Original Article

In vitro Evaluations of Biodegradable Polyacrylamide Grafted Moringa Bark Gum Graft Copolymer (MOG-g-PAAM) as Biomedical and Controlled Drug Delivery Device Synthesized by Microwave Accelerated free Radical Synthesis

Nikhil Rai, Trishna Bal*, Sabyasachi Swain

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, INDIA.

ABSTRACT

Aim: The present work is based on preparation of different grades of *Moringa* Bark Gum (MOG) with Acrylamide (AM) with varying amount of AM (monomer) and APS (redox initiator) using microwave accelerated free radical reaction. **Objectives:** In the current work, the *Moringa* bark gum grafted with acrylamide graft copolymer was tested for tissue engineered polymeric scaffold as well as controlled drug delivery system using metronidazole as a model drug. **Methodology:** The microwave radiation process was used along with redox initiator for the graft copolymerization process and the optimization of the grades was done using % grafting efficiency, intrinsic viscosity. Moreover, the optimized grade GF4 was analyzed with FTIR as well as NMR proving efficient grafting has resulted along with TGA, OCA and XRD. **Results:** The grade GF4 showed 95% drug release for 24 hours with only 1% hemolysis proving non-toxic and SEM images evidence its biodegradability thereby making the grade GF4 suitable for controlled release as well as tissue engineered scaffold. **Conclusion:** The results indicated that the optimized grade GF4 can be utilized as biodegradable polymer having applications in controlled delivery of drugs as well as scaffold for cell proliferation in wound healing and burn therapies.

Key words: Graft Copolymer, Metronidazole, *Moringa* Gum, Microwave Irradiation, Biodegradability, Controlled drug release, Polymeric scaffold, Histopathology.

INTRODUCTION

The use of natural polysaccharides for formulation is advantageous as they are non-toxic, less expensive, biodegradable, biocompatible, renewable and freely available. Recently many natural polysaccharides isolated from plants are widely used for the preparation of drug delivery systems either in their native form or modified forms.[1] The natural plant polymers are having stability problems and thus any surface modifications in them make them useful as polymeric scaffold in tissue engineering as well in drug delivery for potent drugs as these systems slowly swell and erode to release the drugs in a controlled fashion thereby preventing the peak and valley release pattern as expected in conventional delivery.[2] Grafting technique is one efficient process to improve the shelf life, stability and instant biodegradation of natural polymers.[3,4] Graft copolymerization of synthetic polymers on to the natural polysaccharides makes the material more effective for specific uses.[5] Synthesis of grafted copolymers involves free radical mechanism. The free radicals are generated by various ways like use of free radical initiators, use of high energy radiations like γ-rays and microwave radiation. Microwave irradiation method provides highest yield for commercial mass production as well as

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Correspondence:
Dr. Trishna Bal
Assistant Professor, Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi-835215, Jharkhand, INDIA.
Phone: +91-9470141136
E-mail: trishna.bal@gmail.com

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better grafting and reproducible grafting efficiency.[6] Use of microwave irradiation becomes more efficient when the process is assisted with the help of free radial initiator and the process is more ecofriendly as there is no use of any chemical as catalyst for the synthesis.[7] Moreover, the use of green chemistry design for polymer synthesis has eliminated the use of most toxic chemicals creating an environment free from chemical pollution.[8] The different molecular configuration and the functional groups in the natural polymers make them suitable for their modifications and to be applied in biomedical fields as crosslinking of biopolymers improves the quality of the material as tissue implant.[2] The biomedical nature of such modified polymers mainly depends on their abilities to interact with the living cells, cytocompatibility and their macromolecular structure to form the three-dimensional (3D) structure to mimic the cells.[9] Several polysaccharides are utilized for preparation of graft copolymer with vinyl or acryl monomers. The acrylamide monomers have varied applications in the field of drug delivery, flocculation, stabilizing agents etc. [2] Different plant sources like seeds, leaves, bark have plenty mucilage as well as gum content as rich source of polysaccharides. *Moringa* bark gum (MOG) is reported to have gel forming potential for topical preparations [10] as well as the graft copolymer of MOG grafted with polyacrylamide (PAAM) along with the blends of Gum Ghatti are utilized as efficient flocculant systems for sewage cleaning.[11] Moreover, biodegradation is a major issue with various synthetic polymers. Thus, various natural polymers are combined with synthetic polymers to make them biodegradable[12] and thus as an evidence for bacterial biodegradation, the polymers are tested in presence of bacterial culture, as these polymers degrades in presence of bacterial enzymes and thereby render them to be environment friendly. From previous studies, no evidences of microwave accelerated biodegradable graft copolymer of MOG with PAAM (MOG-g-PAAM) are found for being utilized as biodegradable material for controlled drug delivery as well as polymeric tissue scaffold. Based on this concept, the present research with the synthesis of microwave irradiated graft copolymer of *Moringa* bark gum with Polyacrylamide (MOG-g-PAAM) with various characterizations for its use as biodegradable controlled drug delivery device using metronidazole (MTZ) as a model drug.

Experimental

Crude *Moringa* bark gum (MOG) was collected from BIT Mesra, Ranchi campus. The gum collected from injured tree bark was dried and passed through sieve 80 mesh and soaked in distilled water for 9 hrs and then gently heated with stirring till all the materials completely swelled and finally dissolved. The solution so formed was filtered through muslin cloth and concentrated to reduce in volume and the final filtrate was treated with equal quantity of acetone to precipitate polysaccharide. The isolated polysaccharide was dried in an oven at 50°C till to get a free-flowing powder.[12,13] Acrylamide (AM), Ammonium Persulphate (APS) (SD, Fine-Chem Ltd., India) was used as received. Metronidazole (MTZ) was procured from Lupin Pharmaceuticals, India.

Synthesis

The graft copolymer was prepared by using microwave assisted process by varying different concentration of APS and AM using APS as the redox initiator[13,14] as shown in composition Table 1. The time for the reaction was kept constant. Initially the required quantity of polymers (MOG) along with calculated amount of monomer AM were taken in a glass beaker and dissolved in milipore water (100 ml) stirred on a magnetic stirrer maintained at temperature 50°C till all the polymers dissolve and then later APS is added and stirred for another 15 min. The mixture in the glass beaker was microwaved keeping the time constant at 40 sec till the polymer mixture thickens to gel consistency. The reaction vessel was immediately taken out and placed aside in ice bath for cooling. The unreacted MOG, monomer, APS and any other byproducts formed are removed by pouring excess amount of acetone as a solvent to obtain the purified graft copolymer.[15,16] The precipitated material was taken out and dried in a hot air oven maintained at 50^0C. Reaction detail is outlined in Scheme 1. The optimization of the best grade of MOG-g-PAAM is done using % Grafting Efficiency as shown in Equation 1.[17-19] The synthesized graft copolymer was dried in a hot air oven and ground to fine powder.

$$\% \text{ Grafting Efficiency} = \frac{\text{Weight of Graftcopolymer} - \text{Weight of polymer}}{\text{Weight of monomer}} \times 100 \quad (1)$$

Characterization and analysis

The intrinsic viscosity measurement of all the prepared grades of graft copolymer were done using Ubbelodhe viscometer (capillary pore size 0.36 mm, measuring viscosity range from 0.3-1mm^2/sec) at 25°C. Other characterizations of graft copolymer along with the pure polymers and monomer was done by using Fourier Transformation Infrared Spectroscopy (FTIR) (Shimadzu, FTIR-8400S) using KBr pellets from 4000 – 400 cm^{-1}.[16-17] Thermo-gravimetric analysis (TGA) (Model: DTG-60; Shimadzu, Japan) was performed in an inert atmosphere (nitrogen) from 30°C to 400°C at the heating rate of 10°C/min.[17-18] X-ray diffraction

(XRD) was done with PW 1840 diffractometer and PW 1729 X-ray generator, which were used for the study to produce Cu-Ka radiation with the scattering angle (2Θ) varied from 10 to 800.[19] ^{13}C Nuclear magnetic resonance (NMR) was also done for the confirmation of grafting reaction in optimized graft copolymer which was compared with that pure MOG.

Intrinsic Viscosity measurement

Intrinsic viscosity measurements of the aqueous solution of graft copolymer of different concentration (0.1, 0.05, 0.025 and 0.0125% w/v) were carried out with the help of Ubbelohde viscometer with a capillary diameter of 0.36mm (constant 0.001) at 25±0.10C in triplicate. The intrinsic viscosity was calculated by plotting specific viscosity/concentration (ηsp/C) versus concentration (C) and then taking the intercept at C=0 of the fitted straight line.[18-19]

Elemental Analysis

The elemental analysis of MOG and MOG-g-PAAM were performed using an Elemental Analyzer (Make and Model M/S Elementar, Germany Vario EL-III) to determine the carbon, hydrogen, nitrogen and sulphur content.[20]

FTIR

The FTIR spectrum of MOG, Acrylamide and the optimized grade MOG-g-PAAM were recorded in solid state by KBR pellet method using FTIR 8400s (Shimadzu, Japan) in the finger printing regions of IR (400 to 4000 cm^{-1}).

Thermal analysis

The thermal stability of vacuum-dried samples of pure MOG, GF4 and AM was examined using thermal gravimetric analyzer (Shimadzu, Japan, DTG-60) and heated from room temperature to 700°C in a nitrogen atmosphere at a heating rate of 10°C/min.[2]

XRD

The sample of pure *Moringa* bark gum MOG, AM and GF4 were diffracted for 2θ value ranging from 20 to 80° at 2°/min and chart speed of 2°/2 cm/2θ (Bruker AXC D8 Advance, Germany).[21]

^{13}C NMR studies

Solid state ^{13}C NMR spectroscopy was performed for native MOG and GF4. The analysis was performed by inserting about 300 mg of sample respectively in ceramic rotor on a JEOL ECX 400 (Peabody, MA, USA) spectrometer functioned at 75 MHz.[22]

Surface morphology

Surface morphology of native *Moringa* gum (MOG) and the optimized grade of MOG-g-PAAM (GF4) were analyzed by scanning electron microscopy (SEM) in powdered form (Model: JSM-6390LV, Jeol, Japan).

In vitro drug release study

Preparation of tablets

The tablets of MTZ using the optimized grade of graft copolymer GF4 was prepared by hand powdering the graft polymer[21] and then weighing out a 225 mg of polymer along with 25 mg of drug which is 10% of total weight of the polymer and passing the mixture through a 20 mesh sieve and to this mixture around 0.01 mL of water was added just to moisten the mixture and triturated.

In vitro release study

The *in-vitro* dissolution study of MTZ from graft copolymer in the form of tablets was carried out in a basket type 8-station dissolution test apparatus Dissolution apparatus(TDT -08 l), Electrolabs TDT-08 L, Mumbai, India, with stirring speed of 50 rpm using phosphate buffer of pH-7 as dissolution medium under sink conditions at 37°C ± 0.5°C. Periodically, 10 ml of the solution was withdrawn from the dissolution medium and was filtered with a 0.45 µm (µ) membrane filter disc and analyzed by double beam spectrophotometer (UV-1800), Shimadzu Corporation, Japan, at 320 nm[23] for the drug. Same volume of dissolution medium was replaced back after each sampling to maintain sink condition. The kinetic data obtained from the release profiles were also evaluated by fitting into different kinetic models.

Determination of Drug Release Kinetics

The drug release data were fitted to models representing zero order (cumulative amount of drug released vs. time), first order (log percentage of drug unreleased vs. time), Higuchi's (cumulative percentage of drug released vs. square root of time) and Korsmeyer's equation (ln cumulative release vs. ln concentration) kinetics to know the release mechanisms. In Korsmeyer's model the mechanism of drug release was dependent on the value of '*n*', when n = 0.5, 0.5 < *n* < 1, *n* = 1 and *n* > 1 corresponds to Case-I (Fickian) diffusion or square root of time kinetics, anomalous (non- Fickian) diffusion and Super Case-II transport respectively.

Optical contact angle

The contact angle of MOG and MOG-g-PAAM were performed by optical contact angle instrument (Dataphysics, Germany, OCAH230). The film was placed

on the testing bench and a liquid drop was dropped on the film. The contact angle was calculated by using Θ/2 method and the average of 3 readings was used as an experimental value.[24]

Bacterial Biodegradability studies

The graft copolymer with specific weight was tested for the biodegradability study. A Minimal Agar Media (devoid of carbon source) was used as the medium for the biodegradation study and was placed the petridish using *B. subtilis* as the microrganism. The weighed polymeric specimens were placed on the surface of the prepared solidified Minimal agar media in petridish of test and control each. The specimens were maintained at 25°C for 15 days, after which they were examined concerning the growth of the microorganism on the surface of the solidified media and weight loss of the specimen.[12]

Hemocompatibility studies

The hemolytic activity of MOG-g-PAAM (GF4) was investigated according to the standard protocol, 2% RBC suspension (100ml) was treated with 1ml of GF4 (40mcg/ml) solution and incubated for 2 hr at 37°C with gentle shaking. A negative control solution (0% hemolysis) was prepared by adding 0.9% of Sodium chloride solution to the erythrocyte suspension and a positive control solution (100% hemolysis) was prepared by adding 10% (v/v) Triton X 100 to the erythrocyte suspension. Two hours later the RBC suspension was centrifuged at 1,000 rpm for 10 min. The supernatants were assayed for the absorbance of released hemoglobin at 540 nm.[25] The degree of hemolysis was determined by the Equation 2.

$$\%\text{Hemolysis} = 100 \times \frac{[\text{Abs-Abs0}]}{[\text{Abs100-Abs0}]} \qquad (2)$$

Where Ab_s, Abs_0 and Abs_{100} are the absorbance of test samples, the suspension treated with 0.9% NaCl and the suspension of complete hemolysis treated with Triton X-100 (10%, v/v), respectively.

Acute Oral Toxicity studies

Acute oral toxicity study for GF4 was performed as per Organization of Economic Co-operation and Development (OECD) guidelines. A total of 5 nulliparous and nonpregnant five weeks old female mice (Swiss albino strain) were used for this study. The study protocol was approved by the Animal Ethics Committee (CPCSEA approval No: 1972/PH/BIT/24/17/IAEC) of Birla Institute of Technology, Ranchi, India. Mice were placed in polycarbonate cage with food and deionized reverse osmosis water at 20-25°C and 40-70% relative humidity in a 12 hr light/dark series. A single dose of 2000 mg/kg body weight of GF4 was administered by gavages using a stomach tube in the first animal. The same dose was administered in the remaining four animals after survival of first animal. The animals were kept under constant observation up to 4 hr on subsequent dosing and daily thereafter for a total of 14 days. The animals were observed for any mortality up to 14 days.[5] The animals were sacrificed on the 15th day and performed histopathological studies for liver and kidney.

Histological procedure

Excised tissues from local site and liver were fixed in 10% formalin solution for 5 days. All tissues were processed by embedding in paraffin wax and then sectioned at 3 µm thicknesses, mounted on glass slides, deparaffinized and stained with Hematoxylin–Eosin (HE). Images were taken with an optical microscope (Leica, DME).[2]

Assessment of mice subcutaneous tissue response graft copolymer as tissue engineered scaffold

The experiment was conducted following the institutional animal use and care regulations of Birla Institute of Technology, Mesra, Ranchi (Institutional Animal ethical Committee of Birla Institute of Technology, Mesra, Ranchi, Approval number 1972/PH/BIT/24/17/IAEC). Male mice were housed under standard conditions with a controlled temperature of 25°C and a light/dark cycle of 12/12 hr and were divided in two groups i.e. control and test each consisting of 6 animals and hairs on the skin were completely removed using hair removal cream. The exposed skin of the animals was sterilized using povidone iodine. An incision was done on the epithelial skin and sterilized cotton ball pellet as the control and cotton ball pellet coated with solution of graft copolymer and dried and sterilized was used as the test material, were placed in the skin pockets and stitched back. After 15 days, each group was euthanized and the test and control materials were taken out and freeze dried and observed under SEM for the evidence of any cell growth on the scaffolds. Isolated liver, kidney and scaffold applied local tissue were preserved in formalin for histological procedures for determination of any toxicity. After collection, the carcasses were disposed by burial.[2]

RESULTS AND DISCUSSION

Synthesis of MOG-g-PAAM

The MOG-g-PAAM has been synthesized using microwave irradiation technique assisted by redox initiator to

speed up the reaction. Different grades of graft copolymer were synthesized varying the monomer (AM) concentration, redox initiator (APS) and the optimized grade GF4 was selected based on the % grafting efficiency (%GE).[2]

Characterizations

Intrinsic viscosity measurement

Viscosity of the all the grades of graft copolymer are shown in Table 1. The grade GF4 shows the highest intrinsic viscosity. The intrinsic viscosity of a polymeric solution is a measure of the hydrodynamic volume of the polymer in solution which in turn depends on the polymer molecular weight, its structure, nature of the solvent and temperature of the medium. For the polymers which have a low intrinsic viscosity indicates that the hydrodynamic volume is less as it may be due to the smaller branched chains and for the polymers having large intrinsic viscosity, their hydrodynamic volume is also higher as this may be due to the reason for the long linear grafted chains on to the polymer backbone. This may be an advantageous factor as more the hydrodynamic value, there may be more swelling and thus this can be an essential factor in controlling the release rate of drug from the polymeric matrix.[2]

Elemental analysis

The results of the elemental analysis of the pure MOG and that of the optimized grade MOG-g-PAAM are provided in Table 2. The absence and presence of nitrogen in the pure MOG and that of MOG-g-PAAM grafted grade GF4 respectively indicates that PAAM chains have indeed been attached to the backbone of pure MOG polysaccharide.[20]

FTIR Spectroscopy

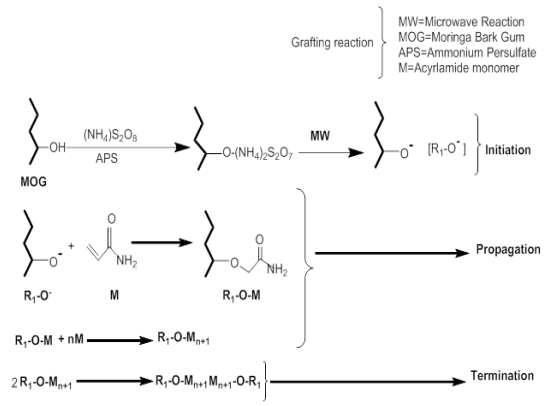
FTIR overlay spectra of MOG, AM, GF4 and PM are shown in Figure 1 and Table 3. The spectral analysis of MOG shows characteristics peaks at 3406.875 cm^{-1} for –OH stretching, 2807.8125 cm^{-1} for C-H stretching band, 1734.375 cm^{-1} C-O stretching due to acetyl, 1420.5112 cm^{-1} O-H bending due to carboxylic acid, 1623.75 cm^{-1} for C=O stretching of carboxylic acid of Glucuronic acid, strong C-O stretching at 1201.87 cm^{-1}, a medium sharp –O-H stretching band at 3679.68 cm^{-1}, at 3009.375 cm^{-1} –C-H alkene stretching.[13,26] In case of AM, the FTIR spectra show N-H stretching at 3350.625 cm^{-1} and 3200.225 cm^{-1}. Also there appears wave number at 1687.5 cm^{-1} due to the presence of secondary amide. The wave number at 1419.375 cm^{-1} may be due to the existence of the symmetrical –COO- vibrations. The spectra of GF4, shows band at 1211.0349 cm^{1} due to O–H stretching, spectra at 1414 cm^{-1} due to –OH stretching , band at 1687 cm^{-1} may be attributed to the C=O stretching of carboxylic acid of glucuronic of the MOG, band at 2807 cm^{-1} and 3000 cm^{-1} may be attributed to the C-H stretching and C-H alkene stretching respectively. Thus, this comparison between MOG, AM and GF4 indicates that grafting has occurred.[27] In case of physical mixture (PM) of drug MTZ along with the optimized powder of graft copolymer GF4 in a ratio of 1:1, all the characteristic peaks of drug MTZ and the active functional groups of graft copolymer GF4 are present as shown in Table 3, indicating that there is no evidences of drug-polymer interactions.

Thermal analysis of optimized grade of grafted co-polymer

Thermal analysis of pure monomer acrylamide (AM), pure gum (MOG) and that of graft copolymer MOG0g-PAAM (GF4) is shown in Figure 2. The thermogravimetric curve of pure AM shows four steps of degradation, with major degradation occurring at above 240°C and this is mainly due to the loss of ammonia with the formation of imide group via cyclisation.[27] The decomposition of the cyclized product only occurs after 380°C. After 380°C, the weight loss was found to be major showing 45%. In case of pure gum MOG, there appears major degradation at 230-325°C with % weight loss of 48%, indicating polysaccharide decomposition. In case of graft copolymer MOG-g-PAAM (GF4), there are three stages of degradation starting at higher temperature than in comparison to pure MOG and AM, with only 4% weight loss at temperature range from 220-260°C, while the second starts at 260-315°C showing weight loss of 13%, which may be due to the

Table 1: Composition table for synthesis of Moringa bark gum grafted with acrylamide (MOG-g-PAAM)

Grades Code	Wt.(gm) MOG	Wt.(mg) acrylamide	Wt.(mg) APS	% Yield	%Grafting	%Grafting efficiency (%GE)	Intrinsic viscosity(cP)
GF1	500	5	100	77.393	766.8	76.68	9.99
GF2	500	5	125	82.506	828.2	82.82	25.2
GF3	500	5	150	81.487	820.8	82.08	21.92
GF4	500	7	125	94.59	1342.6	95.9	35.3



Scheme 1: Reaction Mechanism of formation of graft-copolymer of *Moringa* bark Gum (MOG) with Acrylamide. Ammonium persulphate (NH4)2S2O8) is used as initiator in the initiation step leading to the formation of free radical on the Polymer backbone of MOG and the monomer Acrylamide attaches to the free radical sites on to the *Moringa* gum and further propagates to form Polyacrylamide grafted copolymer in the Propagation step and later terminated by addition of acetone in the termination step

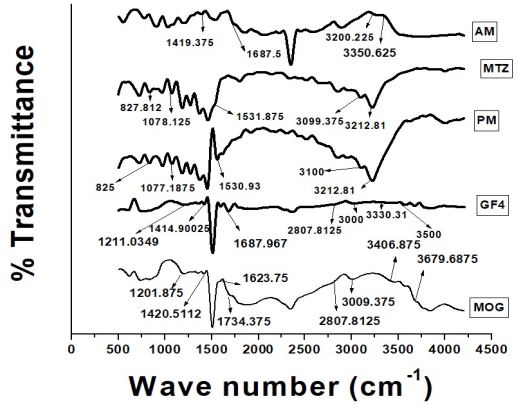


Figure 1: Overlay FTIR spectra of MOG(Moringa Bark gum) GF4 (MOG grafted Acrylamide graft copolymer), MTZ(Pure drug Metronidazole, PM(Physical Mixture of MTZ with GF4), AM (Acrylamide Monomer).

degradation of the amide group because of the grafting process and the last stage ranges more than 355°C, which may be due to the degradation of the polysaccharide backbone. This result indicates that the pure MOG after graft copolymerization has increased its stability, thereby making the polymer suitable for the controlled device.

X-Ray Diffraction

The XRD diffraction pattern of pure MOG shows less broadened peak, indicating amorphous nature as shown in Figure 3.[28] Whereas the diffraction pattern of acrylamide monomer shows very sharp peaks, indicating crystalline nature of the compound. But, after grafting process of the pure MOG with AM, the optimized grade GF4 reveals amorphous nature. Moreover, the diffraction patterns of MTZ as well as the physical mixture of GF4 along with MTZ indicates that there is no drug polymer interaction as the crystalline nature of the drug has been retained in the physical mixture even after combined with the graft copolymer physically.

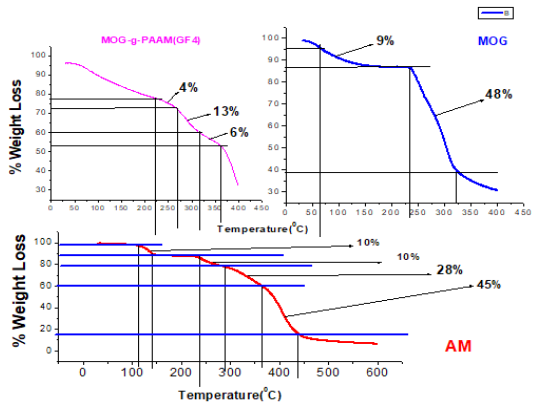


Figure 2: Thermal analysis of MOG, AM and Graft copolymer MOG-g-PAAM (GF4).

^{13}C Solid state nuclear magnetic resonance (^{13}C NMR)

The ^{13}C-NMR resonance of mucilage, revealed the peaks as shown in Figure 4a and b and Table 4. There is a small downfield shift of all the backbone carbons of the polysaccharide in the copolymer in comparison to those of the parent, except for C-1, there is a upshift of 4.529 ppm in the graft copolymer and this may be due to the introduction of the acrylamide moiety onto the polysaccharide.[29]

Surface morphology

Scanning electron microscopy of pure MOG as shown in Figure 5a, exhibits granular surface whereas in case of graft copolymer MOG-g-PAAM (GF4) as shown in Figure 5b, the material has turned to be more fibrillar which may be due of grafting reaction of PAM chains attaching on to the polysaccharide backbone. Thus, the material has become more efficient than in comparison to pure natural MOG and can be easily utilized as polymeric scaffold for various biomedical applications.

Determination of Drug Release and release Kinetics

The results of dissolution studies of all four formulations were carried out for 25 hr and are graphically represented in Figure 6. The release kinetics results are shown in Table 5. The cumulative percentage release vs. time plot for all formulations showed that the drug release from formulations coded GF1 and GF3 was more rapid than GF2 and GF4. It might be due to higher grafting efficiency of GF2 and GF4. From Table 5, it is quite evident that GF1 and GF3 follows Higuchi

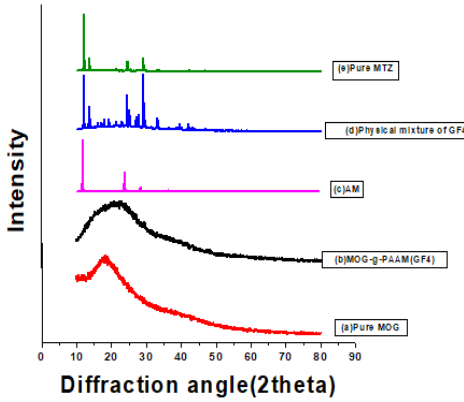


Figure 3: X-ray diffraction pattern of (a) Pure Moring Bark guk (MOG (b) Graft copolymer MOG-g-PAAM (GF4), (c) Pure Acrylamide (AM) (d) Graft copolymer MOG-g-PAAM (GF4) (d) Physical mixture of GF4 with pure durg MTZ (e) Pure Drug MTZ.

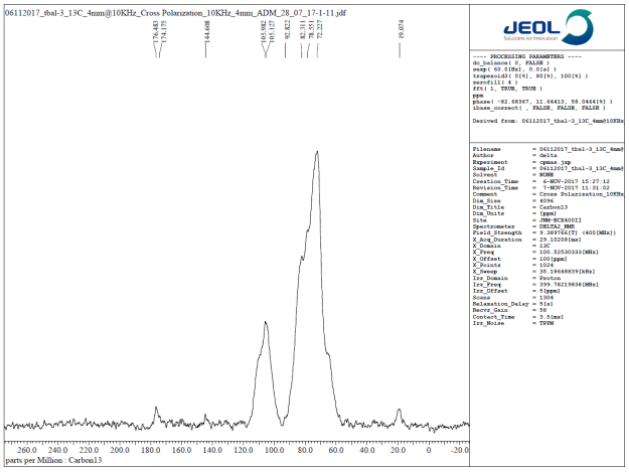


Figure 4a: ^{13}C NMR of Pure *Moringa* Bark Gum (MOG).

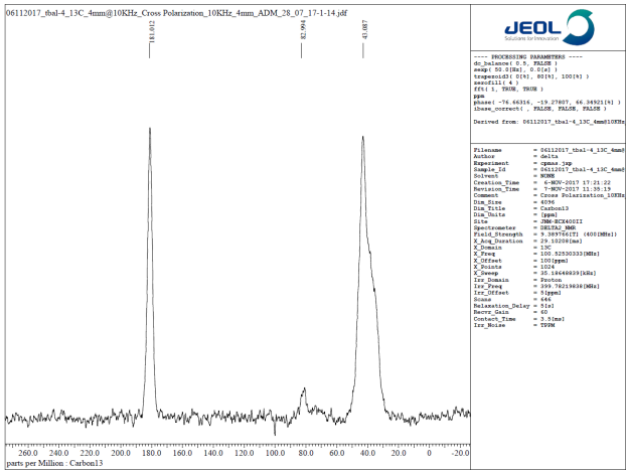


Figure 4b: ^{13}C NMR of Graft Copolymer MOG-g-PAAM (GF4).

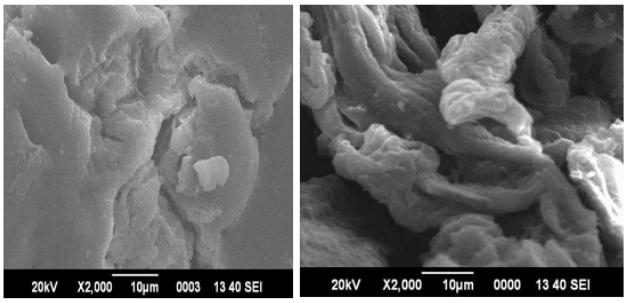


Figure 5: Scanning electron micrographs of: (a) MOG, (b) MOG-g-PAAM.

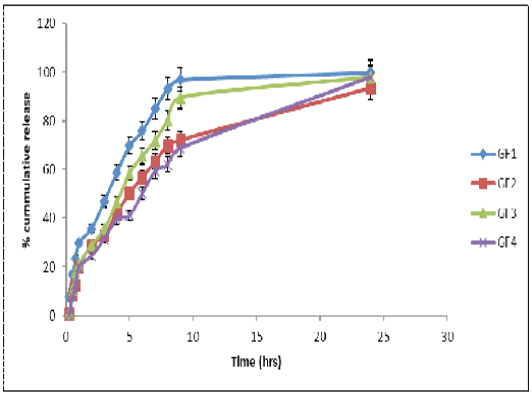


Figure 6: Drug release profile of different grades of graft co-polymers GF1-DF4.

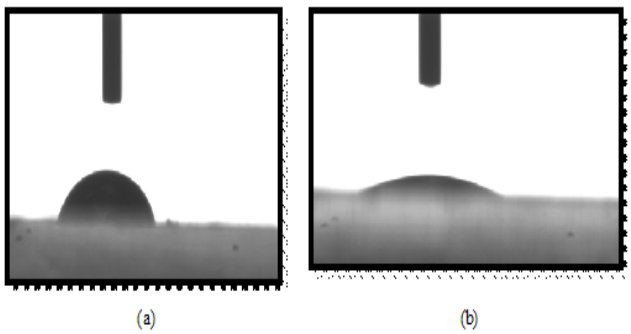


Figure 7: Optical image of: (a) MOG, (b) MOG-g-PAAM.

model and GF2 and GF4 follows Korsmeyer's Peppas model. The optimized sample (GF4) follow the Korsmeyer's release with R^2 value of 0.9815. Moreover, the drug release from GF4 showed 95% release for a period of 24 hours duration with non-fickian transport.

Optical contact angle analysis of optimized grade of grafted co-polymer

The measurement of the contact angle is an important characterization parameter for detection of the hydrophilicity of surfaces of biomedical devices, since these materials are designed to be used in biological environment. As shown in Table 6 and Figure 7, pure MOG shows more contact angle with very less surface energy, whereas in case of graft copolymer MOG-g-PAAM, the contact angle decreases with the increase in surface energy, this may be attributable that after grafting there appears more hydrophilic sites on MOG, thereby making the material more hydrophilic in nature.[24]

Table 2: Elemental Analysis of grafted formulation (GF4) and native gum (MOG)

MOG				MOG-g-PAAM (GF4)			
%C	%H	%N	%S	%C	%H	%N	%S
34.78	7.668	0.000	0.233	39.43	8.153	14.65	0.215

Table 3: FTIR Spectra and their interpretations.

Compound	Wave Number (cm^{-1})	Functional Groups
MOG	3679.68	Medium Sharp O-H stretching
	3406.87	-OH stretching
	3009.37	-CH alkene stretching
	2807.81	-CH aldehyde
	1623.75	-C=O stretching of glucuronic acid
	1734.37	-C=O stretching due to acetyl group
	1420.51	-OH bending due to carboxylic group
	1201.87	Strong –CO stretching
AM	3350.62	-NH stretching
	3200.22	-NH stretching
	1687.50	Secondary amide
	1419.37	-COO- symmetrical vibrations indicating polyacrylamide hydrolysis
GF4	3500.00	Strong broad –OH stretching of Gum
	3330.31	Strong broad –OH stretching
	3000.00	-CH alkene stretching
	2807.81	-CH aldehyde stretching
	1687.96	Secondary amide
	1414.90	Symmetrical -COO- vibrations of the polyacrylamide hydrolysis
	1211.03	-OH band
MTZ	3212.81	-OH stretching
	3099.37	-CH stretching of alkene of GF4
	1531.87	Presence of NO_2, N-O stretching
	1078.12	-C-OH, -C-O stretching
	827.81	-C-NO_2 and -C-N stretching
PM	3212.81	-OH stretching
	3100.00	-CH stretching alkene of the GF4
	1530.93	Presence of NO_2, N-O stretching
	1077.18	-C-OH, -C-O stretching
	825.00	-C-NO_2 and -C-N stretching

Bacterial Biodegradability Study

The biodegradability studies reveal that there was initial increase in weight of GF4 on day 2 which may be due to soaking of water from the media. Later, there was continuous decrease in weight for 12 days due to degradations of GF4 in presence of *B. subtilis*. Polynomial degradability rate was observed and weight loss might be due to loss of carbon dioxide and nitrogen from the acrylamide moiety from optimized sample GF4 as shown in Figure 8.

Hemocompatibility studies

According to the ISO 10993-4, biomaterials are considered to be nonhemolytic if the hemolytic index is less than 2%, slightly hemolytic if between 2% and 5% and hemolytic if that value is over 5%. The degree of hemolysis for GF4 was calculated to be 1% which is less than 2% as shown in Table 7. Thus, it is concluded that GF4 is nonhemolytic and could be used for biomedical applications.[30]

Acute oral toxicity studies and histopathological studies

Oral administration of mice with grafted co-polymer (MOG-g-PAAM) with single dose of 2000mg/kg body weight had no toxic effect. No death occurred during 14-day observation period and no toxic response was found in mice. The animals displayed full of energy, normal behavior and free movement and the mice were sensitive to sound, light and other stimulations. They had no salivation or vomit, no mouth or nose dryness

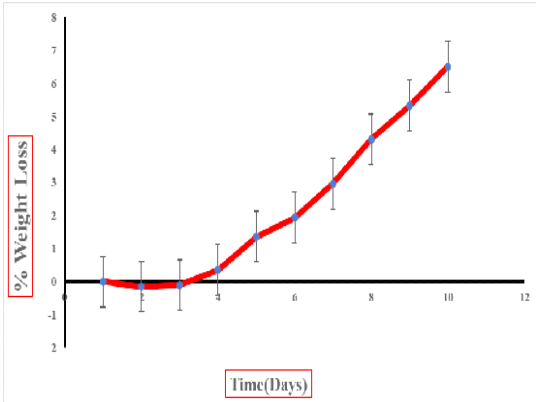


Figure 8: *In vitro* bacterial biodegrad ability studies.

Table 4: ^{13}C-NMR.

Pure Polysaccharide (MOG)	Functional groups	Graft copolymer MOG-g-PAAM (GF4)	Functional groups
176.483	Carbonyl Group	181.012	Amide
174.175	Carbonyl group		
144.608	Aromatic carbon		
105.982	Anomeric carbon		
105.127	Anomeric carbon		
92.822	Secondary alcohols	82.994	Secondary alcohols
82.311	Secondary alcohols	43.087	Alkanes
78.551	Secondary alcohols		
72.227	Alkanes		

Table 5: *In vitro* release kinetics.

Grades	Zero order		First order		Higuchi kinetics		Korsmeyer's equation	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
GF1	3.685	0.6944	0.130	0.35	22.05	0.901	2.0518	0.744
GF2	2.696	0.7489	0.133	0.4342	15.77	0.9294	1.88	0.931
GF3	3.232	0.8033	0.157	0.44	18.606	0.9649	2.2078	0.943
GF4	1.809	0.7419	0.069	0.5641	10.67	0.9362	0.8749	0.981

Table 6: Optical contact angle analysis.

Sample code	Theta	SE [mN/m]
MOG-g-PAM	23.18	67.36
MOG	79.03	36.07

or edema, no running nose or eye secretion. The form, color and shell of excreta (feces, urine) were normal.

There were no significant histopathological changes in orally administered graft copolymer GF4 (MOG-g-PAAM). The light microscopic image of liver treated with GF4 and control are shown in Figure 9A and B showing normal hepatocyte array.[31] No hepatocellular degeneration or necrosis was observed. From light micrograph of test and control mice kidney, as shown in Figure 9C and D, it is evident there no degeneration, bleeding and necrosis when compared to control, with evidence of presence of distal tubules, bowman's space and proximal tubules.[32] Thus, these results indicate that the graft copolymer GF4 do not pose any toxicity to liver and kidney.

However, in this article, it can be visualized that our material GF4, is a versatile material having broader application. This novel material has potential to be applied as biodegradable oral drug delivery devices as well. The applicability of this material needs to be explored widely. In-depth toxicity study and applicability in some higher animal models like dog, monkey and human need to be explored. Full phase clinical trial need

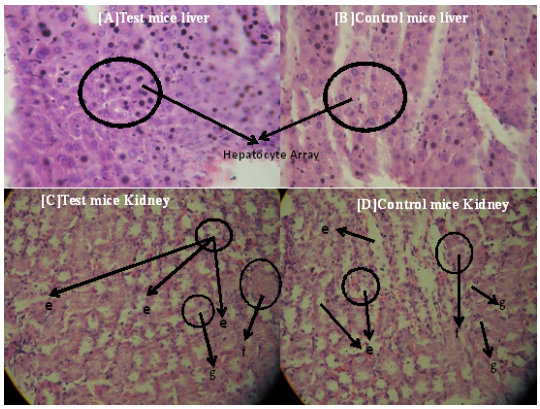


Figure 9: [A] and [B] Photomicrographs of histology of Test and control mice liver area stained with hematoxylin and eosin on day 15 after surgery captured by Leica Microscope at 40X respectively shows that the hepatocyte array is complete in both cases and in [C] and [D] Photomicrographs of histology of test and control kidney, icons representing e, f and g represent distal tubules, Bowman's space and Proximal tubules respectively.

be observed to check the effect of material in the long run.

Assessment of response of graft copolymer scaffold towards mice tissue and Histological investigations

After a period of 15 days, efficient healing was observed evidenced by presence of hair follicles and presence of mature fibrous tissue. There is a clear indication towards the facility of moist environment at the site of wound which prompted rapid epithelization. The scanning

Table 7: Hemocompatibility study of GF4.			
Sample name	**Contents**	**Absorbance at 540nm**	**Degree of hemolysis (%)**
Positive control	2% RBC suspension, 10% TRX and 0.9% NaCl solution	0.111	1 %
Negative control	2% RBC suspension and 0.9% NaCl solution	0.015	
Test	2% RBC suspension, GF4 (40mcg/ml) and 0.9% NaCl solution	0.016	

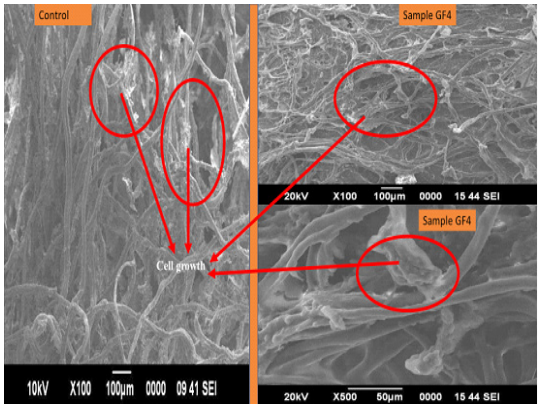


Figure 10: SEM image of tissue growth on control as well as graft Copolymer GF4.

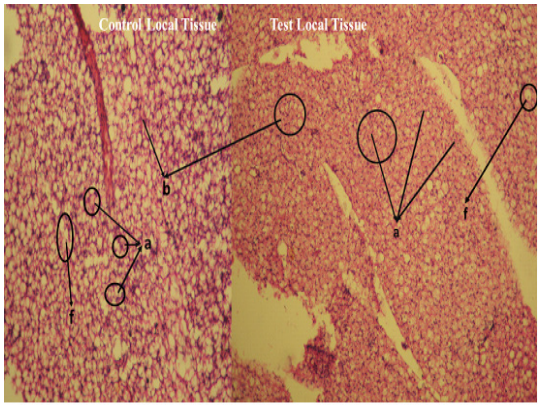


Figure 11: Photomicrographs of histology of control local tissue area and test tissue stained with hematoxylin and eosin on day 15 after surgery captured by Leica Microscope at 40X resoectuvely and a, b and f represent spondle shaped figreblst, cpllagen and foreign body giant cells.

electron microscopy of the control cotton ball and the test cotton ball are shown in Figure 10 to detect the growth of cells on to the polymer which can be used as tissue scaffold. The figure indicate that the cells have proliferated and differentiated onto the cotton ball as well as onto the cotton ball coated with GF4[33] which also suggested that the inserted material are highly biodegradable in nature.

The histology slide of the local tissue for both control as well as test animal was investigated for detection of any obvious toxicity as shown in Figure 11, from which it is quite evident that denser growth of spindle shaped fibroblasts[34] and collagen was observed in case of test tissue than in comparison to control tissue.[33] The increased quantity of these cells in the local test tissue indicates that wound healing is rapid as collagen enhances the growth of extracellular matrix and thus its increase indicates rapid wound healing in the local test tissue. Foreign body giant cells observed in minor quantity in case of test shown in Figure 11, an inflammatory response is most common at the interface of both tissue and scaffold which is previously reported in several literature.[35,36] These results ensure the non-toxic nature of the graft copolymer GF4 as well as its efficiency to be utilized as a template material for tissue growth respectively.

CONCLUSION

From the forgone discussion, it is concluded that the microwave irradiated graft copolymer GF4 proves to be an effective controlled delivery polymeric system. The intrinsic viscosity measurements indicated the sample being more swellable due to presence of longer chains. The various analytical characterizations using C13-NMR, TGA, FTIR of the pure MOG as well as GF4 indicates that grafting has occurred successfully and this was further confirmed by the elemental analysis which evidenced the presence of significant elements mainly nitrogen on GF4, as pure MOG was devoid of any nitrogen. Thus, all the analytical studies confirmed that efficient grafting has taken place. Also, the optical contact angle results indicated that the newly formed graft copolymer GF4 shows better hydrophilic properties as compared to the pure MOG, which may be due to the inclusion of monomer acrylamide (AM) onto the polymer backbone. Further, the bacterial biodegradability studies indicated the graft copolymer being highly biodegradable and thus it may be concluded that the material is environment friendly. Also, the drug release from all the grades of graft copolymer indicated that the release took place for more than 24 hr and optimized grade GF4 exhibited highest release with anomalous non-fickian diffusion release kinetics. The oral toxicity studies showing histology of liver and kidney indicates the non-toxicity of the test material. Also the *in-vitro* tissue growth on the graft copolymer sample was suc-

cessful and photomicrographs of histology slides of the local tissues indicated the sample being non-toxic. Thus, all these versatility makes the material sufficiently suitable as controlled release polymeric matrix as well as tissue engineering scaffold.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

PAM: Polyacrylamide; **MOG:** *Moringa* Bark Gum; **AM:** Acrylamide; **APS:** Ammonium per sulfate; **NMR:** Nuclear magnetic resonance; **FTIR:** Fourier Transform infrared spectroscopy; **XRD:** X ray diffraction; **PM:** Physical mixture of drug with the graft copolymer(MOG-g-PAM); **MTZ:** Metronidazole.

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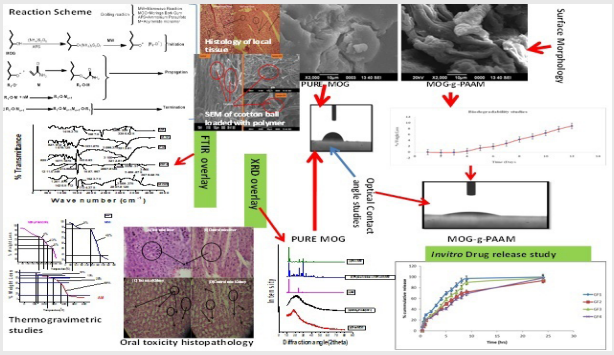
SUMMARY

The use of natural polysaccharides for the preparation of drug delivery system is advantageous as they are inexpensive and abundantly available and at the same time biodegradable in nature. But these natural polysaccharides are unstable in nature and thus grating with different types of synthetic monomers makes them suitable for use as more stable polymeric system for release of drugs. Thus, the present study is mainly focused on the preparation of different grades of graft copolymer of Moringa bark gum (MOG) with acrylamide (AM) as the monomer and ammonium per sulfate as the redox initiator in presence of microwave irradiation to obtain Polyacrylamide grafted Moringa bark gum graft copolymer (MOG-g-PAM). The optimization of the best grade GF4 was done using percentage grafting efficiency(%GE).To get a better confirmation on the polymer chain length, intrinsic viscosity studies of all the grades showed that GF4 has highest intrinsic viscosity, which is mainly attributed for the presence of longer polymer side chains on GF4 which increases the hydrodynamic volume and thus makes it better candidate for controlled release. The analytical characterization of GF4 using Fourier Transform Infrared spectroscopy (FTIR), Nuclear magnetic spectroscopy (NMR) and X-ray diffraction (XRD) indicated that grafting of the acrylamide onto the backbone of the Moringa bark gum was successful. The comparison of thermogravimetric analysis (TGA) of GF4 with pure MOG, and AM indicates that optimized sample GF4 is thermally more stable than in comparison to pure MOG. Also the optical contact angle of GF4 shows less contact angle with increase in surface energy when compared to that of pure MOG. The hemocompatibility and biodegradation studies indicated GF4 to be highly biodegradable and hemocompatible. Histology slides of liver and kidney for oral toxicity studies indicated the material is non-toxic. The assessment of tissue proliferation on GF4 as polymeric scaffold was done by use of Scanning electron microscopy which showed that numerous cells proliferated on the scaffold. Moreover, the assessment of local tissue toxicity, where the polymer sample was inserted showed numerous collagen cell with fibroblasts, indicating wound healing process. The drug release from the polymeric system showed controlled release with 95% drug release for 24hours with non-fickian transport with value of n = 0.87.Thus the material can be used for both drug delivery systems effectively.

PICTORIAL ABSTRACT



About Authors

Nikhil Rai, Department of Pharmaceutical Sciences and Technology, BIT, Mesra, Ranchi.

Trishna Bal, Department of Pharmaceutical Sciences and Technology, BIT,Mesra, Ranchi.

Sabyasachi Swain, Department of Pharmaceutical Sciences and Technology, BIT, Mesra, Ranchi.

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