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Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery

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ABSTRACT

Acrylamide grafted copolymer of locust bean gum was prepared by microwave irradiation using ceric ammonium nitrate as redox initiator. The grafting process was optimized in terms of irradiation time, amount of initiator and acrylamide by using constant amount of native locust bean gum. The grafted gum was characterized by Fourier transform infrared spectroscopy (FT-IR), ^{13}C nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), X-ray diffraction study (XRD), differential scanning calorimetry (DSC), elemental analysis, contact angle, viscosity, molecular weight, swelling and biodegradability studies. The grafted gum was found to be biodegradable and non-toxic. It was further used to prepare controlled-release matrix tablet of buflomedil hydrochloride. The in vitro release profile of the tablet showed the rate controlling property of acrylamide grafted locust bean gum was similar to that of hydroxypropyl methylcellulose (HPMC-K15M).

1. Introduction

Biopolymer grafting and its application have gained a great attention in drug delivery arena. The polymer is essentially modified to tailor to target drug delivery applications, in conjunction with the aim to increase therapeutic effects, reduce adverse effects, improve patients' compliance and develop new therapeutic strategies. Natural polymers are preferred for medical application over synthetic polymers because of their biodegradability, low cost, easy availability and non-toxicity (Bhardwaj, Kanwar, Lal, & Gupta, 2000; Vijan, Kaity, Biswas, Isaac, & Ghosh, 2012). However, they possess drawbacks such as uncontrolled hydration, microbial contamination, and drop in viscosity during storage. The properties of natural polymers can be modified through hybridization with the synthetic polymers via blending, grafting, and curing.

'Blending' refers to physical mixing of two (or more) polymers to obtain the requisite properties. 'Grafting' is a method where monomers are covalently bonded onto a parent polymer chain. Curing proceeds with polymerization of an oligomer mixture to form a coat which adheres to a substrate by physical forces (Bhattacharya & Misra, 2004). A graft copolymer is a macromolecular chain with one or more species of block connected to the main chain as side chain(s). Thus, it can be described as, having the general structure, where the main polymer backbone, commonly referred to as the trunk polymer, has branches of another polymeric chain emanating from different points along its length (Zohuriaan-Mehr, 2005).

The Ce (IV) induces graft copolymerization of vinyl monomers onto polysaccharide substrates (Adhikary, Tiwari, & Singh, 2007; Mishra & Bajpai, 2006). The main constraint of graft copolymerization is the formation of concurrent homopolymer thereby resulting in low grafting yield. Apart from the redox initiator-induced graft copolymerization, microwave-assisted graft copolymerization has also been employed. The microwave irradiation is characterized by rapid transfer of energy in the bulk of a reaction mixture. The microwave-assisted graft copolymerization requires a very short reaction time and proceeds even in the absence of any redox initiator (Singh, Sethi, Tewari, Srivastava, & Sanghi, 2003).

Natural gums are polysaccharides consisting of multiple sugar units linked together to create large molecules. Gums are frequently produced by higher plants for protection against injury. They are heterogeneous in composition. Upon hydrolysis, they yield simple sugar units such as arabinose, galactose, glucose, mannose,

Abbreviations: LBG, locust bean gum; Am-g-LBG, acrylamide grafted locust bean gum; Am, acrylamide; CAN, ceric ammonium nitrate; BH, buflomedil hydrochloride; HPMC, hydroxypropyl methylcellulose; FTIR, Fourier transform infrared; XRD, X-ray diffraction; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; KBr, potassium bromide; UV–vis, ultraviolet–visible; C, carbon; N, nitrogen; O, oxygen.

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Table 1
Synthesis details of acrylamide grafted LBG.

Batch code	Amount of LBG (g)	Amount of CAN (mg)	Irradiation time (min)	Amount of Am (g)	%GE	Elemental analysis		
						%C	%H	%N
G1	1	300	5.0	5.0	84.806	45.94	6.341	14.94
G2	1	300	5.0	10	98.356	45.59	6.495	16.41
G3	1	300	2.5	5.0	90.946	45.91	6.408	15.13
G4	1	300	2.5	10	101.083	45.87	6.407	16.87
G5	1	150	5.0	5.0	73.314	44.27	6.199	13.65
G6	1	150	5.0	10	87.841	46.15	6.704	16.47
G7	1	150	2.5	5.0	78.304	45.38	6.528	14.92
G8	1	150	2.5	10	91.461	47.33	6.847	16.40
LBG	–	–	–	–	–	40.74	5.778	0.942

xylose or uronic acids (Rana et al., 2011). Locust bean gum (LBG) is a high molecular weight branch polysaccharide and is extracted from the seeds of carob tree *Ceratonia siliqua*. It is a non-starch polysaccharides consisting of galactose and mannose in the ratio of 1:4 and hence they are known as galactomannan (Parvathy, Susheelamma, Tharanathan, & Gaonkar, 2005). LBG consists of a (1,4)-linked β-D mannopyranose backbone with branch points from their 6-positions linked to α-D-galactose. The mannose elements from a linear chain linked with branched galactopyranosyl residues at varying distance of parent chain as a function of the plant origin (Sharma, Dhuldhoya, & Merchant, 2008). The molecular weight of LBG ranges between 300,000 and 1,200,000 Da. It is less soluble in water and needs heating to dissolve. Being non-ionic, its aqueous solubility is not affected by pH or ionic strength of the liquid medium. LBG is dispersible in either hot or cold water, forming a sol having a pH of 5.4–7.0, which may be converted to a gel by the addition of small amounts of sodium borate.

With a view to prepare value added products, the exploitation of chemically modified LBG is attractive. So far, microwave assisted grafting has been widely applied in processing of a variety of polysaccharides namely gellan gum (Vijan et al., 2012), alginate (Sen, Singh, & Pal, 2010), xanthan gum (Kumar, Singh, & Ahuja, 2009), artemisia seed gum (Zhang, Zhang, Yuan, & Wang, 2007), cashew gum (Silva, Paula, & Feitosa, 2007), guar gum (Singh, Tiwari, Tripathi, & Sanghi, 2004) and many more. However, microwave assisted grafting of LBG is till not reported. Recently, we have investigated interpenetrating polymer network of LBG and poly (vinyl alcohol) as a mode to modify the property of native LBG for controlled-release drug delivery application (Kaity, Isaac, & Ghosh, 2013). So, as a continuation of the previous work, we herein present the work on microwave assisted synthesis, characterization and application of acrylamide grafted locust bean gum (Am-g-LBG) for controlled-release drug delivery. The grafted gum was used for controlled delivery of highly water-soluble drug, buflomedil hydrochloride (BH) with the aim to reduce its dose and dose related toxicities. The usual oral dose of BH is 300–600 mg/day and its plasma half life is 2–3 h (Dubourg & Scamuffa, 1981).

2. Materials

Locust bean gum (LBG) was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India. Ceric ammonium nitrate (CAN) and acrylamide (Am) were obtained from Merck Specialties Private Limited, Mumbai, India. Buflomedil hydrochloride was obtained as gift sample from Fresenius Kabi Oncology Limited (Kalyani, West Bengal, India). Acetone (density = 0.789–0.791 g/mL) was bought from Rankem India Private Limited, Mumbai, India. All other chemicals used were of reagent grade and were used as supplied. Throughout the experiment, Millipore water was used.

3. Methods

3.1. Preparation of acrylamide grafted LBG

The graft copolymer derived from Am and LBG was prepared by free radical induced, microwave-assisted polymerization method (Vijan et al., 2012). Briefly, a specified amount of acrylamide (Table 1) was mixed with 30 mL of water and added to aqueous dispersion of LBG (0.0083 g/mL) and stirred for about 1 h. A specified amount of CAN (Table 1) was dissolved in 30 mL of water and added to the above dispersion. The dispersion was irradiated by microwave (laboratory scientific microwave system, Catalyst systems, India) at 480 W for different time periods using alternate one minute heating and one minute cooling cycle. The irradiated sample was then left overnight at ambient temperature and then precipitated using acetone. Ethanol (anhydrous) was used to remove the unreacted acrylamide monomer from the precipitate. The precipitate was further washed with 30% aqueous ethanol to remove unreacted homopolymer and other reagents. The grafted gum thus prepared was vacuum dried at 40 °C to a constant weight and converted to fines. The grafting efficiency (GE) was calculated using following equation (Athawale & Lele, 1998):

$$\mathrm{GE}\,(\%) = \left(\frac{W_1 - W_0}{W_2}\right) \times 100$$

where, W_0, W_1 and W_2 denote the weight of LBG, graft copolymer and Am, respectively.

3.1.1. Full factorial design

Factorial design is very widely used experimental design in which different levels of a given factor are combined with all levels of every other factor in the experiment. In the present study, two-level, three-factor, full factorial design (8 batches) was used for the optimization of acrylamide grafting onto LBG. The amount of acrylamide, ceric ammonium nitrate and microwave exposure time were selected as the independent variables and percentage of nitrogen (%N) in grafted gum and its viscosity and contact angle were selected as the responses. The %N was expected to increase in modified gum due to high nitrogen content acrylamide grafting. The grafting of amide onto LBG was envisaged to be accompanied by changes in viscosity and hydrophilicity of the native gum.

Each independent variable was investigated at two levels, high level (+1) and low level (−1). Polynomial models including interactions and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA) approach. The results (Tables 1 and 2) of response generated using the experimental designs were analyzed by factorial models using Design Expert software (Version 7.0.0, Stat-Ease, Inc., Minneapolis).

Table 2
Molecular weight, viscosity, contact angle, surface energy and swelling characteristics of Am-g-LBG and native LBG.

Batch code	Molecular weight ($\times 10^6$ g/mol)	Viscosity @ 100 rpm (poise)	Contact angle (°)	Surface energy (mN/m)	% equilibrium swelling	
					pH 1.2	pH 6.8
G1	2.9387	1.13	57.11	48.29	161.85	234.72
G2	4.7567	0.64	55.08	51.03	217.59	275.18
G3	3.8330	0.71	58.52	48.69	192.63	251.43
G4	5.7347	0.90	45.19	56.44	221.27	283.47
G5	11.0740	1.03	58.39	48.73	145.82	209.83
G6	8.6189	0.64	56.49	49.89	174.11	246.61
G7	7.9606	1.01	60.24	47.61	152.35	227.55
G8	11.3490	0.67	50.16	53.60	206.37	265.82
LBG	12.3820	2.78	65.28	44.62	139.33	141.21

3.2. Characterization of grafted gum

3.2.1. Fourier transform infrared spectroscopy

FTIR was performed to characterize the presence of specific functional groups in the materials. 0.5–1 mm thick films of LBG and all graft copolymers were prepared and analyzed by ATR-FTIR using transmittance mode. Infrared spectra were obtained in the range of wave number from 4000 to 650 cm^{-1} during 64 scans, with 2 cm^{-1} resolution (FTIR-8400S, Shimadzu, Japan).

3.2.2. Solid state ^{13}C NMR spectroscopy

A Bruker AMX 300 spectrophotometer (Germany) operating at 75 MHz was used for ^{13}C solid state NMR analysis of LBG and Am-g-LBG. Approximately 300 mg of samples were inserted into the ceramic rotor of NMR spectrophotometer.

3.2.3. Scanning electron microscopy

The morphology of the LBG and Am-g-LBG was examined using a SEM system (JMS-6390, JEOL, Tokyo). The samples were gold coated to increase the conductivity of the electron beam. An accelerating voltage of 10 kV and a working distance of 12 mm were used.

3.2.4. Powder X-ray diffraction

X-ray powder diffractometry (PXRD) of native gum and grafted gum were recorded using X-ray diffractometer (Bruker AXS D8 Advance, Bruker, Germany). The X-ray source was Cu, with wavelength 1.5406 Å and Si (Li) PSD detector employed. The diffractometer was run at a scanning speed of 2°/min, a chart speed of 2°/2 cm per 2θ and an angular range fixed between 3° and 80°.

3.2.5. Differential scanning calorimetry

DSC thermograms of LBG and different batches of Am-g-LBG were obtained using DSC-60 (Shimadzu, Japan) via heating 3–5 mg samples from 10 °C to 300 °C under nitrogen purge (50 mL/min) at a heating rate of 10 °C/min.

3.2.6. Elemental analysis

The elemental analysis of native gum and all the graft copolymers (Am-g-LBG 1 to Am-g-LBG 8) was performed by using a PCHN 2400 microanalyser (PerkinElmer, USA). The carbon, hydrogen and nitrogen contents were calculated.

3.2.7. Contact angle and surface energy measurement

Wetting ability of native LBG and Am-g-LBG was determined by contact angle measurement, using a video based contact angle metre OCA 20 (DataPhysics, Germany), attached to a camera. Before measurement, the samples were prepared by casting 1% (w/v) aqueous solution on glass slides and vacuum drying at 45 °C. The wetting liquid used was Millipore grade distilled water (liquid surface tension (γ_1) = 72.8 mJ/m^2). The contact angle value was the average of five replicates. Surface energy was calculated using equation of state, Schultz Method-2, by means of Data Physics SCA20 software (Version 2.01).

3.2.8. Viscosity measurement

The viscosity of LBG and Am-g-LBG (2% (w/v) aqueous solution) was determined by a programmable Brookfield viscometer (Model: Brookfield CAP-2000+ viscometer, Brookfield Engineering Labs, Inc., Middleboro, MA, USA) at 32.7 °C. The samples were dissolved in water (for native gum, sample was heated at 80 °C) and conditioned at 32.7 °C. The spindle (Spindle no. S-01) was rotated at varying rpm and the corresponding shear rate, shear stress and viscosities were recorded.

3.2.9. Molecular weight analysis

The molecular weight of sample was determined using gel permeation chromatography technique (1100 series, Agilent Technologies, Germany) by means of a refractive index detector. A PL aquagel-OH mixed column (7.5 mm × 300 mm; 8 μm; Agilent Technologies, United Kingdom) was used with mobile phase consisted of 0.1% (w/w) sodium azide (Ajax Finechem, Australia) dissolved in de-ionized water. The flow rate of mobile phase and column temperature was kept at 0.5 mL/min and 30 °C respectively. Dextrans (Sigma Aldrich, Germany) with molecular weights of 150,000, 410,000, 670,000, 1,400,000 and 2,000,000 Da were used as standards. 1 mg/mL of standard or sample solution was filtered through a cellulose nitrate membrane (pore diameter = 0.45 μm, Sartorius, Germany) before analysis. At least triplicates were carried out for each batch of sample and the average results were reported.

3.2.10. Swelling study

Equilibrium swelling measurements of both LBG and different grades of Am-g-LBG were performed in two different media. A small, previously weighted piece of the material (W_1) was immersed in 50 mL buffer (pH 1.2 and pH 6.8) and left to swell for 2 h. Then, the swollen piece was recovered and the excess water was removed carefully with tissue paper and reweighed (W_2) to an accuracy of ±0.01 mg on an electronic microbalance (Mettler, model AT120, Greifensee, Switzerland). The swelling characteristics of sample were calculated as:

$$\text{Swelling index} = \left[\frac{W_2 - W_1}{W_1}\right] \times 100$$

where, W_2 and W_1 are the swollen and dry weights of the native gum/Am-g-LBG, respectively.

3.2.11. Acute oral toxicity study

Acute oral toxicity study of Am-g-LBG was performed as per the "Organization of Economic Co-operation and Development (OECD) guideline for the test of chemicals" 425, adopted "17 December 2001". Five nulliparous and non-pregnant five weeks old female mice (Swiss albino strain) were taken for this study. The study protocol was prior approved by the Animal Ethics Committee (CPCSEA

approval no.: 621/02/ac/CPCSEA) of Birla Institute of Technology, Ranchi, India. Mice were housed in polycarbonate cage with food and deionized reverse osmosis water ad libitum at 20–25 °C and 40–70% relative humidity in a 12 h light/dark cycle. A single dose of 2000 mg/kg body weight of Am-g-LBG was administered by gavages using a stomach tube to the first animal. The same dose was administered to the remaining four animals after survival of the first animal. The animals were kept under the continuous observation up to 4 h after dosing. The observation was continued up to 14 days. The mortality rate was evaluated by visible observation and reported accordingly (Vijan et al., 2012). Serum biochemical studies on 30th min, 4th h, 1st, 3rd, 7th and 14th day were performed. The animals were sacrificed on the 15th day and histopathological studies on liver, kidney, lung and stomach were performed.

3.2.12. Biodegradability study

Sample films of Am-g-LBG (5% gum solution was casted on petri dish and dried) was inoculated with *Aspergillus niger* on a medium and incubated at ambient temperature (25–37 °C) for 21 days. Samples were cut (2.5 cm × 2.5 cm) and faced on the surface of mineral salts agar in a Petri dish containing no additional carbon source. Before placing the samples, agars surfaced were cultivated with *A. niger* from tapioca slices. Thereafter, the films were examined for evidence of colony growth (Vijan et al., 2012).

3.3. Preparation of controlled-release tablets of BH

The optimized grafted gum (300 mg) was taken for the preparation of BH (300 mg) tablet. The grafted gum was first ground to fines and mixed with PVP-K 30 (120 mg) and BH (300 mg). The blend was passed through 22 mesh size sieve. The blend was lubricated with talc (15 mg) and magnesium stearate (15 mg). The tablets were compressed at an average weight of 750 mg using a rotary tablet machine with 13 mm single punch diameter (Cadmach, Ahmadabad, India). Hardness was found to be in the range of 4–5 kg/cm^2. Similar formulation was prepared by replacing Am-g-LBG with a standard rate controlling polymer, hydroxypropyl methylcellulose (HPMC) for comparison with the grafted gum.

3.4. In vitro drug release study

Drug release from the tablet formulation was investigated in pH 1.2 medium for the initial 2 h, followed by in phosphate buffer of pH 6.8. This experiment was performed in triplicate using a tablet dissolution tester (Electro Lab, TDT-08L, India) equipped with eight baskets (glass jars) at the stirring speed of 50 rpm. Tablets were placed in 900 mL of dissolution medium maintained at 37.5 ± 2 °C. At regular intervals of time, sample aliquots were withdrawn, replaced with equal fresh medium and analyzed using UV spectrophotometer (UV-1800, Shimadzu, Japan) at the wavelength maxima of 282 nm.

The cumulative release percentage (CPR) at each time point for both tablets containing HPMC and Am-g-LBG were taken to calculate the similarity factor (f_2). The f_2 value was calculated by the following equation:

$$f_2 = 50 \log \left[\left\{ 1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right\}^{-0.5} \times 100 \right]$$

where t is time, n is number of time points, R_t and T_t are the CPR of reference and test sample at time t.

3.5. Drug release kinetics

The drug-release data were fitted into various empirical equations like zero order ($Q_t = kt$), first order ($\ln Q_t = \ln Q_0 - kt$), Higuchi ($Q_t = kt^n$), Hixson–Crowell ($Q_0^{1/3} - Q_t^{1/3} = kt$) and power-law ($M_t/M_\infty = kt^n$) equations, where M_t/M_∞ is the fraction of drug release at time t, k is the release rate constant, n is the diffusion exponent that denotes the drug-release mechanism (Korsemeyer, Gunny, & Peppas, 1983), Q_0 and Q_t are amounts of drug released at time zero and t, respectively. A least squares regression method was used to determine the values of n and k when applicable. Using power-law equation, values of $n = 0.43$ or less indicate Fickian transport, n values of 0.43–0.85 indicate anomalous or non-Fickian drug transport, and n values greater than 0.85 denotes super case II transport mechanism of tablet matrices.

4. Results and discussion

4.1. Synthesis of acrylamide grafted LBG

Synthesis of acrylamide grafted LBG was carried out by graft copolymerization of acrylamide onto LBG. Ceric ammonium nitrate is a common reagent employed to initiate free radical graft copolymerization. The proposed reaction scheme is shown in Fig. 1. The reaction proceeds first by having ceric ion attacks the LBG macrochains and forms a LBG–ceric complex. The ceric (IV) ions in the complex are then reduced to ceric (III) ions by oxidizing hydrogen atom and thereby creating a free radical onto LBG backbone. A critical amount of redox initiator is required for free radical formation. The grafting of Am onto LBG is effected by having free radical reacted with the monomer. In the presence of Am, the LBG free radical is chemically coupled to the monomer unit via covalent bond. The reaction is terminated through combination of two radicals. The reaction sample can be subjected to microwave irradiation to induce rapid energy transfer in its bulk thereby shortening the reaction time. Microwave is considered as a catalyst which synergises with ceric ions in graft copolymerization.

Table 1 presents details of grafting process. Three different variables were selected to optimize the GE using the factorial design. Their mathematical relationship with GE was generated using MLRA and was expressed as:

$$\text{GE } (\%) = 88.33 + 5.46A - 2.11B + 6.35C$$

where, A is amount of CAN (mg), B is irradiation time (min) and C is amount of Am (g). The three dimensional plot is shown in supplementary file (Fig. S1a, S1b and S1c). The positive impact of CAN and Am was due to formation of higher number of free radical sites and attachment of Am side chains. The negative impact of irradiation time on GE was due to frequent chain breakage under microwave irradiation. ANOVA analysis indicated that the factorial model was significant ($P < 0.05$) having R^2 value of 0.99. The adjusted (0.98) and the predicted (0.96) R^2 values were in reasonably good agreement. The higher value of adequate precision (36.65) indicated an adequate signal. Hence it can be said that the model can be used to navigate the design space.

4.2. Fourier transform infrared spectroscopy

Fourier transform infrared spectra (ATR mode) of LBG and optimized Am-g-LBG (G4) are shown in Fig. 2. A strong O—H stretching peak of hydroxyl group was observed in LBG at about 3300 cm^{-1}, and it was due to hydrogen bonding involving the hydroxyl groups on the gum molecules (Parvathy et al., 2005). The C—H stretching vibrations were observed at 2950 cm^{-1}, and additional characteristic bands of LBG appearing at 1363 and 1143 cm^{-1} were attributed

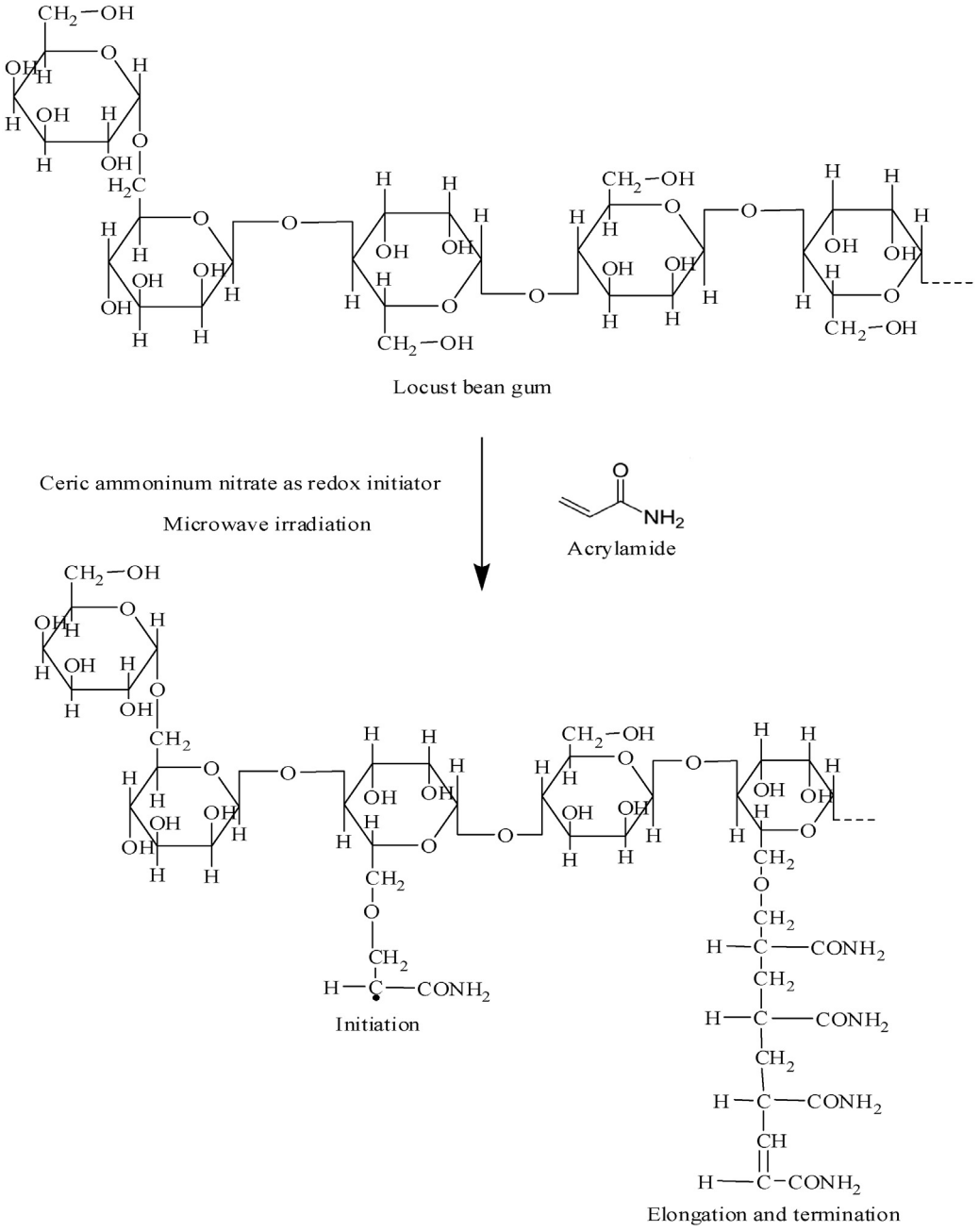

Fig. 1. Proposed reaction scheme of formation of Am-g-LBG (Kajjari, Manjeshwar, & Aminabhavi, 2011).

to C—H and O—H bending vibrations, respectively. The broad peak at about 1000–1100 cm^{-1} was mainly attributed to C—O—H stretching/bending, and was also identified in all modified batches (Fig. S2, supplementary file) with reduced peak intensity. Marked changes were observed in spectra of Am-g-LBG compared to LBG. The bands at 1653 and 1589 cm^{-1} were attributed to amide-I (C—O stretching) and amide-II (N—H bending) conferred by Am (Mundargi, Patil, & Aminabhavi, 2007). The peak at 2800–3510 cm^{-1} in Am-g-LBG was attributed to overlap of N–H stretching band of amide group and O—H stretching band. A shouldering at about 1450 cm^{-1} was due to the C—N stretching. Peak at 1020 cm^{-1} was due to CH—O—CH_2 group which occurs during grafting reaction between OH group of C_2 and π bond of acrylamide (Vijan et al., 2012). FTIR analysis indicated that LBG was successfully modified into Am-g-LBG. This modification was further confirmed by solid state ^{13}C NMR study.

4.3. Solid state NMR

In the ^{13}C NMR spectra of native LBG (Fig. 3a), four distinct peaks were observed. The absorption peak at $\delta = 61$ ppm corresponded to —CH_2OH group of glucose. The intense absorption peak at $\delta = 71$ ppm was ascribed to aromatic carbons of sugar moieties. The absorption peak at $\delta = 100$ ppm might be attributed to ring anomeric carbons. The very low frequency signal at about 173 ppm was due to —COOH group and an indication of presence of sugar acid as impurity. Sen, Singh, and Pal (2010) reported that acrylamide had three major peaks, amide carbonyl carbon peak at $\delta = 179$ ppm, and

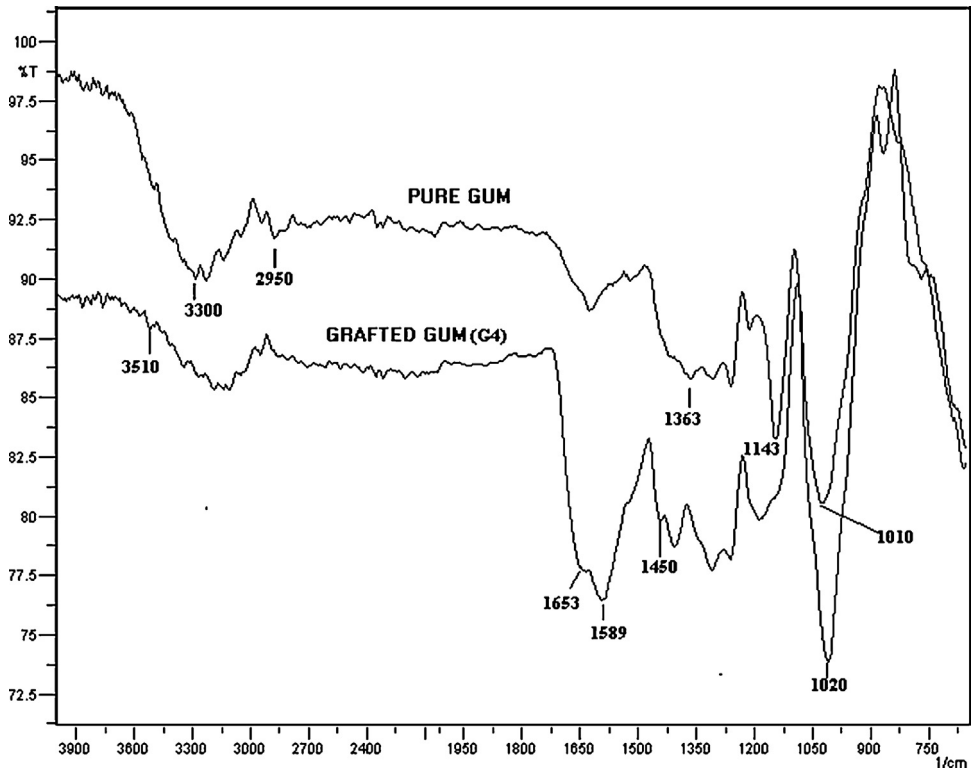

Fig. 2. FT-IR spectrum of pure LBG and optimized Am-g-LBG (G4).

two sp^2 hybridized carbon atoms (i.e. CH_2=CH—) peaks at δ = 130 and δ = 133 ppm.

In the ^{13}C NMR spectrum of Am-g-LBG (Fig. 3b), the peak at δ = 41 ppm was attributed to (—CH—CH2—CH—)$_n$ groups which formed during the polymerization reaction of acrylamide. The presence of very intense peak at δ = 179 ppm indicated carbon atoms of —CONH2 group. Absorption peak at δ = 70 ppm indicated aromatic carbon —C—OH groups. Absorption peak at δ = 100 ppm indicated ring anomeric carbon. Absorption peak at δ = 70 ppm indicated carbon which was attached with the oxygen of ring —C—OH group. Peaks at δ = 133 ppm and 130 ppm represented sp^2 carbons of acrylamide. Those sp^2 carbons, which were attached with oxygen atom, get converted to the sp^3 hybridized carbon atom (δ = 70 ppm). The summative NMR data evidenced the formation of Am-g-LBG.

4.4. Scanning electron microscopy

The SEM image of the native gum and the representative grafted gum is shown in Fig. 4. The gum, in its native form (Fig. 4a), was more powdery and fibrous in nature. Following acrylamide grafting (Fig. 4c and d), it became lumpy and undulant with sharp breaking points. These sharp edge breaking points gave an indication that the modified gum was brittle. Acrylamide is characterized by crystalline structure and polyhedral shape (Fig. 4b) (Kumar et al., 2009; Singh, Nath, & Guha, 2011). Upon grafting, the characteristic of both acrylamide and LBG disappeared and was leaded to formation of granular grafted gum particles. The size of the grafted gum granules was larger than that of native LBG.

4.5. Powder X-ray diffraction

The outcome of XRD analysis on LBG and representative acrylamide grafted LBG (G4) was shown in Fig. S3 (supplementary file). Native gum gave a more intense peak than that of grafted gum. This suggested that amorphous nature of the native gum increased after grafting. The amorphous materials generally had higher water solubility. Successful grafting led to an increase in amorphous nature of modified gum and this was reflected by changes in water contact angle and swelling features of LBG (Table 2).

4.6. Differential scanning calorimetry

DSC curves of different batches of Am-g-LBG and native LBG are shown in Fig. 5. In case of native LBG, a broad endothermic peak at around 79.27 °C was observed. This was due to loss of moisture. Though the native gum was dried at 110 °C for 1 h before conducting the study, the endothermic peak of moisture was till appeared in the thermogram. An endothermic peak at 253.74 °C with heat of fusion of 69.56 J/g was observed in native gum thermogram. This could be associated with degradation of polymer backbone. Graft polymer showed a distinct feature in DSC curve (Fig. 5). The transition midpoint temperatures (T_m) of different batches of Am-g-LBG varied from 233 °C (G4) to 242 °C (G5) and were shown in Table 3. This variation in T_m was due to different formulation parameters. It can be seen in Table 3 that the T_m value for grafted gums was less than that of the native gum. T_m is an indicator of thermostability and generally the higher the value of T_m, the more thermodynamically stable is the macromolecule (Vijan et al., 2012). The reduction in thermodynamic stability was due to polymer chain breakage during microwave irradiation and addition of acrylamide side chains. The formulation with the highest %GE showed the minimum T_m value. Having the highest amount of grafted Am in its backbone, the polymer degraded easily.

Glass transition temperature (T_g) of all the batches of grafted gum was distinctively observed but for native gum it was hard to detect (Fig. 5). T_g value was less for those batches of grafted gum having lower viscosity. Viscosity decreased with increase of %GE. This result corroborated the findings obtained from contact angle, viscosity and molecular weight studies.

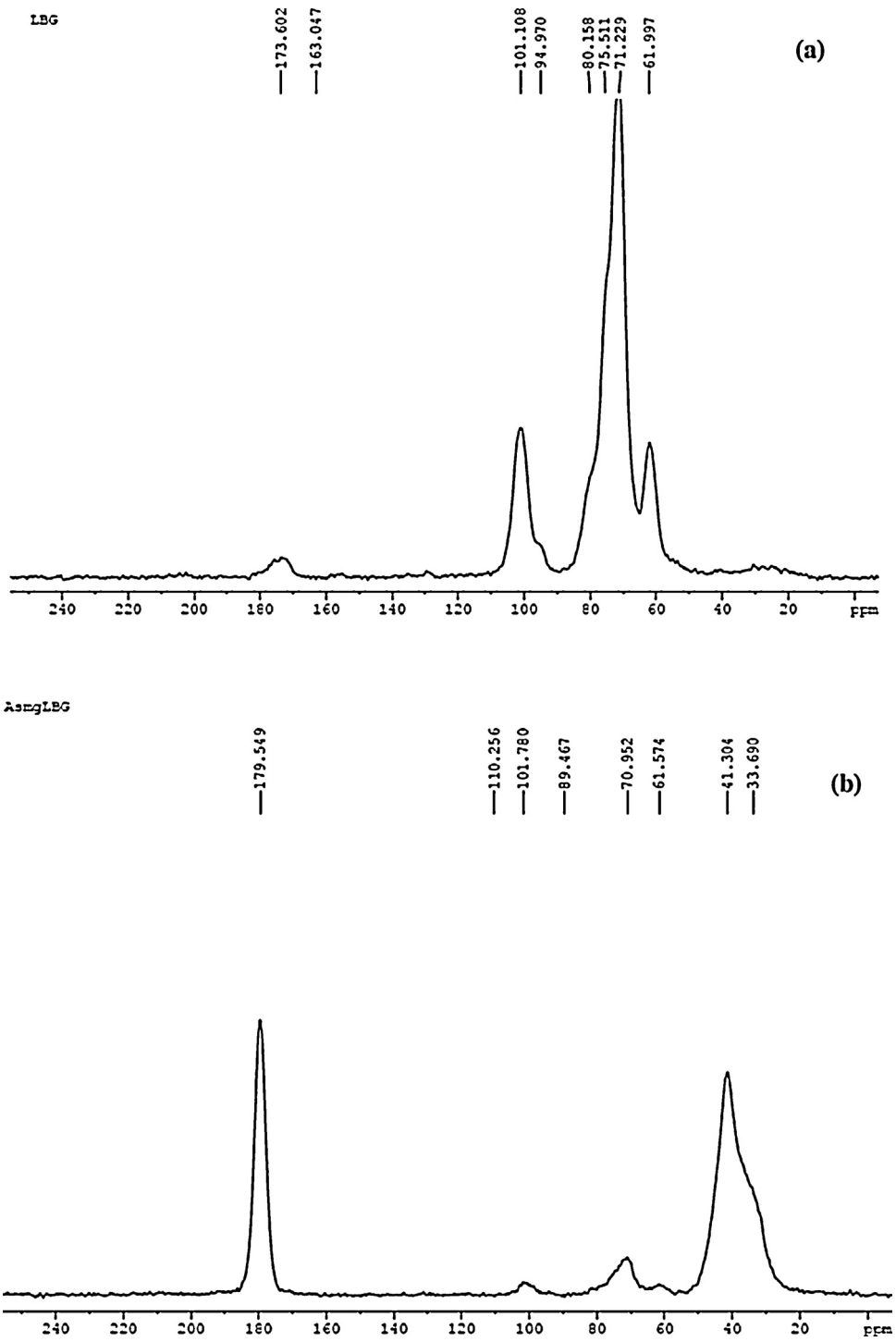

Fig. 3. Solid state ^{13}C NMR of (a) locust bean gum and (b) acrylamide grafted locust bean gum (G4).

Table 3
Thermodynamic parameters derived from DSC of various batches of Am-g-LBG.

Batch code	Transition midpoint temperature T_m (°C)	Endothermic Heat of fusion ΔH_m^0 (J/g)	Glass transition temperature T_g (°C)
G1	237.46	68.27	210.20
G2	239.44	36.57	198.83
G3	240.30	60.25	199.02
G4	233.07	68.4	206.29
G5	242.03	44.38	210.65
G6	240.01	45.83	198.23
G7	238.03	68.77	208.91
G8	238.95	37.26	202.59
LBG	253.74	69.56	–

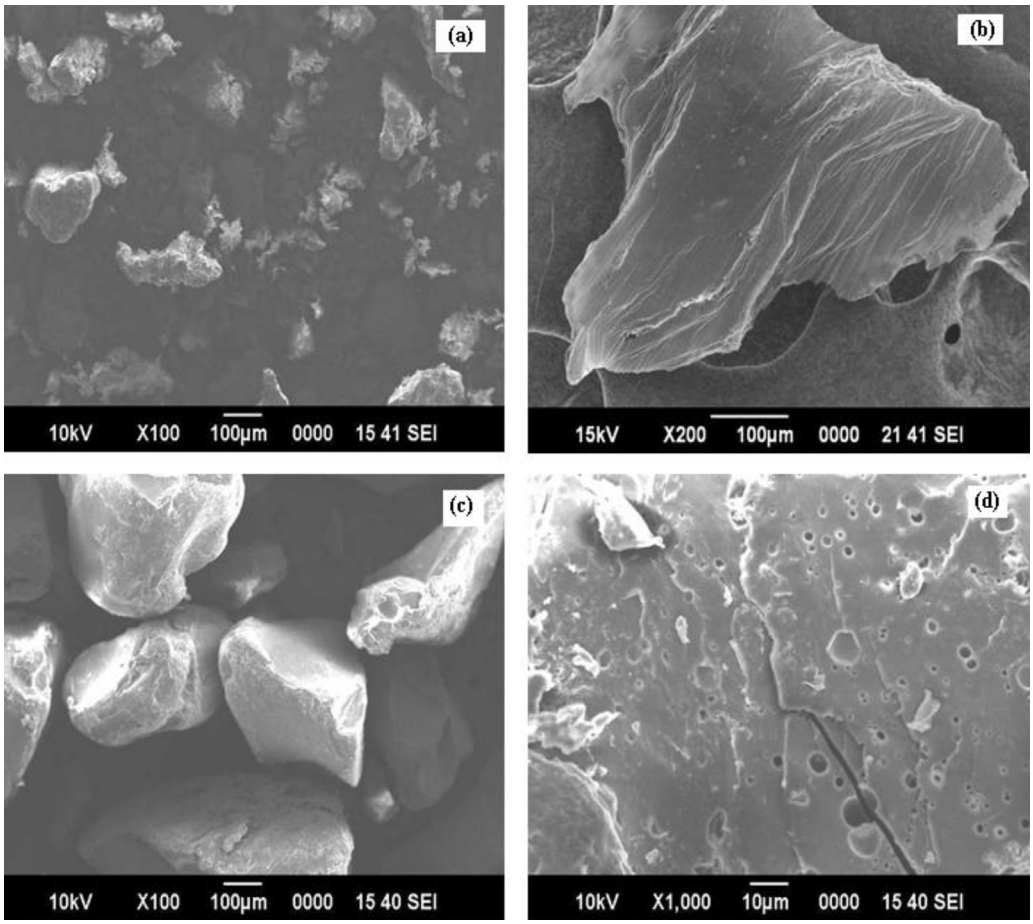

Fig. 4. SEM images of (a) locust bean gum, (b) acrylamide, (c) and (d) representative acrylamide grafted locust bean gum.

From the DSC study, it was found that the heat of fusion (ΔH_m^0) for all the batches of grafted gum was less than that of native LBG. The value of ΔH_m^0 was less for those batches having lower viscosity. This finding also suggested that acrylamide grafting onto LBG backbone lowers the solubility *viz*. viscosity.

4.7. Elemental analysis

The elemental analysis results for LBG and eight different batches of grafted gums are shown in Table 1. A very little amount of nitrogen was presented in LBG (0.942%) due to impurities. An increase in %GE was accompanied by a sharp increase in nitrogen content in all the Am-g-LBG batches. A significant increase of nitrogen % in all eight batches of Am-g-LBG confirmed the attachment of acrylamide onto the backbone of the LBG. The highest % N value in case of G4 (16.87%) was attributed to maximum grafting propensity.

Mathematical relationship generated using MLRA for %N was expressed as:

$$\%N = 15.59 + 0.94C$$

where C is amount of Am (g). The three dimensional plot is shown in supplementary file (Fig. S1d, supplementary file). The effects of CAN amount and irradiation time on %N were negligible since Am was the only source of nitrogen. An increase in %N with %GE indicated that increase in %N was due to the grafting of Am onto the LBG backbone as unreacted monomer and polymer (polyacrylamide) were removed from grafted gum. From ANOVA study, it was observed that the factorial model was significant ($P < 0.05$) having R^2 value of 0.82. The adjusted (0.79) and the predicted (0.68) R^2 values were in reasonably good agreement. The higher value of adequate precision (7.42) indicated an adequate signal. Hence it can be said that the model can be used to navigate the design space.

4.8. Contact angle and surface energy measurement

We performed this study to identify the change of wetting ability of the grafted gum against that of LBG. The higher the surface energy, water contact angle will decrease and polymer interaction with water molecule will increase. The result of this study is shown in Table 2 and Fig. S4 (supplementary file). All batches of grafted form showed a sharp increase in wetting ability in water than that of native LBG (contact angle 65.28°). The water contact angle decreased and surface energy increased gradually with %GE (Tables 1 and 2). Among all grafted gum batches, G4 showed highest %GE and minimum contact angle (45.19°). When Am-g-LBG came in contact with water molecules, it easily broke the cohesive force of water molecules due to presence of highly polar groups on the surface. The more grafted batches had lower contact angle because of their high affinity towards water molecules. The surface energy was also found to be high in case of highly grafted batches. This might be also a cause of grafted gum having higher affinity for water molecule.

Mathematical relationship generated using MLRA for contact angle was expressed as:

$$\text{Contact angle} = 55.15 + 1.62B - 3.42C + 2.44BC.$$

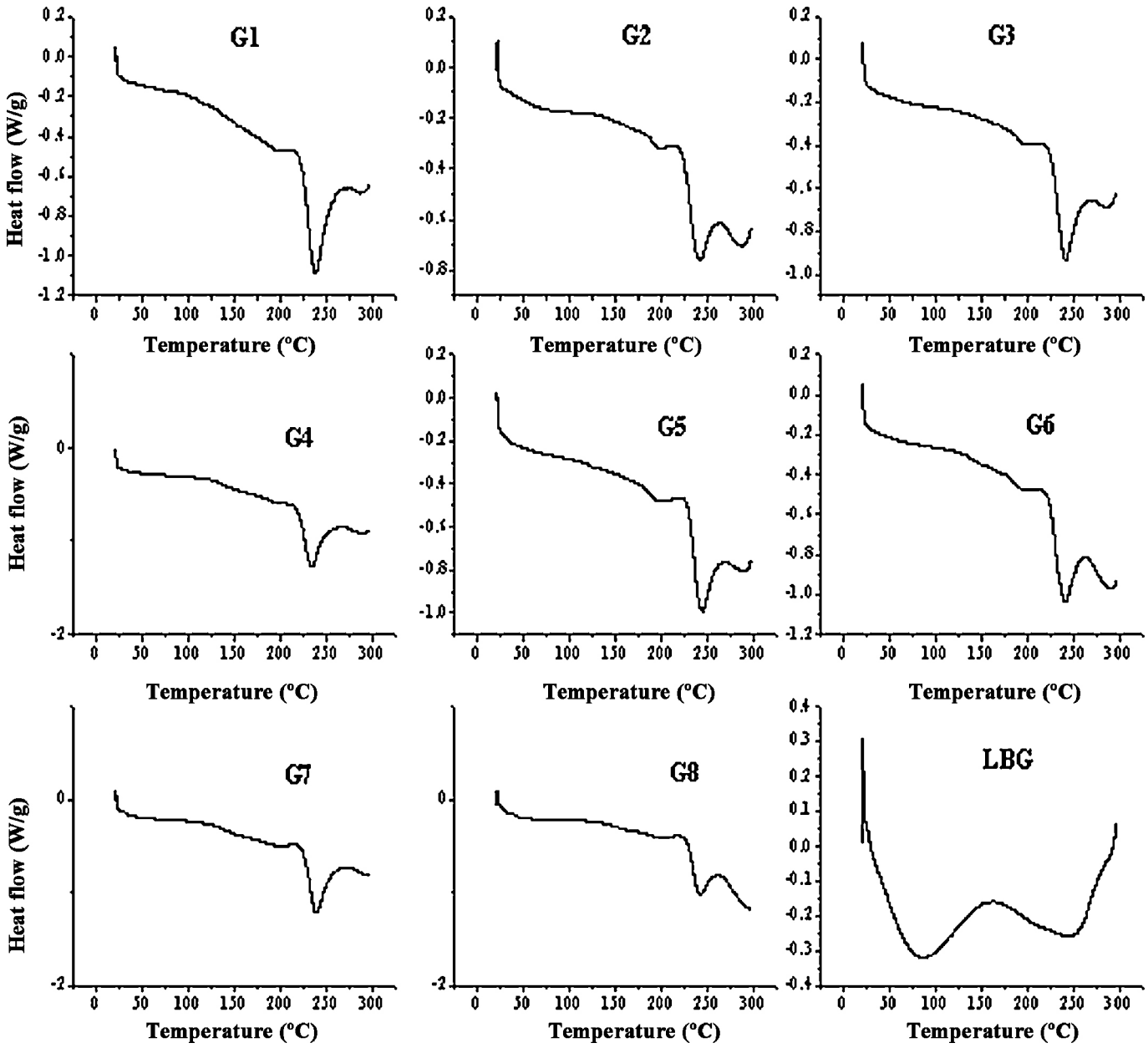

Fig. 5. DSC thermogram of different batches of acrylamide grafted locust bean gum and native locust bean gum.

where B is irradiation time (min) and C is amount of Am (g). The three dimensional plot is shown in supplementary file (Fig. S1g). From ANOVA study, it was observed that the factorial model was significant ($P < 0.05$) having R^2 value of 0.91. The adjusted (0.84) and the predicted (0.65) R^2 values were in reasonably good agreement. The higher value of adequate precision (8.37) indicated an adequate signal. Hence it can be said that the model can be used to navigate the design space.

4.9. Viscosity analysis

The result of viscosity analysis is shown in Table 2. The results suggest that native gum had the highest viscosity (2.78 poise). The viscosity of all batches of Am-g-LBG was less than that of the native gum. LBG is a galactomannan polymer and longer galactose side chain (mannose:galactose = 4:1) brought about higher viscosity (Englyst & Commings, 1988). LBG generally exists as highly branched random coils (Lundin & Hermansson, 1995). The presence of galactose branch point provides stiffness and reduced flexibility in galactomannans which might increase their resistance to flow in dispersion state. For the grafted gums, the reduction of viscosity was due to the breakage of galactose branch points of the native gum under drastic irradiation by microwave. Among the grafted gums, the batches which were having less %GE (G1, G5 and G7) showed higher viscosity than the others. Among the highly grafted batches (G2, G4, G6 and G8), G4 showed the highest viscosity (0.90 poise). This was due to direct effects of grafts on viscosity.

Mathematical relationship generated using MLRA for viscosity was expressed as:

$$\text{Viscosity} = 0.84 - 0.13C + 0.054AC - 0.091BC - 0.079ABC.$$

where A is amount of CAN (mg), B is irradiation time (min) and C is amount of Am (g). The three dimensional plot is shown in supplementary file (Fig. S1e and S1f). From ANOVA study, it was found that the factorial model was significant ($P < 0.05$) having R^2 value

of 0.97. The adjusted (0.94) and the predicted (0.83) R^2 values were in reasonably good agreement. The higher value of adequate precision (13.27) indicated an adequate signal. Hence it can be said that the model can be used to navigate the design space.

4.10. Molecular weight analysis

The result of gel permeation chromatography (GPC) is shown in Table 2. Most of the batches of grafted gum were eluted at about 7.5–8 mL of elution volume. From the results it can be said that the native gum was having the highest molecular weight (12.382×10^6 g/mol) (Fig. S5b, supplementary file). However, in case of grafted gum, molecular weight was reduced. In case of optimized batch (G4), molecular weight was found to be 5.7347×10^6 g/mol (Fig. S5a, supplementary file). This drastic reduction in molecular weight was due to the polymer chain disruption in course of microwave irradiation. Few batches having less % GE (G5) showed higher molecular weight. This was due to the presence of native LBG chains in the system. However, in case of G8, there was an increase in molecular weight though its GE was 91.461%. This was due to the presence of high amounts of grafted polymer segments in the system. The relation between log transformed value of viscosity and molecular weight was linear ($R^2 = 0.98$) which satisfied the Mark–Houwink equation (Paul & Lodge, 2007).

A numerical optimization tool of Design Expert software using the desirability approach was used to obtain grafted gum with desired response. In this study, optimization of independent variables was performed with a goal of maximizing grafting efficiency, %N, viscosity and minimizing water contact angle so that the polymer matrix can be sufficiently hydrophilic for solvent imbibition and swell substantially without dissolving the matrix. The numerical optimization tool provided us with different sets of optimal solutions. Among these solutions, the optimal batch with the highest desirability (0.820) was G4. Hence, G4 was considered as the optimum acrylamide grafted locust bean gum batch.

4.11. Swelling study

From the swelling study (Table 2), it was observed that the native LBG showed least swelling in both pH 1.2 and pH 6.8 media (139.33% and 141.21%, respectively). In both the media, LBG did not show any significant difference in swelling behaviour. This was due to its non-ionic nature. Natural gums generally on hydration swell rapidly forming the viscous gel layer on the surface, which increases the diffusion path length and slows down the solvent imbibition. Moreover, as it is a galactomannan, it has lesser tendency to interact with water molecule due to its steric bulkiness and random coil nature (Lundin & Hermansson, 1995). However, the grafted gums showed higher swelling extents in both the media than that of the native gum. The optimized batch (G4) showed highest swelling in both the media (221.27% and 281.47%). Graft copolymerization of vinylic monomer increased swelling power of native polysaccharide due to the introduction of free hydrophilic groups. Due to those hydrophilic groups, strong interchain hydrogen bonding takes place between the grafted side chains of acrylamide. This strong hydrogen bonding favours the formation of a three dimensional network that can hold more water in it (Singh et al., 2011).

In general, grafting is used to decrease the aqueous solubility of a native polysaccharide viz. increasing contact angle, viscosity as well as molecular weight. In the present study we observed opposite behaviour of the grafted gum. The reasons may be as follows.

The cold water less soluble LBG typically has a mannose to galactose ratio (M/G) nearly equal to 4. In LBG, both random, blockwise, and ordered galactose distributions are present as reported by Daas, Schols, and de Jongh (2000). The functional properties of different galactomannans depend on both the M/G ratio and the distribution pattern of galactose unit along chain backbone. Specially, as far as water solubility is concerned, the higher the degree of substitutions of the galactomannan, the more easy it is to form true solutions. Another explanation for this is that the galactose side chains promote solubility because they introduce an entropic (perhaps steric) barrier to ordered packing of mannan chains. The hydrophobic effect is itself entropic, we can regard the mannan chains to be relatively hydrophobic and galactose units are more hydrophilic (Picout, Ross-Murphy, Jumel, & Harding, 2002). The grafting sites are present in both galactose as well as mannose subunits. Grafting of acrylamide in galactose subunit may increase the entropic and/or steric barrier to the ordered packing of mannan chains which lead to increase the solubility of grafted LBG than the native gum. When polyacrylamide, a water soluble polymer, grafted onto mannan chains, the hydrophobic nature of gum, which is attributed by the mannan subunits, will be reduced. This may help to increase the solubility of native LBG. Similar findings were reported for xanthan (Adhikary & Singh, 2004) and sodium alginate (Wunderlich et al., 2000).

4.12. Acute oral toxicity study

From oral toxicity study, no mortality was found within the observation period of 14 days after dosing. The haematological and blood chemistry data collected at different time points of study period were presented in Table S1 (supplementary file). No abnormalities were found in all haematological and blood chemistry parameters. Moreover, the histopathological sections of kidney, liver, lung and stomach (Fig. S6, supplementary file) did not show any abnormalities on 15th day of toxicity study. As per the "Organization of Economic Co-operation and Development (OECD) guideline for the test of chemicals" 425, adopted "17 December 2001" Annexure-4, further test was unnecessary as the LD_{50} value was deemed to be greater than 2000 mg/kg dose of Am-g-LBG. The test product was classified as "Category 5" and "zero" toxicity rating as per the Globally Harmonized System (GHS), since LD_{50} value was greater than 2000 mg/kg dose.

4.13. Biodegradability study

The biodegradability study shows fungal growth (Fig. 6) on Am-g-LBG film. The microscopic study revealed apparent fungi growth on 0, 3, 7, 14 and 21 days in all the Petri dishes. The fungi growth consumed carbon from Am-g-LBG and this formed the basis for its biodegradability.

4.14. In vitro drug release study and release kinetics

The in vitro drug release study was performed to evaluate the rate controlling property of the grafted gum. The release profile was compared with a standard rate controlling hydrophilic polymer, hydroxypropyl methylcellulose (HPMC) (Fig. 7). It was observed that in each time point there was no significant difference in cumulative percentage release (CPR) between the grafted gum and HPMC. After 10th h the grafted gum showed $81.07 \pm 2.01\%$ drug release whereas HPMC showed $71.77 \pm 0.92\%$ drug release. However, after 24 h the grafted gum showed $98.3 \pm 1.03\%$ and HPMC showed $93.3 \pm 1.63\%$ drug release.

The f_2 value is a well established parameter for comparison of dissolution profiles between two formulations. Two dissolution profiles are considered to be identical when $f_2 = 100$. An average difference of 10% at all measured time point results in a f_2 value of 50. The standard of f_2 value from 50 to 100 indicates similarity between two dissolution profiles. Another way of saying this is

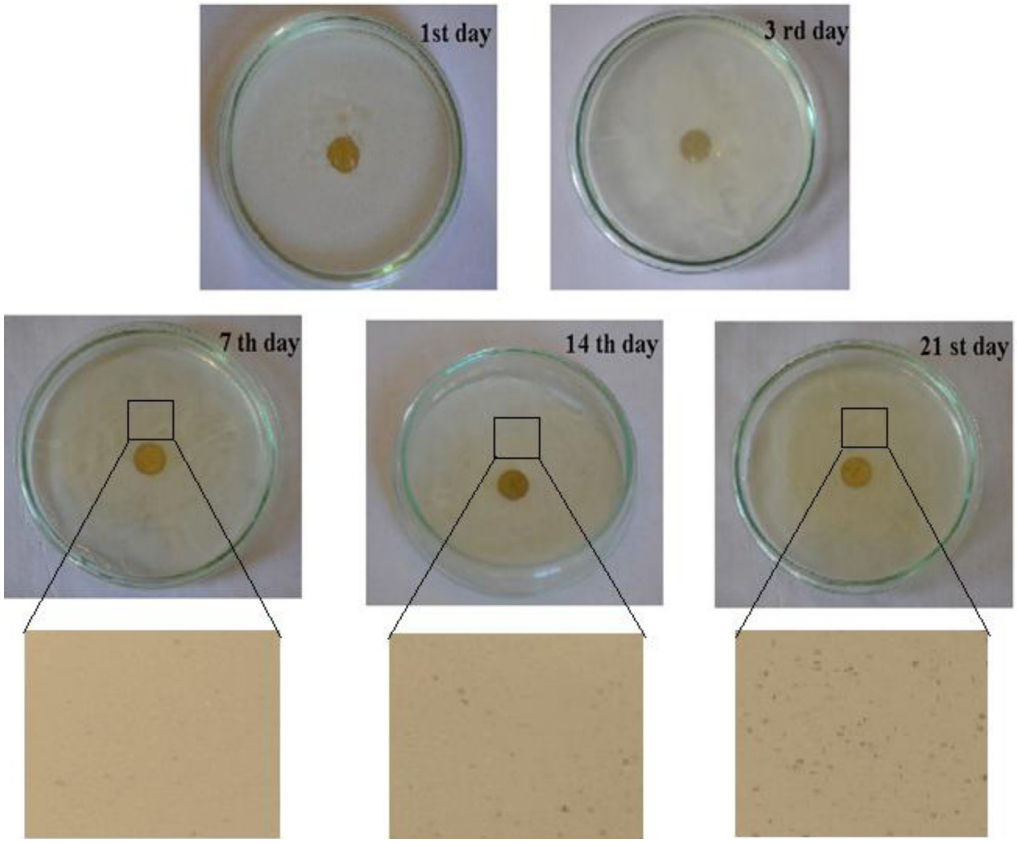

Fig. 6. Gradual growth of *A. niger* in minimal salt agar media by utilizing the grafted gum as carbon source.

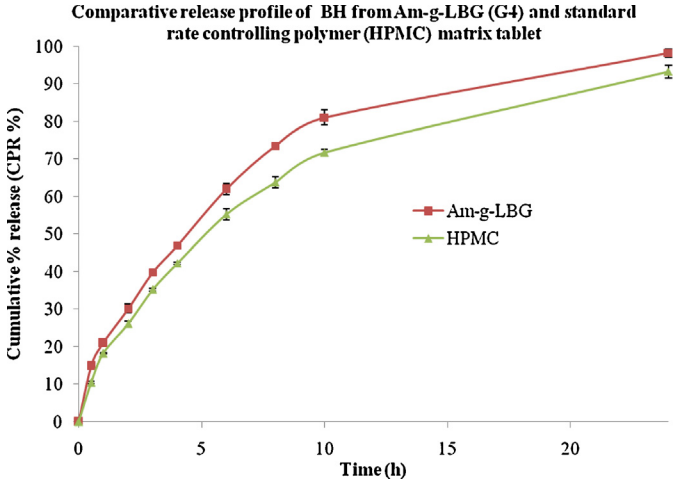

Fig. 7. Comparative release profile of BH from Am-g-LBG (G4) and standard rate controlling polymer (HPMC-K15M) matrix tablet.

that on average if difference at each sampling time is 10% or less then f_2 value will be between 50 and 100. The f_2 value was found to be 60.37 when the CPR values of HPMC and Am-g-LBG containing tablets were compared. Am-g-LBG showed similar rate controlling property as HPMC. It can also be used as a rate controlling polymer for controlled release drug delivery.

Kinetic modelling (Table S2, supplementary file) of release data indicated that the drug release from matrix of both cases was non-Fickian, a diffusion controlled process without subsequent dissolution of the matrix as the n values of Am-g-LBG and HPMC were 0.810 and 0.844, respectively. Among all empirical equations, the release data best fitted with Higuchi model. Am-g-LBG can be used as rate controlling hydrophilic polymer for controlled-release formulations.

5. Conclusion

Am-g-LBG was synthesized by microwave irradiation using ceric ammonium nitrate as a redox initiator. The G4 batch was found to be the optimized batch. The grafted gum was biocompatible and biodegradable in nature. This optimized grafted gum was further used to formulate controlled-release matrix tablet of buflomedil hydrochloride (BH). The release pattern was compared with HPMC matrix tablet of BH. The release of drug from both the matrix tablets followed non-Fickian diffusion controlled drug release process. Hence, it can be concluded that Am-g-LBG can be used as a rate controlling hydrophilic polymer for controlled-release application.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.07.037.

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