesized copolymers. Synthesised graft copolymers showed significant swelling index, water retention capacity, viscosity, hydration capacity than the pure gum polymers. Compressed tablets showed hardness (5.5 to 6.6 kg/cm^2), friability (less than 1%). thickness (3 mm) and complied weight variation test as per compendial standards. Further tablets were coated with Eudragit RS 100 demonstrated small amount of drug release in 5 hr and maximum amount of drug released in colonic region. Hence, after reaching colonic region drug release extended upto 12 hr due to satisfactory drug release by copolymers. **Conclusion:** Grafted copolymers were biodegradable, non-toxic and showed higher swelling index then pure polymers. These copolymers can be used as drug release retardant in the tablet formulation.

Keywords: Grafting, Fenugreek gum polymer, Mesquite gum polymer, Helminthiasis, *Drosophila melanogaster*, Elemental analysis.

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INTRODUCTION

Helminthiasis, a group of parasitic infections caused by helminths (worms), remains a significant global health concern, particularly in developing countries.[1] The treatment of helminthiasis requires effective drug delivery systems that can target the worms in the gastrointestinal tract, particularly the colon, where many helminths reside. Conventional drug delivery systems face several challenges in effectively delivering anthelmintic drugs to the colon, including poor drug solubility, enzymatic degradation and systemic side effects. Therefore, the development of colon-targeted drug delivery systems using grafted natural polymers has emerged as a promising strategy for enhancing the therapeutic efficacy of anthelmintic drugs and minimizing their adverse effects.[2]

Helminthiasis affects millions of people worldwide, causing significant morbidity and mortality. Anthelmintic drugs, such as mebendazole, albendazole and praziquantel, are commonly used for the treatment of helminthiasis.[3] Colon-targeted drug delivery systems offer several advantages for the treatment of helminthiasis. By specifically targeting the colon, these systems can increase drug concentration at the site of infection, improving the contact time with the worms and enhancing therapeutic outcomes. Moreover, colon targeting can minimize systemic side effects associated with high drug concentrations in other tissues and organs. Various strategies have been explored

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to achieve colon targeting, including pH-dependent systems, time-dependent systems and microbially-triggered systems.[4]

Natural polymers have gained considerable attention as promising carriers for drug delivery applications due to their biocompatibility, biodegradability, low toxicity and abundance in nature.[5] However; natural polymers suffer with some limitation such as susceptibility to enzymatic degradation, uncontrolled the drug release, reduction of viscosity during storage and temperature instability. These aforesaid limitations can be overcome by polymer grafting techniques and optimize drug delivery characteristics.[6]

Several natural polymers have been investigated for grafting applications in colon-targeted drug delivery systems for helminthiasis.[7] Chitosan, a polysaccharide derived from chitin, is one of the most widely studied natural polymers due to its biocompatibility, biodegradability and colon-specific properties.[8] Grafting chitosan with various functional groups can further enhance its drug delivery capabilities. Other natural polymers, such as guar gum, pectin and alginate, have also shown promise as grafting substrates for colon-specific drug delivery systems.[9,10]

Fenugreek Gum (FGP), a galactomannan, is extracted and purified from the endosperm of ripe seeds of *Trigonella foenum-graecum,* is nontoxic, readily soluble in water and biodegradable. It contains high percentage of mucilage, D-galactose and D-mannose in 1:1 and 1:1.2 ratios (galactomannan) as similar to locust bean, guar and tara gum. Due to its associated properties and galactomannan polymeric backbone the Fenugreek gum possess the required properties for the grafting with Acrylamide.[11]

Mesquite Gum (MGP), an amber-coloured, tasteless extrudate obtained from mesquite trees (*Prosopis* sp.). The mononmers that constituted mesquite gum include D-galactose, D-mannose, D-glucuronate, L-arabinose and D-xylose. Mesquite gum has a significant emulsifying, encapsulating and film-forming potential. The pharmaceutical applications of mesquite gum are not limited to binder in tablets as it is reported to be stabilizer in suspensions. Due to its associated properties, these gums are under-utilized as polymers in pharmaceutical dosage form and no data is available on its grafting with synthetic polymers to enhance its functional properties. Conventional mebendazole tablets may have undesirable systemic effects since they release the medication along the Gastrointestinal (GI) tract. Mebendazole's selective local action in the colon may help prevent systemic adverse effects and helminthiasis may be effectively treated with even at a lower dose of mebendazole. The grafting of the these natural gums with acrylamide increased the swelling index and hence can be used for the development of extended release formulations.[12]

Based on the above facts it's planned to develop colon targeted mebendazole tablets for the treatment of helminthiasis using acrylamide grafted fenugreek and mesquite gum polymers.

MATERIALS AND METHODS

Mebendazole was a gift sample from Alcon Biosciences private Limited Vapi, India. Fenugreek seeds and Mesquite gum was purchased from local market. All other Excipients and chemicals used were of analytical grade. Diluent lactose was procured from Rajesh chemicals Mumbai. Ceric ammonium nitrate and microcrystalline cellulose were obtained from Loba Chemical Mumbai. HPMC, Lactose, Magnesium stearate, Aerosil was obtained from Ozone® International India. *Drosophila melanogaster* (code W1118) was obtained from National Centre for Biological Sciences (NCBS), Bangalore and *Aspergillus niger* was obtained from V. G. Shivdare arts, commerce and science, Solapur (Maharashtra, India).

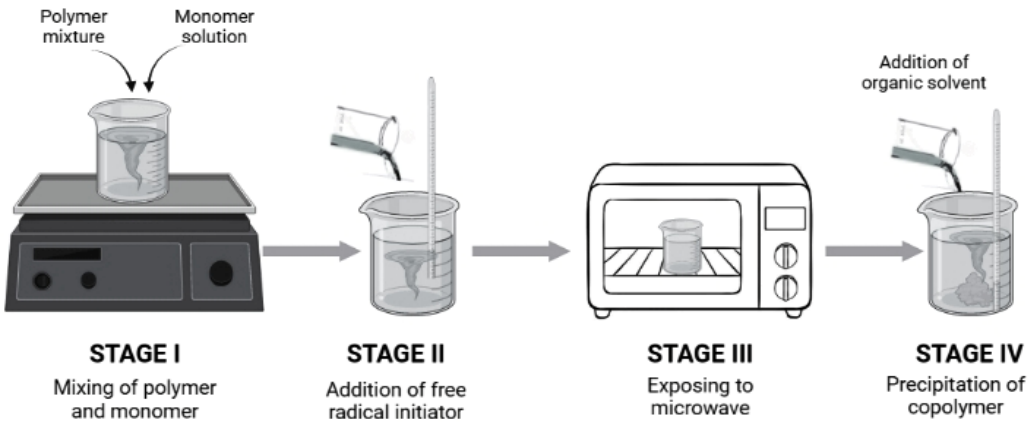

Figure 1: Stages involved in microwave-assisted synthesis of graft copolymers (Adopted from Chandakavathe B. N. *et al* 2022).

Table 1: Components for synthesis of graft copolymer.

Code	Gum polymer (g)	Ceric ammonium nitrate (mg)	Acrylamide (g)	Microwave time (min)
G1	1	200	5	5
G2	1	400	5	5
G3	1	600	5	5
G4	1	800	5	5
G5	1	200	10	5
G6	1	400	10	5
G7	1	600	10	5
G8	1	800	10	5

Where G: Grafting formulation.

Methodology

Purification of mesquite gum

Mesquite Gum (MGP) was hydrated with required amount water and heated to 40°C for 2 hr while being stirred occasionally. The heated mixture is cooled and filtered using muslin cloth. From the obtained filtrate pure gum was precipitated by adding excess amount of acetone. Obtained precipitate was washed several times with excess quantity of acetone. The precipitate was separated and taken in petri plates, dried in an oven at 40°C until all water removed. Thereafter, dried gum polymer crushed, sieved (#60) and preserved in hermetically sealed amber-coloured bottle.[12]

Isolation of fenugreek seed gum polymer

Fenugreek seeds were crushed in mortar using pestle and crushed material was soaked in distilled water at room temperature for 12 hr. Further mixture was boiled on water bath until the slurry was obtained and cooled the slurry by keep it in refrigerator for overnight to settle down un-dissolved materials. Upper clear solution was decanted and rest was concentrated it upto 1/3rd of its original volume at 60°C. The dispersion was cooled and gum was precipitated using three times as much acetone. Further, obtained precipitate was washed repeatedly using acetone and dried until constant weight was noted and then eventually pulverised and sieved.[11]

Synthesis of Acrylamide Grafted Mesquite Gum Polymer (AAm-g-MGP) and Fenugreek Gum Polymer (AAm-g-FGP)

The purified fenugreek and mesquite gum were separately grafted with acrylamide. Grafting was carried as per the procedure reported by *Bhagwat et al. 2020*, briefly, 1 g of purified gum dispersed in 100 mL of distilled water, 5 or 10 g of acrylamide in 30 mL of distilled water and varying concentrations of CAN in 30 mL of distilled water in separate containers. The conical flask containing acrylamide solution was added to flask containing pure gum (polymeric) dispersion and swirled for 1 hr at 300 rpm using magnetic stirrer. Another flask containing CAN solution was incorporated into above made mixture (polymer dispersion+acrylamide solution) to initiate the generation active sites on the polymeric backbone and kept it overnight, the quantities used are listed in Table 1. On next day, the mixture was exposed to microwave irradiation (Scientific microwave synthesizer (CATA - R, Catalyst systems, Pune, India) at 6 powers for 5 min. Cooled resultant mixture was precipitated using thrice quantity of acetone and precipitated copolymer was dried and preserved in hermetically sealed container. Further, it was pulverised and passed through sieve # 20. The powdered gum was preserved in hermetically sealed container for further use.[13] The grafting of acrylamide onto the fenugreek was carried similar to that of mesquite gum polymer. The stages involved in the synthesis of grafted copolymers is depicted in Figure 1.

Elemental analysis, DSC and FT-IR study

Confirmation of grafting

To verify the grafted polymer structure, data from elemental analysis, DSC and Fourier Transform Infrared (FTIR) spectroscopy were investigated. In order to obtain FT-IR spectra, the pure and grafted gum polymers was collected in a sample holder cavity and scanned between wave-number 4000-400 cm^{1} (Perkin Elmer FT-IR, model-1615 spectrophotometer). In order to examine the thermal characteristics, 1 go of the sample was placed in an aluminium sample holder and heated between 25 and 35°C at a rate of 10°C/min (Mettler, Stare SW 14.00 T6). For calculation of the C, H and N elements by using elemental analyser (Thermo Finnigan) and here the temperature was increased to 105°C and helium gas was utilised.[13] As a calibrating technique, K Factors.

Compatibility Study

In order to determine grafted polymer and drug compatibility, the physical mixture of mebendazole and grafted polymers were subjected for FT-IR and results were compared with mebendazole spectra alone.

Table 2: Composition of extended release mebendazole tablets.

Ingredient	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)
Mebendazole	100	100	100	100	100	100	100	100	100
AAm-g-FGP	12.5	12.5	12.5	25	25	25	50	50	50
AAm-g-MGP	12.5	25	50	12.5	25	50	12.5	25	50
Lactose	265	252.5	227.5	252.5	240	215	227.5	215	190
Talc	5	5	5	5	5	5	5	5	5
Magnesium Stearate	5	5	5	5	5	5	5	5	5
Total	400	400	400	400	400	400	400	400	400

All quantities mentioned are in mg.

Evaluation of Grafted Polymers

Determination of swelling index

The method described by Patel *et al.* 2008[14] was used to calculate the swelling index for pure and grafted polymers. 100 mL graduated glass cylinders containing 1 g of pure and grafted polymer were filled with distilled water and the volume occupied by the pure gum polymer and grafted polymers was recorded as the beginning Volume (VO). After 24 hr, the final Volume for swelled pure and grafted (VT) was again recorded[13] and the swelling index was calculated using the equation.

$$\text{Swelling index} = \frac{V_T - V_O}{V_O} \times 100$$

Where V_O and V_T are the initial and final volume in mL, respectively.

Determination of Water Retention Capacity (WRC)

After the swelling index was determined, the swelled pure and grafted materials were placed on muslin fabric and tightly squeezed to release all of the held water. A graduated glass measuring cylinder was used to collect the drained water and the volume inside was recorded. WRC was computed by deducting the original volume of mucilage from the volume of drained water. The amount of water retained per unit volume of a polymer is considered the polymer's water retention capacity.[15]

$$\text{Water retention capacity} = \frac{\text{Original volume of mucilage (mL)}}{\text{Volume of drained water (mL)}}$$

Determination of Hydration Capacity (HC)

For determination of HC, weighed amount of pure and grafted powder (1 g) were suspended separately in a tared centrifuge tube having 10 mL of distilled water and centrifuged for 10 min at 1000 rpm (Remi, Mumbai). After 10 min, the supernatant was decanted, weights of centrifuge tubes were noted and concentration of HC was determined[15] using the following formula:

$$\text{Hydration capacity} = \frac{\text{Weight of hydrated sample (g)}}{\text{Weight of the dry sample (g)}}$$

Determination of Viscosity

The viscosity of the 1% aqueous dispersion of pure and grafted copolymers in distilled water was determined using a Brookfield viscometer with spindle number s62 at 50 rpm (DLVE, U.S.A.). The viscosity was expressed in centipoises.[14]

Biodegradability

By utilizing *Aspergillus niger* as the test organism, the biodegradation of the grafted polymer was investigated. In order to achieve the study, a minimal agar medium without a carbon source was made and placed into petri dishes. Using a nichrome wire loop, *Aspergillus niger* suspension was introduced into the prepared sterile medium and the copolymer film was prepared by solvent casting method; the obtained thin film was placed onto the micro-organism inoculated medium. The plates were incubated at room temperature and after 8, 14 and 21 days, films were analysed for colony growth.[13]

Toxicity testing in *Drosophila melanogaster*

Preparation of culture media

The 80 g of corn flour, 70 g jaggery, 9 g of agar and 15 g of yeast powder (15 g) were all separately weighed and kept aside. 1 L of purified water was in a pressure cooker and heated to about 40°C. To avoid lumps, corn flour was gradually added to the warm water while being constantly stirred. Yeast powder, jaggery and agar were added as well as the remaining ingredients. The cooker was then closed without a whistle and heated on high flame for 5 min. After cooling, the lid cooker was gently opened, its contents were thoroughly mixed to prevent lumps and the cooker was once again sealed with a whistle. After 25 min of heating on low flame and later it was cooled. After carefully mixing the ingredients, 1.25 g of methyl para hydroxy benzoate was dissolved in sufficient quantity of ethanol poured into media and finally 4.4 mL propionic acid was added to it. The prepared media was introduced into fly vials at a depth of about 2.5 cm when it was in a molten state.[16]

Evaluation of effect of grafted copolymers on lifespan of *D. melanogaster*

Synthesized grafted copolymers in introduced to the medium vial in a dispersion of 0.5 mL at concentrations of 0.5% and 1% w/v. For the purpose of comparison, a control vial without grafted gum polymer was made and kept. 20 *D. melanogaster* flies (W1118) were introduced in each vial at a 24 hr old age and the number of flies was counted each day. For each treatment vial, this process was done three times. The number of days needed for 50% of the flies to survive was used to compute the average lifespan of the insects and the number of days needed for 5% of the insects to survive was used to determine the maximum lifespan. This information was utilised to develop survival curves.[17]

Preparation of mebendazole extended-release tablets

Wet granulation method was used to make various batches of formulations. All ingredients were weighed and sifted through 60 mesh screen. Weighed material was properly mixed by using polythene bag. Mebemdazole, AAm-g-FSM, AAm-g-MGP and lactose placed in the morter to it 5% W/V starch paste was added till damp mass was obtained. The obtained damp mass/ cohesive mass was passed through sieve number 22 (wet screening). Obtained granules were dried; dried granules were sifted through 44 (dry screening). Finally, talc and magnesium stearate were added as lubricant and glidant. Rotary compression machine was used to compress the granules. Each tablet has 100 mg of mebendazole and other ingredients as shown in Table 2, with a total weight of 400 mg. By dipping method, the compressed tablets were coated with Eudragit RS 100 polymer, with chloroform as a solvent.[18]

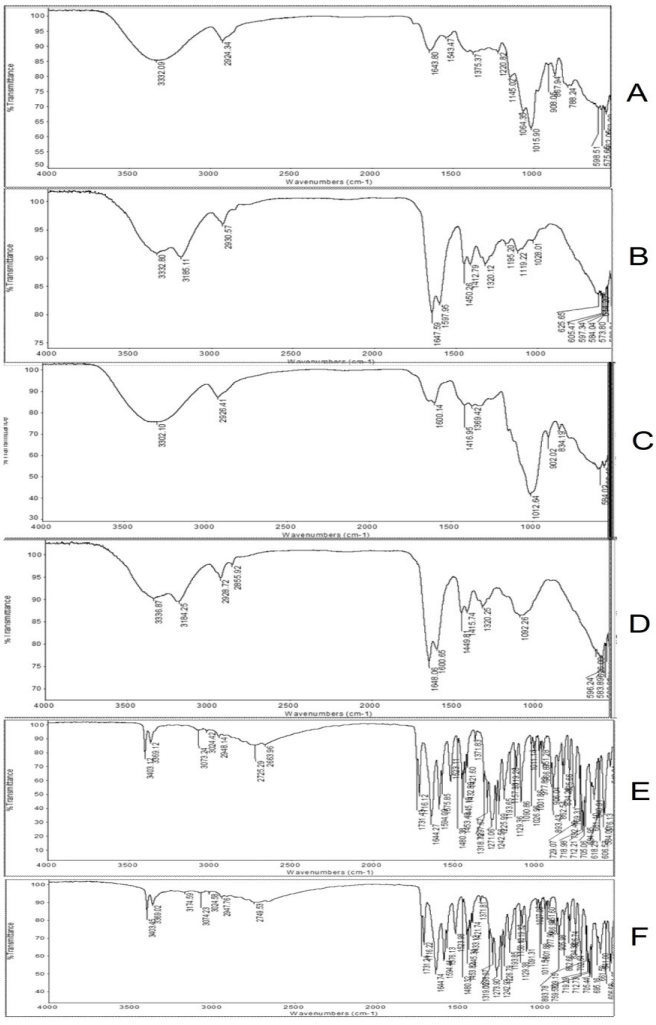

Figure 2: FT-IR spectra of (A) FGP, (B) AAm-g-FGP, (C) MGP (D) AAm-g-MGP (E) Mebendazole and (F) Physical mixture (Mebendazole+grafted polymers).

In vitro dissolution study

The USP Dissolution Test Apparatus Type-II Basket was used for the dissolution test. The bowl was filled with 900 mL of pH 1.2 acid buffers and rotated at 100 rpm. The dissolution medium was kept at 37±5°C for 2 hr and samples (5 mL) were taken out every hour. To maintain sink environment the same amount of fresh medium was added. After 2 hr, the acid pH 1.2 buffer was changed to phosphate buffer pH 7.4 to serve as a dissolution medium. The temperature was kept at 37±5°C for the remaining hours and samples (5 mL) were taken out every hour and fresh medium was added every time. Using a UV/visible spectrophotometer, the absorbance of the resultant solutions was determined at lambda max 234 nm. It was calculated to find the percentage of drug released.

Stability Studies

A 6-month stability study was conducted on the selected mebendazole tablet formulations at different conditions to evaluate physical appearance, friability, hardness, percent amount drug content and *in vitro* release studies, samples were taken on days 0, 90 and 180.

RESULTS AND DISCUSSION

Fourier transforms infrared spectroscopic analysis

Drug-polymer Compatibility and confirmation of grafting of AAm-g-FSM

The pure polymer, drug mebendazole and grafted copolymer i.e., AAm-g-FGP and AAm-g-MGP subjected for FT-IR study to find out any interaction. Pure FGP shows the characteristics peaks at a wave number of 3332.09, 2924.34, 1643.80, 1543.47, 1375.37 and 1220.82 cm^{-1} corresponding to -OH stretching, CH stretching, C=O stretch and C=C respectively shown in Figure 2 (A). However, AAm-g-FSM shows peaks at 3185.11, 2930.57, 1597.95 and 1450.26 which are attributed to O-H stretch, C-C bending and C-H stretch respectively. The band at 1674.59 appeared due to C-O stretching of amide.

Unsubstituted bands in the region of 3100-3500 cm attributed to N-H amide stretching. The bands in the region of 1650-1560 cm assigned to N-H bending of amide. So that, FTIR analysis confirmed that successful grafting of amide group over FGP. It is shown in Figure 2 (B).

Drug-polymer compatibility and confirmation of grafting of AAm-g-MGP

Drug, pure polymer, and grafted co-polymers were subjected FT-IR study to find out any interaction. IR spectrum bands at 3302.10, 2926.41, 1600.14, 1416.95, 1369.42, 1012.64 and 902.02 cm^{-1}. Which are assigned to C=O stretch, C=O stretch, OH Bending. C-H stretch, C=O stretch. FT-IR analysis confirms the successful grafting of mesquite gum. AAm-g-MGP showed peaks at 3336.87, 3184.25, 2928.72, 2855.92, 1648.06,1600.65 and 1449.81cm-1 which are attributed to O-H Stretching, C-H stretching, C-C stretch, C=O stretch, C-H bending. C-H bend in plane respectively Figure 2 (C).

The band at 1600.65 appeared due to C=O stretching of amide. FT-IR analysis confirmed the successful grafting of amide group over mesquite backbone Figure 2 (D).

IR spectrum of Mebendazole showed prominent bands at 3403.12, 3369.12, 3073.24, 3024.42, 2948.14, 2663.96 cm^{-1} and 1731.43, 1644.27, 1594.09, 1193.65 which are assigned to N-H stretch, C-H stretch, C=O Stretch, C-C stretch C-N stretch and C-O stretch, CH_2waggingrespectively shown in Figure 2 (E).

Notably physical mixture (mebendazole+AAm-g-MGP+AAm-g-FSM) retained all the characteristic peaks of mebendazole at 3403.45, 3369.02, 3174.59, 3074.23, 2947.76 and 2749.53 cm^{-1} corresponding to N-H stretch. C-H stretch, C-O Stretch, C-C stretch and aromatic C-H starching respectively. This indicates the absence of drug-excipients interaction. An IR spectra of physical mixture is shown in Figure 2 (F).

Differential Scanning Calorimetry

DSC is a method that reveals information about the physical and chemical changes that occur in polysaccharides during heat processing, producing distinctive curves for a particular polysaccharide. A broad endothermic peak at 54.65º and an exothermic peak at 340.15º were visible on the DSC thermogram of physical mixture (Figure 3). Our findings concur with those of Malviya *et al.* 2019, who found that the thermogram of pure gums had an exothermic peak at 300ºC and an endothermic peak at 96ºC. The early breakdown and gum dehydration were attributed for the endothermic shift. Water that has been physically adsorbed, hydrogen-bounded, or crystallised can all be considered detached water.[19]

According to Malviya *et al.* 2019, the first endotherm is attributed to polymer degradation because the amide group is converted to an imine and ammonia is released, while the second transition is related to the imine breakdown.

X-ray Diffraction study

X-ray diffraction patterns of mebendazole and physical mixture at room temperature from 2ϴ=5º to 70º. The grafted gums shows partial crystalline nature, like all other natural polymers. The XRD pattern of grafted copolymers also shows no distinct peak indicates amorphous nature. The x-ray diffraction pattern of mebendazole and physical mixture (mebendazole+grafted

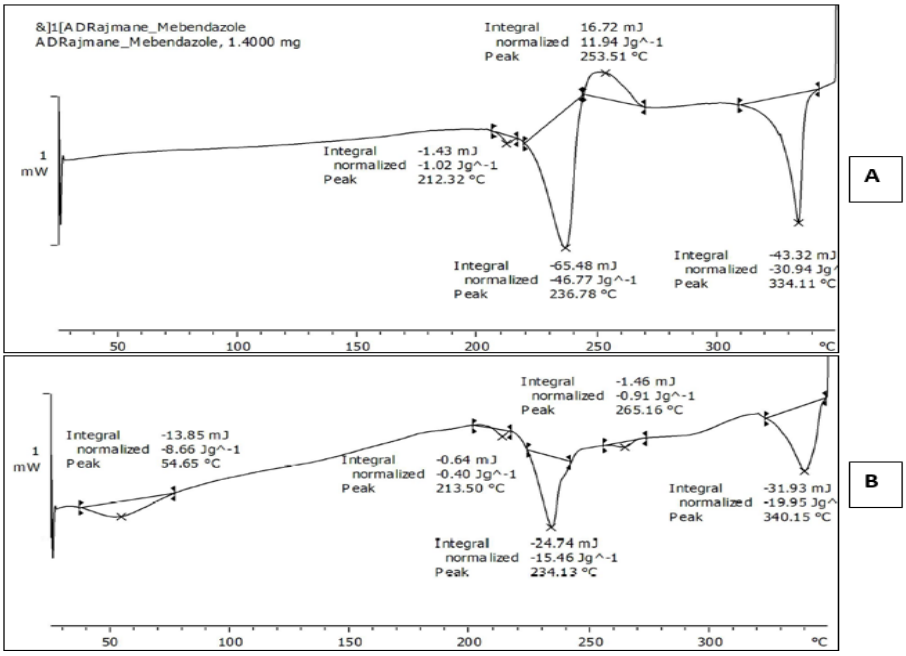

Figure 3: DSC thermogram of (A) Mebendazole and (B) physical mixture (mebendazole+AAm-g-MGP+AAm-g-FSM).

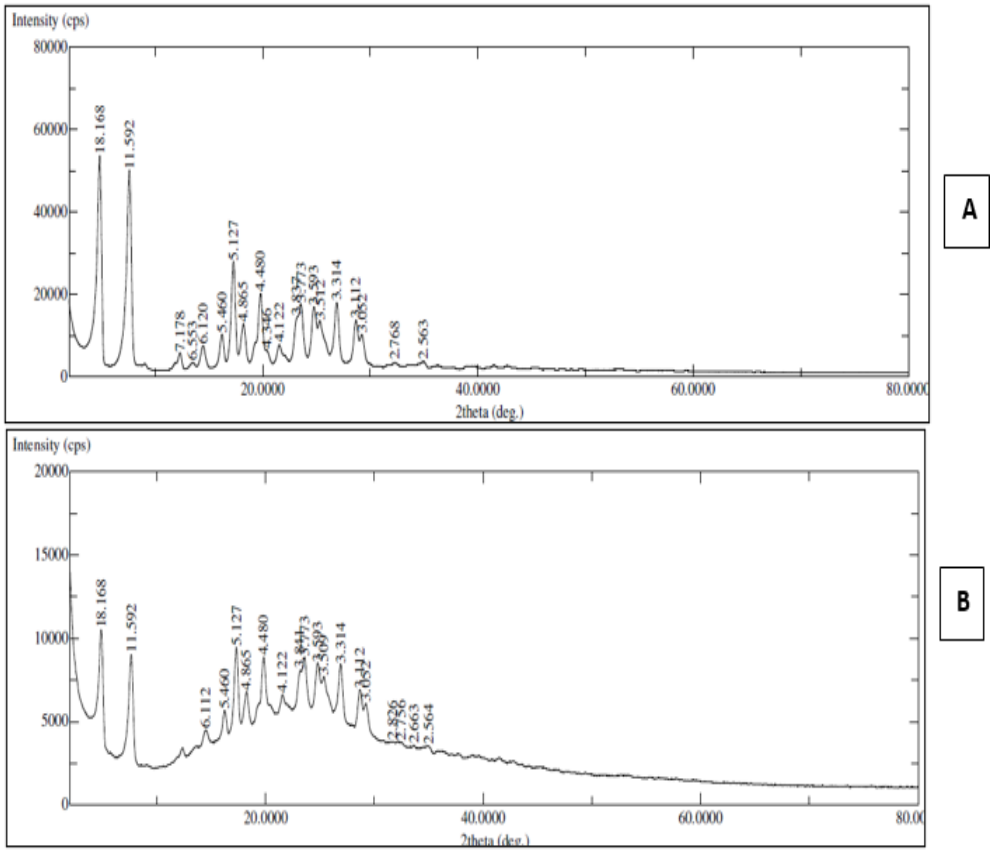

Figure 4: Spectra of X-RD of (A) mebendazole, (B) physical mixture (mebendazole+grafted polymers).

polymers) shown in Figure 4. XRD pattern of mebendazole shows strong peak at 18.168, 11.592, 5.127 and 4.480º showing crystalline nature of drug. XRD pattern of physical mixture also shows strong diffraction peak at 6.112, 5.460, 4.480 and 3.331º shows that crystalline nature of the material.

Elemental analysis

Elemental analysis of MGP and grafted AAm-g-MGP were 29.326% of carbon, 6.878% of hydrogen, 4.822% of nitrogen and 38.486% of carbon, 5.411% of hydrogen, 16.766% of nitrogen respectively. Similarly, FSP and grafted AAm-g- FSP were 28.226% of carbon, 6.215% of hydrogen, 5.231% of nitrogen and 34.545% of carbon, 6.256% of hydrogen, 15.956% of nitrogen respectively. From the C, H and N data it can be confirmed that grafted copolymers has significantly more nitrogen content than pure polymers. This could be as a result of the hydrogen atom in the replaceable hydroxyl groups being replaced with the $-CH_2CONH_2$ group. The amide group substitution on MGP is supported by the findings of the elemental analysis.

Evaluation of Grafted Copolymers

Swelling Index

The Swelling index of AAm-g-MGP was found to be much higher (112.33±36.55%) than MGP (62.14±11.26%) whereas, AAm-g-FGP was 102.46±7.56%, which is higher than that of pure fenugreek gum 58.66±6.12%. This significant swelling property demonstrates the increase in gel or solid volume brought on by the interpenetration of a liquid. The swelling behaviour of grafted gum directly influences dissemination of drug from swollen gel matrix structure. Hence, drug release retardation various devices such as hydrogels, tablets, microspheres and so on.[19]

Water Retention Capacity (WRC)

Here, water retention capacity of AAm-g-MGP was 2.53±0.25 and pure gum was 1.44±0.42 and 2.53±0.25 and pure gum was 3.22±0.68. In order to evaluate the grafted natural gums as superabsorbent, the improved water holding capacity is crucial. The drug release property depends upon the water holding capacity grafted polymers. The higher water retention of the grafted polymer without getting eroded decides its ability to sustain the drug release. The extra polymeric network and hydrostatic interaction that results in the high cross linking that keeps the absorbed water for a long period of time.[20]

Hydration Capacity (HC)

Grafting of MGP has improved the hydration capacity more than 50% (7.69±0.1) as compare to MGP and decreased MSC (2.8±0.42%) than pure polymers (Hydration Capacity=1.49±0.091). The HC of grafted copolymers was higher

compared to un-grafted polymers, thus more HC improves the water holding capacity of grafted polymers. Therefore more the HC and WRC it significantly gives more swelling index hence more swelling index that in-turn drug release retardant property.[20]

Viscosity

It is resistance to flow of liquids is the meaning of viscosity. The viscosity of pure gum polymers and grafted copolymers was determined by using the Brookfield viscometer.[21] Viscosity of 1% aqueous dispersion of pure and grafted gum Aam-g-MGP was found to 252.34±2.56 and 489.86±6.84 centi poise respectively whereas, pure fenugreek gum displayed 156.25±3.56 cP and AAm-g-FGP was 325.12±9.65 cP at at 50 RPM, spindle number s62.

Biodegradability

Both the Aam-g-MGP and Aam-g-FGP thin films were placed in petri dishes with carbon source-free agar medium that had been contaminated with *Aspergillus niger. Aspergillus niger* growth in petri dishes was examined and colonies were counted at 8, 14 and 21 days to reveal the *Aspergillus niger* growth on films. Colony-forming units were found on AAm-g-MGP at $3.125x10^6$ CFU/mL and on AAm-g-FGP film at $2.356x10^7$ CFU/mL. By forming such massive colonies of organisms in medium in the absence of carbon, *Aspergillus niger* had used the carbon present in the grafted copolymers for growth and colony development thus validated the biodegradable nature of both grafted copolymers.[20]

Toxicity

The toxicity assessment of grafted polymeric gum on *Drosophila melanogaster* (*D. melanogaster*) has been investigated for the first time. Calculated and compared to unaffected (control) flies were the mean and maximum lifespans of *D. melanogaster* flies exposed to 0.5% and 1% of AAm-g-MGP and AAm-g-FGP, respectively. Fly lifetime in the control group was 64 days on average, while it was 62 and 61 days for those exposed to 0.5% and 1% of fenugreek grafted copolymer, respectively and 60 and 59 days for mesquite grafted copolymer. Figure 5 demonstrates that the maximum lifespan reduction was found to be 3.12 % (62 days) and 4.68% (61 days) for flies exposed to 0.5% and 1% of AAm-g-FGP respectively. However, 6.25% (60 days) and 7.81% (59 days) for flies exposed to 0.5% and 1% of AAm-g-MGP respectively. The statistical analysis did not show any significant changes between the flies treated to 0.5% and 1% of grafted copolymers (p value=0.98). At concentrations of 0.5% and 1%, the toxicity assessment investigation did not reveal any notable harmful effects of both the grafted copolymers on *D. melanogaster* life span.[22,23]

Pre-compression evaluation of prepared formulation

For the prepared granules, pre-compression characteristics such the angle of repose, Carr's index and Hausner's ratio were measured. Granules showed good flow property, as evidenced by the angle of repose, Carr's index and Hausner's ratio values, which ranged from 23.70º to 29.54º, 10.42% to 12.94% and 0.91 to 1.14, respectively. The flow nature of granules characteristics contribute to the tablets consistent mass, which is necessary before the tablets are compressed. The experimental results of bulk density and tapped density revealed that the packing of granules was good. Table 3 presents the pre-compression results in detail.

Evaluation of mebendazole extended-release tablets

The formulation components were weighed in accordance with Table 3 and granules were made and tested for various pre-compression parameters. Further, prepared granules were compressed using a 12-station compression machine.

Table 4 provides post-compression evaluation results for compressed tablets from various batches. The prepared tablets were tested using a range of post-compression assessment criteria. All the batches of compressed tablets compiled the weight variation test as per Indian Pharmacopoieal (IP) standards. The tablet measured 3 mm in thickness and showed 5.50 to 6.60 kg/

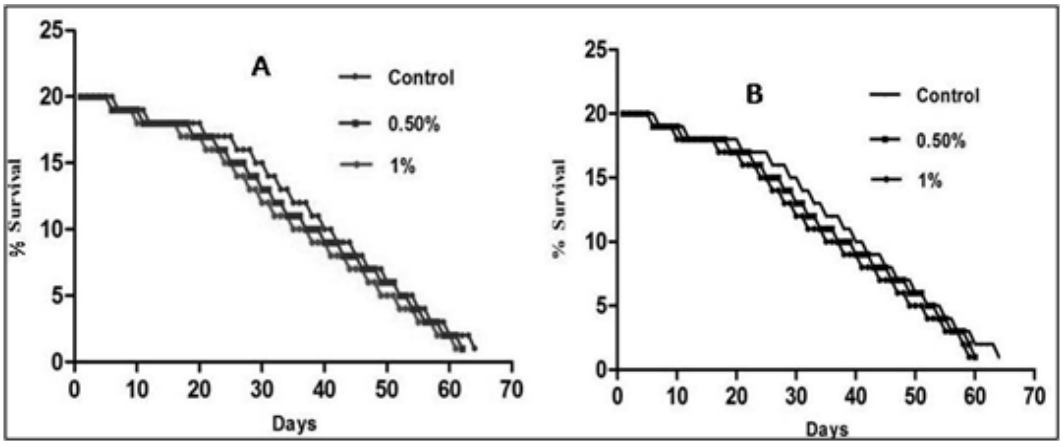

Figure 5: Toxicity studies of 0.5% and 1% AAm-g-FGP (A) and AAm-g-MGP (B) on *D. Melanogaster.*

Table 3: Results of pre-compression evaluation of various tablet formulations.

Formulation Code	Bulk density (g/mL)	Tap density (g/mL)	Carr's index (%)	Hausner's ratio	Angle of repose (°)
F1	0.48±0.022	0.52±0.063	10.69±0.032	0.92±0.034	23.70±0.65
F2	0.47± 0.013	0.51±0.045	10.68±0.022	0.91±0.022	24.65±0.93
F3	0.55±0.074	0.61±0.084	10.42±0.046	1.12±0.044	25.35±0.58
F4	0.45±0.033	0.51±0.043	11.76±0.064	1.12±0.055	26.12±0.58
F5	0.46±0.015	0.52±0.062	11.53±0.023	1.10±0.024	27.50±1.25
F6	0.50±0.054	0.56±0.052	11.71±0.054	1.12±0.031	28.56±1.25
F7	0.43±0.027	0.56±0.014	12.94±0.045	1.07±0.052	29.54±1.45
F8	0.50±0.058	0.57±0.053	12.22±0.068	1.12±0.044	27.66±0.95
F9	0.55±0.078	0.60±0.083	11.33±0.036	1.14±0.034	26.65±1.25

Where F: Formulation, Mean±SD, (n=3).

Table 4: Result of post-compression evaluation of various batches of tablets.

Formulation Code	Weight (mg)	Hardness (kg/cm²)	Friability (%)	Thickness (mm)	Drug content (%)
F1	400±0.67	5.60±0.45	0.49±0.14	3.00±0.11	96.26±0.32
F2	401±0.34	5.60±0.40	0.30±0.13	3.00±0.12	97.37±0.54
F3	400±0.39	5.50±0.35	0.24±0.18	3.00±0.11	94.88±0.80
F4	402±0.15	5.50±0.36	0.20±0.14	3.00±0.12	95.65±0.52
F5	400±0.45	6.60±0.50	0.20±0.12	3.00±0.12	99.19±0.11
F6	401±0.67	5.50±0.26	0.20±0.14	3.00±0.12	96.23±0.32
F7	400±0.36	5.50±0.20	0.25±0.15	3.00±0.14	94.08±0.54
F8	403±0.25	5.50±0.25	0.50±0.18	3.00±0.16	97.90±0.44
F9	400±0.56	6.10±0.30	0.25±0.15	3.00±0.12	95.48±0.56

Where F: Formulation, Mean±SD, (n=3).

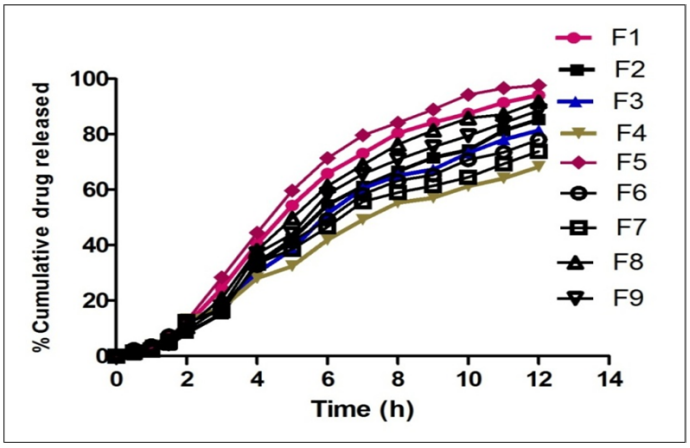

Figure 6: *In vitro* drug dissolution profile of F1 to F9 formulations.

cm² in hardness. Studies on thickness and hardness provided information on the mechanical strength tablet. The physical integrity of the tablet was demonstrated by the percent friability of all formulations, which was well within recommended limit of IP (less than 1%). All of the prepared tablets had a drug content percentage between 94.08% and 99.19%, this assured drug uniformity in all the batches of tablets.[24]

In vitro drug dissolution study

USP-I dissolution apparatus used to carry out an *in vitro* drug dissolution test with all compressed batches (F1 to F9). The study was carried out in a pH 1.2 buffer for the 1st 2 hr and then in a pH 7.4 phosphate buffer for the remaining hours. The influence of raising the polymer concentration was seen in the drug release profiles of all batches.

The *in vitro* drug release study shows that 93.15, 85.34, 82.43, 82.31, 83.67, 81.16, 80.16, 78.44, 77.56, % of drug was released from F1 to F9 tablet formulations respectively, at the end of the 12th hr. The grafted copolymer of mesquite and fenugreek polysaccharides might have under gone degradation in the pH 7.4 lead to release of remaining amount of drug from the tablet formulation. The drug release profile of all batches of the formulation is presented in Figure 6.

Drug release kinetics

The drug mebendazole release mechanism and kinetics were ascertained by the utilisation of the Korsmeyer-Peppas model, Higuchi model, zero order kinetics and first order kinetics. Since the r^2 values of the various formulations ranged from 0.979 to

0.997, they were found to follow the Higuchi model. Mechanism of drug release is anomalous transport, or non-Fickian diffusion, as evidenced by their good fit with Korsmeyer-Peppas models (r^2 between 0.981 and 0.988 for n values less than 1). This suggests that the drug release is dependent on the swelling and diffusion mechanisms.[25-27]

Stability studies

The 6-month stability study on the F5 mebendazole tablet formulations involved testing under varied environmental conditions (30±2ºC/65±5%). Formulation F5 consistently demonstrated optimal performance across all tested parameters. Following a 6 month stability assessment, the tablets exhibited remarkable stability characteristics: no color change, a hardness of 6.50±0.50 kg/cm², drug content maintaining at 98.16±0.18% and a sustained drug release of 85.20±0.32%. These results underscore the robust stability profile of batch F5, emphasizing its potential as a reliable and effective mebendazole delivery system.

CONCLUSION

The present study results revealed that the mixtures of AAm-g-FGP and AAm-g-MGP co-polymers are biodegradable, biocompatible and its incorporation in colon targeted formulation gives extended drug release. AAm-g-FGP and AAm-g-MGP co-polymers can efficiently be used for the development of an extended drug delivery system. However, further research is warranted to optimize these copolymers for its potential in improving extended release colon targeted drug delivery systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

NCBS: National Centre for Biological Sciences; ***D. melanogaster*:** *Drosophila melanogaster;* **MGP:** Mesquite gum polymer, **FGP:** Fenugreek gum polymer, **AAm-g-MGP:** Acrylamide grafted mesquite gum polymer; **AAm-g-FGP:** Fenugreek gum polymer; **CAN:** Ceric ammonium nitrate; **HPMC:** Hydroxy propyl methyl cellulose; **RPM:** Rotations per minute; **USP-I:** United States pharmacopoeia; **CFU:** Colony forming units; **hr:** Hours; **mL:** Milliliter.

SUMMARY

The present study addresses the limitations of natural polymers in drug delivery systems, such as uncontrolled drug release, enzymatic degradation and thermal instability. The several researchers grafted purified gums with acrylamide using ceric ammonium nitrate as a free radical initiator and obtained high performance biomaterials. Elemental analysis and FT-IR spectroscopy confirmed successful grafting. The synthesized graft copolymers exhibited enhanced properties, including swelling index, water retention capacity, viscosity and hydration capacity compared to pure gum polymers. Mebendazole tablets, prepared using moist granulation, met compendial standards for hardness, friability, thickness and weight variation. Coating the tablets with Eudragit RS 100 resulted in controlled drug release, with a small amount released in 5 hr and maximum release in the colonic region over 12 hr. This study demonstrated the potential of grafted natural polymers for developing high-performance biomaterials in drug delivery applications.

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