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Microwave assisted synthesis and characterization of acrylamide grafted gellan, application in drug delivery

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ABSTRACT

The synthesis of acrylamide-grafted-gellan gum was carried out by microwave-assisted free radical polymerization using cerric ammonium nitrate (CAN) as redox initiator. A series of graft copolymers, varying in amount of acrylamide, CAN and microwave irradiation time was prepared. The modified gum was extracted with 20% (v/v) methanol to remove the homopolymer formed during polymerization reaction. These graft copolymers were characterized by FTIR, ^{13}C NMR, CHN, SEM, rheological studies and DSC studies. Comparison of grafting parameters such as grafting efficiency, percentage grafting and percentage conversion were carried out among various series of graft copolymers and then correlating it with elemental analysis, DSC, viscosity results. The acute oral toxicity study of grated gum was evaluated as per OECD guideline. Tablets were prepared by incorporating antidiabetic drug metformin hydrochloride (MTF) in grafted gum along with excipients. In vitro studies were performed on prepared tablet formulations showing release up to 8 h.

1. Introduction

Natural polymers are preferred over synthetic polymers because of their biodegradability, low cost, easy availability and non toxicity (Bhardwaj, Kanwar, Lal, & Gupta, 2000). However they also possess certain drawbacks like uncontrolled hydration, microbial contamination, drop in viscosity during storage, etc. The chemical combination of natural and synthetic polymers modifies properties of these natural polymers by hybridization. In recent years, controlled drug delivery formulations and the polymers used in these systems have become much more sophisticated, with the ability to do more than simply extend the effective release period for a particular drug. For example, intelligent or smart polymers play an important role in drug delivery since they may dictate not only where a drug is delivered, but also when and with which interval it is released (Soppimath, Aminabhavi, Dave, Kumar, & Rudzinski, 2002). 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). Graft copolymerization of synthetic polymers onto polysaccharide backbone offers one of the best ways to use polysaccharides for controlled release delivery. Graft copolymerization is an easier method to modify the structure of natural polymers and thus makes them attractive biomaterials in controlled release applications since native polysaccharides may not be suitable in controlled drug delivery systems due to their substantial swelling and rapid enzymatic degradation in biological fluids (Soppimath et al., 2002). Graft copolymerization introduces hydrophobicity and steric bulkiness, which considerably protects the matrix and carbohydrate backbone to retard the drug release. In simpler words grafting can be described as a polymer modification process, which is an irreversible covalent attachment process. The main constraint of graft copolymerization is the formation of concurrent homopolymer 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 an efficient method, which results in rapid transfer of fixed energy in the bulk of the 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). Among the synthetic polymers, acrylics occupy a significant position in polymer science. One of the natural polymers that have evoked a great interest of researchers is gellan gum.

Abbreviations: GG, gellan gum; CAN, cerric (IV) ammonium nitrate; MTF, metformin hydrochloride; AAM-g-GG, acrylamide grafted gellan gum; ^{13}C NMR, 13 carbon nuclear magnetic resonance; FTIR, Fourier transform infra-red; 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; t_{50}%, time at which 50% drug is released; OECD, Organization of Economic Co-operation and Development.

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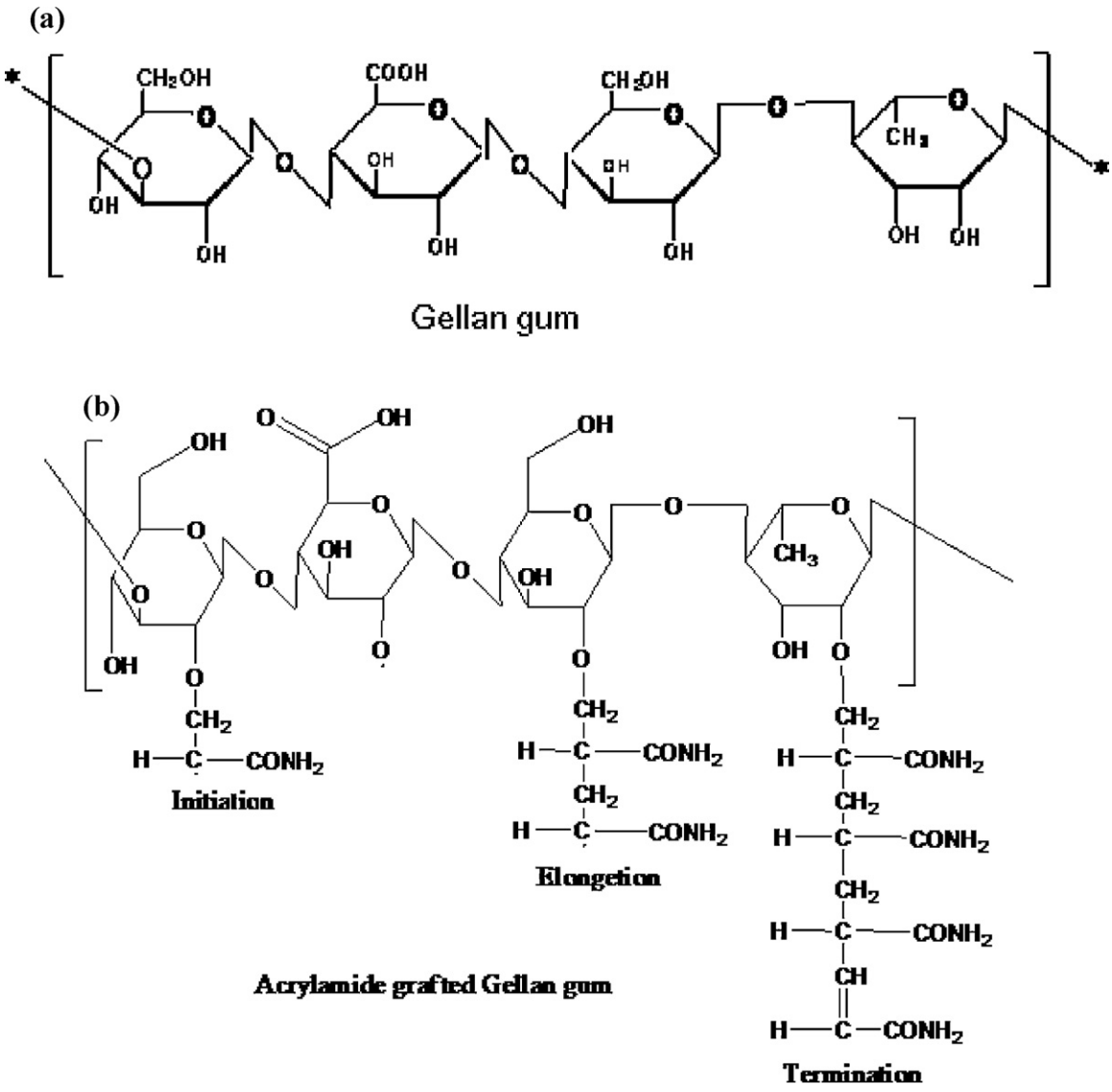

Fig. 1. (a) Structure of gellan gum and (b) AAm grafted gellan gum (AAm-g-GG).

Gellan gum (GG) has been used in ophthalmic drug delivery (Matricardi, Cencetti, Ria, Alhaique, & Coviello, 2009), oral sustained delivery (Agnihotri, Jawalkar, & Aminabhavi, 2006; Kedzierewicz, Lombry, Rios, Hoffman, & Maincentet, 1999; Miyazaki, Aoyama, Kawasaki, Kubo, & Attwoodet, 1999), controlled-release hydrogel with scleroglucan (Coviello et al., 1999), and floating in situ gelling (Rajinikanth, Balasubramaniam, & Mishra, 2007). It is an anionic deacetylated exocellular polysaccharide gum with high molecular weight, produced as a fermentation product by pure culture of *Pseudomonas elodea* (aerobic, gram negative, non pathogenic bacterium). It has a tetrasaccharide repeat unit consisting of two glucose residues, one glucuronic acid residue, and one rhamnose residue (Bhardwaj et al., 2000) (Fig. 1(a)).

Metformin HCl (MTF) is an orally administered biguanide, which is widely used in the management of type-2 diabetes, a common disease that combines defects of both insulin secretion and insulin action. It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis. It has three different actions: it slows the absorption of sugar in our small intestine; it also stops our liver from converting stored sugar into blood sugar; and it helps our body use our natural insulin more efficiently. It is a hydrophilic drug and is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of a single 500 mg dose is reported to be 50–60%. An obstacle to more successful use of metformin therapy is the high incidence of concomitant gastrointestinal symptoms, such as abdominal discomfort, nausea, and diarrhea that especially occur during the initial weeks of treatment. Also the compound has relatively short plasma elimination half-life of 2–4 h. Side effects and the need for administration, about two or three times per day when larger doses are required, can decrease patient compliance. Sustained release (SR) formulation that would maintain plasma levels of drug for 8–12 h might be sufficient for once daily dosing for metformin. SR products are needed for metformin to prolong its duration of action and to improve patient compliance.

In the present study, the graft copolymerization of acrylamide (AAm) on to GG (Fig. 1(b)) was carried out using redox-initiator induced and microwave-assisted graft copolymerization. The acrylamide grafted GG so prepared, was characterized by FTIR, ^{13}C NMR, CHN, SEM, and DSC studies. The acute oral toxicity study in rodents was carried out according to OECD guideline. The graft copolymer was evaluated for modification of release rate by preparing the matrix tablets of MTF. In vitro release studies were performed on prepared tablets formulations in simulated gastric fluid at 37 °C

to investigate their application in successful oral dosage formulations. The release data were fitted to the empirical equations to understand the release kinetics and mechanism of drug release. Tablet formulations were characterized by FTIR, DSC and XRD. To the best of our knowledge, systems studied in the present work are not reported earlier.

2. Experimental

2.1. Materials

Metformin HCl (99.68% purity) was received as gift sample from Stadmed Pvt. Ltd., Kolkata, India. Gellan gum was purchased from SRL India Ltd., Mumbai, India. Cerric ammonium nitrate (CAN) and acrylamide were obtained from Merck Specialties Pvt. Ltd., Mumbai, India. Acetone (M.W. – 58.08 g/mol and density – 0.789–0.791 g/mL) was bought from Rankem India Pvt. Ltd., Mumbai, India. All other chemicals used were of reagent grade and were used as such. Throughout the experiment, Millipore (MilliQ®) water was used.

2.2. Preparation of acrylamide-grafted-gellan gum

The graft copolymer derived from AAm and GG was prepared by microwave-assisted, free radical induced polymerization method. Briefly, 1.0 g of GG was dispersed in 120 mL of water. Specified amount of acrylamide (Table 1) was mixed with 30 mL of water and added to above and stirred for about 1 h. Specified amount of CAN (Table 1) was dissolved in 30 mL of water and added to above dispersion (Mundagri, Patil, & Aminabhavi, 2007). The dispersion was irradiated by microwave in laboratory scientific microwave system (Catalyst systems, India) at 480 W for different time periods following a 1 min heating and 1 min cooling, to prepare eight different batches (Table 1). It was left for overnight and then precipitated using acetone. It was further washed with 20% (v/v) aqueous methanol (Silva, Paula, & Feitosa, 2007), distilled water 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 (%G), grafting efficiency (%GE) and % conversion (%C) were calculated using following equations (Athawale & Lele, 1998).

$$\%\,\text{grafting}\,(\%G) = \left(\frac{W_1 - W_0}{W_0}\right) \times 100$$

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

$$\%\,\text{conversion}\,(\%C) = \left(\frac{W_1}{W_2}\right) \times 100$$

where W_0, W_1 and W_2 denote the weight of GG, graft copolymer and AAm, respectively.

To ensure complete removal of polyacrylamide from the grafted gum, approximately 500 mg of the unextracted sample was stirred overnight in 40 mL of 30% ethanol/70% H_2O (v/v) to remove polyacrylamide homopolymer. Mixture was centrifuged at 3200 rpm for 10 min. Supernatant was transferred to a weighted evaporation dish and dried in 105 °C hot air oven overnight to give soluble sample with weight designated as $Wt_{soluble}$. The tube containing the insoluble portion was placed in 105 °C vacuum oven and dried overnight. This was termed as insoluble sample. Its weight was expressed as $Wt_{insoluble}$ and its nitrogen content was N_{ext}. Elemental analysis of both extracted grafted gums (20% methanol and 30% ethanol) was carried out to verify nitrogen concentration in both the copolymers (Shogren, Willett, & Biswas, 2009).

Thus corresponding grafting parameter for comparison was calculated as

$$\text{Graft efficiency} : f \times \left(\frac{N_{ext}}{N_q}\right).$$

Wt_q, product weight after removal of acrylamide by extraction with ethanol; N_q, nitrogen content after removal of acrylamide by extraction with ethanol; N_{ext}, nitrogen content after removal of homopolymer polyacrylamide by extraction with 30% ethanol; and f = insoluble weight fraction as % $100 \times Wt_{insoluble}/Wt_{soluble} + Wt_{insoluble}$.

Grafting efficiency is based on the polymerized monomer, i.e. the ratio of insoluble polyacrylamide to total polyacrylamide.

2.3. Characterization of grafted gellan gum

2.3.1. Elemental analysis

The elemental analysis of the graft copolymers was performed using a Perkin Elmer CHN 2400 microanalyser to determine the carbon, hydrogen and nitrogen content.

2.3.2. Infrared spectral analysis

FTIR spectra of GG, AAm grafted GG (AAm-g-GG) were obtained using FTIR-8400S (Shimadzu, Japan) to predict the possible changes of functional groups of grafted gum as compared to native gum. A small amount of each material was mixed with KBr (1 wt.% sample) and compressed into tablet. The scanning range selected was 550–4000 cm^{-1}. Metformin HCl and formulation were analyzed by FTIR to predict the possible interactions between drug and modified polymer by same process.

2.3.3. Solid state NMR

A Bruker AMX 300 spectrophotometer operating at 75 MHz for ^{13}C solid state NMR was used for analysis of gellan gum and AAm-g-GG.

2.3.4. Viscosity measurement

The viscosity of gellan gum and AAm-g-GG (0.1%, w/v) was determined by a programmable Brookfield viscometer (Model DV-II+ Pro, Brookfield Engineering Labs, Inc., Middleboro, MA) at 32.7 °C. The samples were dissolved in hot water and conditioned at 32.7 °C. The spindle (Spindle no. CPE 41) was rotated at 0.5 rpm.

2.3.5. Thermal analysis

DSC thermogram of GG, AAm grafted GG (AAm-g-GG) and drug loaded tablet formulation were carried out under N_2 flow (50 mL/min) using Shimadzu DSC-60 at a heating rate of 10 °C/min and sample mass of 3–5 mg. The recorded DSC curve is a second heating. The first curve was taken up to 110 °C and this temperature was kept for 20 min followed by cooling up to ambient temperature (Silva et al., 2007).

The stability of macromolecules and of molecular associations is quantified by the standard free energy ΔG^0 or the difference in Gibbs free energy between different states (native grafted gum and depolymerized grafted gum). At equilibrium (when $\Delta G^0 = 0$), ΔG^0 is related to the equilibrium constant (K) between the two states by;

$$\Delta G^0(T) = -\text{RT} \ln K(T)$$

where R is universal gas constant and T the absolute temperature in Kelvin. ΔG^0 is the sum of two contributions

$$\Delta G^0(T) = \Delta H^0(T) - T\Delta S^0(T)$$

where ΔH^0 and ΔS^0 are the enthalpy and entropy changes at the temperature at which ΔG^0 are being evaluated.

Table 1
Synthetic details and elemental analysis of acrylamide-grafted-gellan gum.

Batch code	Amt. of CAN (mg)	MW irradiation time (min)	Amt. of AAm (gm)	%GE	%G	%Conversion	Viscosity (cP) @ 0.5 rpm	Elemental analysis		
								%C	%H	%N
GG	–	–	–	–	–	–	561.7	33.95	6.43	0.00
F1	100	1	5	–	–	–	–	–	–	–
F2	100	5	5	10.58	52.91	30.58	611.3	40.73	7.23	10.88
F3	100	1	10	6.04	60.04	16.04	587.7	38.61	6.85	7.71
F4	100	5	10	6.80	68.06	16.80	589.2	42.96	7.56	7.97
F5	300	5	10	98.08	980.83	180.08	1283.8	42.84	7.60	15.39
F6	300	1	5	56.01	280.09	76.01	1049.2	41.84	7.60	14.22
F7	300	5	5	45.37	226.86	65.37	1124.7	41.69	7.17	12.02
F8	300	1	10	60.63	606.37	70.63	1190.4	41.18	7.75	14.36

%GE, % grafting efficiency; %G, % grafting; % Conversion; %C, carbon percent; %H, hydrogen percent and %N, nitrogen percent.

When a macromolecule changes its thermodynamic state, a heat capacity change (ΔC_p) is observed. This change is due to the fact that heat required to raise the temperature of grafted gum is lesser than that of depolymerized grafted gum. Heat capacity changes are primarily due to the change of its physical state. Assuming a constant temperature-independent ΔC_p, ΔG^0 is described by:

$$\Delta G^0(T) = [\Delta H^0(T_R) + \Delta C_p(T - T_R)] - T\left[\Delta S^0(T_R) + \Delta C_p \ln\left(\frac{T}{T_R}\right)\right]$$

$$\Delta G^0(T) = \Delta H^0(T_R) - T\Delta S^0(T_R) + \Delta C_p\left[(T - T_R) - T\ln\left(\frac{T}{T_R}\right)\right]$$

where T_R is the reference temperature and T is the temperature of interest.

The physical state transition occurs at a characteristics temperature called transition midpoint, T_m. Assuming two-state transition, ΔG^0 is equal to zero if T_R is equal to T_m (i.e. $K = 1$). As a result ΔS^0 (T_m) is just ΔH^0 $(T_m)/T_m$. ΔG^0 (T) thus becomes:

$$\Delta G_0(T) = \Delta H_m^0\left(1 - \frac{T}{T_m}\right) + \Delta C_p\left[(T - T_m) - T\ln\left(\frac{T}{T_m}\right)\right]$$

where ΔH_m^0 is the value of ΔH^0 at T_m. This equation is often called "modified Gibbs–Helmotz equation".

To characterize the thermodynamic stability of modified gum means to determine ΔG^0, ΔH^0 and ΔS^0 at a given temperature and obtain ΔC_p to predict the change of these three parameters with temperature. The enthalpy and entropy of the depolymerized grafted gum at a temperature can be calculated according to the following relations (Kirchhoff's law):

$$\Delta H^0(T) = \Delta H_m^0 + \Delta C_p(T - T_m)$$

$$\Delta S_0(T) = \Delta S_m^0 + \Delta C_p \ln\left(\frac{T}{T_m}\right)$$

where ΔS_m^0 is $\Delta H_m^0/T_m$, the entropy of depolymerized grafted gum at T_m.

DSC measures the reduction of heat capacity of the molecule of interest as a function of temperature. The transition is recognized as a sharp endothermic peak centered at T_m and the maximum in C_p occurs directly at T_m. Integration of C_p versus T curve yields the transition enthalpy (ΔH_m^0) and the shift in the baseline yields the ΔC_p. DSC is the only method for the determination of ΔH_m^0.

DSC thermogram of metformin HCl was recorded without second heating.

2.3.6. Scanning electron microscopy

The morphology of the GG and AAm-g-GG was examined with an SEM system (JMS-6390, JEOL, and Tokyo, Japan). The samples were coated using gold to increase the conductivity of the electron beam. The operating conditions were an accelerating voltage of 10 kV, the working distance were 12 mm at spot size of 45.

2.3.7. Acute oral toxicity study

Acute oral toxicity study of acrylamide grafted gellan gum (AAm-g-GG) 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 nulliporous 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 sufficient food and deionized reverse osmosis water was available to them ad libitum at 20–25 °C and 40–70% relative humidity in a 12 h light on/off cycle. A single dose of 2000 mg/kg body weight of AAm-g-GG 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.

2.3.8. Biodegradability study of grafted gellan gum

Sample films of acrylamide grafted gellan gum was inoculated with *Aspergillus niger* on a medium and incubated at surrounding 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.

2.4. Preparation of controlled release tablets of metformin HCl

All seven formed grafted gellan gum (400 mg) were taken for the preparation of sustained release metformin 500 mg tablet. The gum was swelled with minimum amount of hot water to form dough like mass, to which PVP-K 30 (80 mg) and MTF (500 mg) was added and passed through 18 mesh size to form granules. These granules were dried at 40 °C temperature and passed through 22 mesh size. The granules were lubricated with talc (10 mg) and magnesium trisilicate (10 mg). The tablets were compressed at an average weight of 1 g using a rotary tablet machine with 13 mm single punch diameter (Cadmach, Ahmadabad, India). Hardness was found to be in the range of 5–6 kg/cm^2.

2.4.1. Powder X-ray diffraction

X-ray powder diffractometry (PXRD) of MTF and formulation were recorded using X-ray diffractometer (Bruker AXS D8 Advance,

Germany Configuration: Vertical, Theta/2 Theta geometry) X-ray source was of Cu, wavelength 1.5406 Å detector: Si (Li) PSD. The diffractometer was run at a scanning speed of 2°/min and a chart speed of 2°/2 cm per 2θ and the angular range fixed was from 3° to 80°.

2.4.2. In vitro drug release studies and release mechanism

In vitro drug dissolution rates from all seven batches of formulated tablets were carried out using USP dissolution test apparatus (TDT-08L, Electrolab, Mumbai, India), at 900 mL phosphate buffer (pH 6.8), maintained at 37 ± 0.5 °C with a stirrer rotation speed of 100 rev/min (USP method II). 5 mL of aliquot was withdrawn at specified intervals using a pipette. MTF released from tablet at different time was measured spectrophotometrically at the λ_{max} value at 232 nm. The in vitro drug release data were fitted to various release kinetic model viz. zero order, 1st order, Higuchi model and Hixson–Crowell. These models fail to explain the drug release mechanism due to the swelling (upon hydration) along with gradual erosion of the matrix. Therefore the dissolution data were fitted to the well known exponential equation, i.e. Koresmeyer–Peppas model.

Zero order model: $Q_t = K_0 t$ (Wyatt, 1999).
First order model: $\ln Q_t = \ln Q_0 - K_1 t$.
Higuchi model: $Q_t = K_p t^n$ (Higuchi, 1963).
Hixson–Crowell: ${Q_0}^{1/3} - {Q_0}^{1/3} = K_{HC} t$.
Koresmeyer–Peppas model: $Q_t = K_H t^{1/2}$ (Koresmeyer, Gurny, Doelker, Buri, & Peppas, 1983).

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, Case-II transport and Super Case-II transport (Costa & Loba, 2001), respectively.

3. Results and discussion

3.1. Synthesis of acrylamide grafted gellan gum

Synthesis of acrylamide grafted gellan gum was carried out by the graft co-polymerization of acrylamide onto gellan gum. Cerric ammonium nitrate is usually employed for initiating free radical graft copolymerization. In the earlier study, grafting of acrylamide onto various natural polysaccharides was prepared using cerric (IV) induced free radical graft copolymerization. Recently microwave assisted graft copolymerization of acrylate monomer onto guar gum (Singh, Tiwari, Tripathi, & Sanghi, 2004), chitosan (Singh, Tiwari, Tripathi, & Sanghi, 2006), starch (Huang & Chen, 1999), artimesia gum (Zhang, Zhang, Yuan & Wang, 2007) and Xanthan gum (Kumar, Singk, & Ahuja, 2009) have been reported. Table 1 presents the different synthetic conditions of microwave-assisted, cerric induced graft copolymerization, % grafting, % grafting efficiency and % conversion of grafted gums. Table 2 presents the nitrogen content and grafting efficiency after extraction with 20% (v/v) aqueous methanol and 30% (v/v) aqueous ethanol for all the different batches of grafted gum. It is clear that there was no significant difference in the nitrogen content and grafting efficiency between the extraction with 20% (v/v) aqueous methanol and 30% (v/v) aqueous ethanol. Therefore, it may be concluded that both extraction procedure are equally successful to remove the homopolymer formed during the polymerization reaction. For the optimization of the synthetic conditions, amount of CAN, acrylamide and microwave irradiation time were taken as independent variables by keeping the other parameters constant. From Table 1 it is clear that amount of CAN has major contribution for the higher grafting efficiency irrespective of other variables. The reactive vicinal group, where the grafting is initiated on GG backbone is anomeric —CHOH. The overall reaction mechanism is that, cerric ion attacks the GG macrochains and forms a GG–cerric complex. The cerric (IV) ions in the complex are then reduced to cerric (III) ions by oxidizing hydrogen atom and thereby creating a free radical onto GG backbone. So, a threshold amount of redox initiator is required for the formation of the free radical. The grafting of AAm onto GG was then initiated by the free radical reacting with the monomer. In the presence of AAm, the GG free radical is chemically coupled to the monomer unit, thereby resulting in a covalent bond between AAm and GG to create the chain reaction for propagation. Finally, termination was achieved through a combination of two radicals. The microwave irradiation results in rapid transfer of energy in the bulk of the reaction mixture, which reduces reaction time therefore it acts as a catalyst and gives a synergistic activity. This phenomenon corroborates the results of the cerric (IV) hyphenated microwave (Ce (IV)-MW) assisted graft copolymerization. The synthetic conditions for the formulation F1 were not enough to initiate the grafting. In case of F6 the % grafting is higher than that of F7, though microwave irradiation time was more in case of F7 but other parameters were (amount of CAN and amount of acrylamide) same. This may be due to the fact that in case of F6 and F7 a saturation of free radical points on polymer back bone occurs in a certain time period of microwave irradiation. After that a further irradiation leads to the breakage of propagated chains on the free radical sites.

3.2. Elemental analysis

The elemental analysis results for GG and seven different batches of grafted gums are shown in Table 1. There is no nitrogen in GG. The significant value of nitrogen % in all seven batches of AAm-g-GG confirms that acrylamide grafted onto the backbone of the GG. The higher values of %N in case of F5 attribute the maximum % grafting, proves that the higher value of all the independent variables were responsible for the maximum yield.

3.3. Infrared spectral analysis

Infrared spectra of GG and grafted GG are shown in Fig. 2(a) and (b), respectively. Gellan gum showed characteristics peaks at 1660 cm^{-1} for carbonyl group indicating C=O stretching, strong bond at 3633 cm^{-1} for OH group, band at 2924–3300 cm^{-1} for C—H stretching, band at 1406 cm^{-1} for methyl C—H bonding and band at 891.11 cm^{-1} for C—O stretching for alkyl ether.

Some differences were observed in spectra of graft products compared to gellan gum. Acrylamide grafted gellan gum shows characteristic peaks at 3738–3190 cm^{-1} due to NH_2 group, addition of acrylamide which was grafted onto gellan gum. An additional peak from 1672 to 1759 cm^{-1} can also be observed due to HN bending. Peak at 1041–1093 cm^{-1} is also seen due to CH—O—CH_2 group which occurs due to grafting reaction between OH group of C_2 and π bond of acrylamide.

Infrared spectra of metformin hydrochloride and tablet formulation are shown in Fig. 2(c) and (d), respectively. Metformin shows the characteristic peaks at 3370 cm^{-1} and peaks within 3177–3294 cm^{-1} for N—H asymmetric stretching and N—H symmetric stretching, respectively. Peak at 2974 cm^{-1} and 2814 cm^{-1} indicates CH_3 asymmetric stretching and CH_3 symmetric stretching, respectively. Peaks at 1622 cm^{-1} and 1474 cm^{-1} correspond to C=N stretching and CH_3 asymmetric deformation, respectively. All these peaks present in the tablet formulation, which proves the compatibility between the drug and grafted polymer (AAm-g-GG).

Table 2
Measurement of nitrogen content and grafting parameters after extraction with 20% (v/v) aqueous methanol and 30% (v/v) aqueous ethanol.

Batch	After extraction with 20% methanol		After extraction with 30% ethanol	
	Nitrogen content (%)	% grafting efficiency	Nitrogen content (%)	% grafting efficiency
F2	10.88	10.58	10.21	10.57
F3	7.71	6.04	7.09	6.36
F4	7.97	6.80	7.81	7.10
F5	15.39	98.08	15.03	93.65
F6	14.22	56.01	12.23	53.01
F7	12.02	45.37	11.79	45.03
F8	14.36	60.63	14.11	55.01

3.4. ^{13}C Solid state nuclear magnetic resonance

The ^{13}C nuclear magnetic resonance spectra of the gellan gum and AAm-g-GG are shown in Fig. 3(a) and (b), respectively. It is being observed from the δ values of ^{13}C NMR spectrum of GG [Fig. 3(a)] that it has five distinct peaks. The absorption peak at $\delta = 18$ ppm is for the $-CH_3$ group of Rhamnose. The absorption peak at $\delta = 62$ ppm corresponds to $-CH_2OH$ group of glucose. The absorption peak of high intensity at $\delta = 75$ ppm is for aromatic carbons of sugar moieties.

The absorption peak at $\delta = 103$ ppm may be due to ring anomeric carbons. The peak at $\delta = 174$ ppm is consistent with $-COOH$ group of glucuronic acid residue of gellan gum, thus indicating presence of sugar acid.

Sen, Singh, and Pal (2010) reported that acrylamide had three major peaks. The peak at $\delta = 179$ ppm was for the amide carbonyl carbon. Peaks at $\delta = 130$ and $\delta = 133$ ppm were for two sp^2 hybridized carbon atoms (i.e. $CH_2{=}CH-$).

In the ^{13}C NMR spectrum of AAm-g-GG [Fig. 3(b)], there are additional bands present, compared with base polysaccharide (GG). The peak at $\delta = 41$ ppm is for $(-CH-CH_2-CH-)_n$ groups those have been formed during the polymerization reaction of acrylamide. The presence of very intense peak at $\delta = 179$ ppm indicates carbon atoms of $-CONH_2$ group. The shape of these bands suggests that it is composed of multiple signals as well as the signals for $-C{=}O$ in glucuronic acid moiety. Absorption peak at $\delta = 75$ ppm indicates the aromatic carbon $-C-OH$ groups.

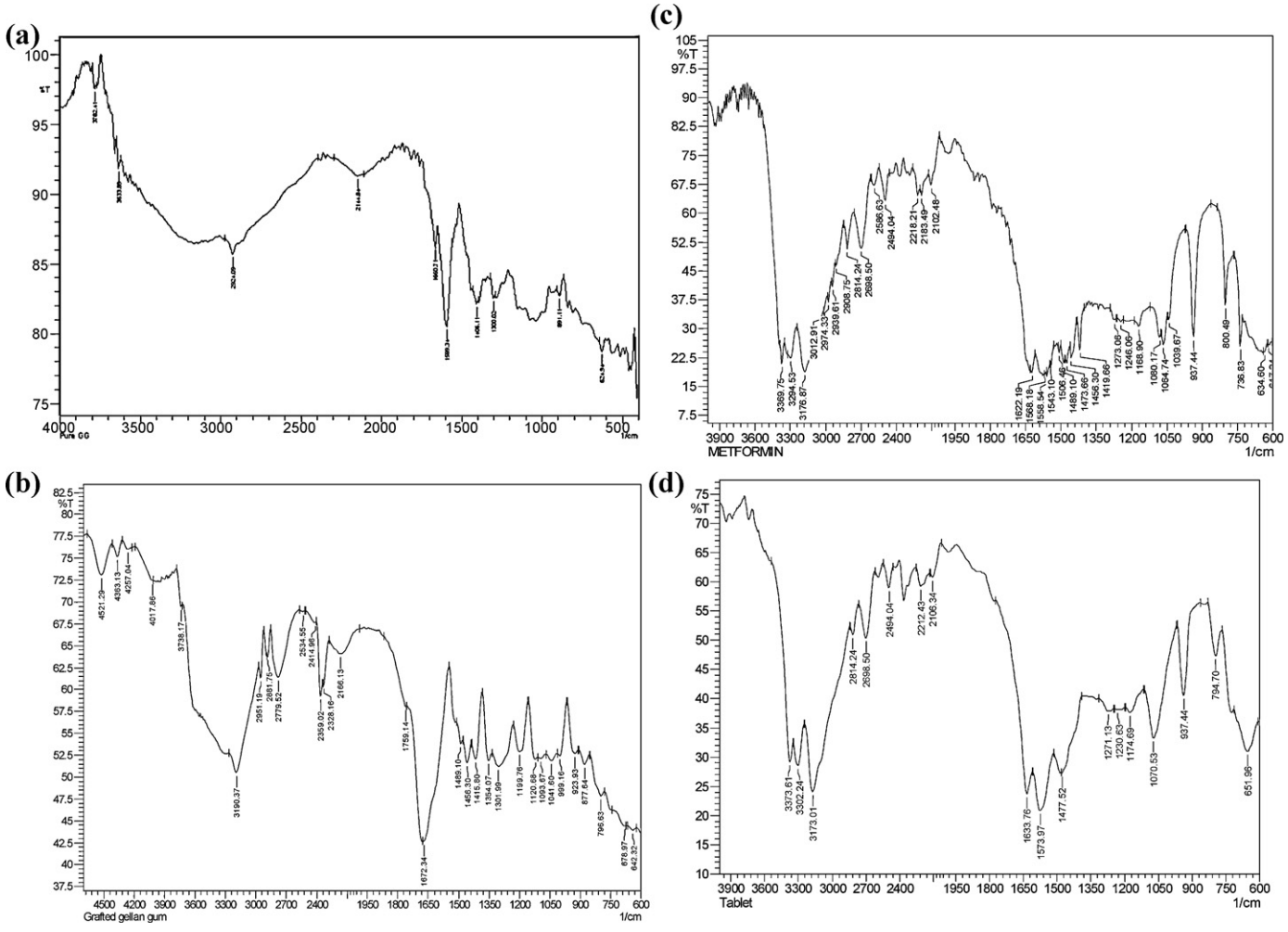

Fig. 2. FTIR spectrum of (a) gellan gum (GG1), (b) acrylamide-grafted-gellan gum (AAm-g-GG), (c) metformin hydrochloride and (d) tablet formulation.

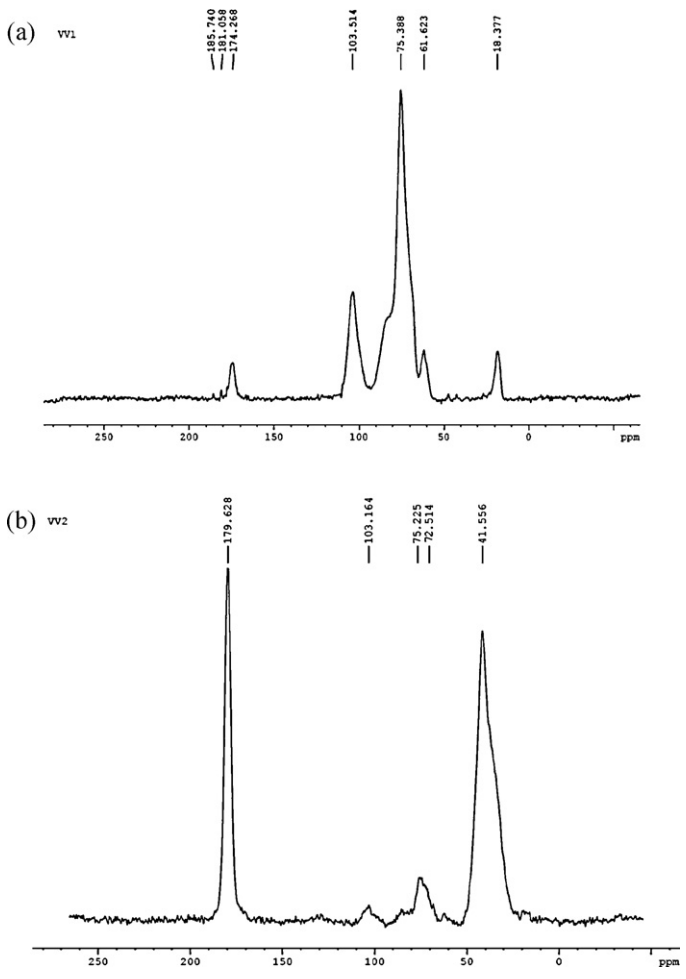

Fig. 3. Solid state ^{13}C NMR spectra of (a) gellean gum (GG) and (b) acrylamide grafted gellen gum (AAm-g-GG).

Absorption peak at $\delta = 103$ ppm indicates ring anomeric carbons. Absorption peak at $\delta = 18$ ppm indicates presence of $-CH_3$ groups of rhamnose but the low resolution suggests the contrary. Absorption peak at $\delta = 72$ ppm indicates the carbon which is attached with the oxygen of ring —C—OH group. Peak at $\delta = 133$ ppm and $\delta = 130$ ppm which represented sp^2 carbons of acrylamide gets converted to the sp^3 hybridized carbon atom attached with oxygen ($\delta = 72$ ppm), thus proving grafting reaction.

3.5. Viscosity study

Viscosity results of different batches of AAm-g-GG and native gum (GG) are shown in Table 1. It is clear that the viscosity of the native gum is less as compared to grafted gum. The viscosity of F2, F3 and F4 batches did not significantly increase due to the less grafting efficiency. The maximum viscosity was achieved from F5 batch, which complies with the maximum grafting efficiency of this modified gum. The viscosity of a fluid can be defined as the resistance generated during flow. In case of linear chain polymer, the viscosity will be less as compared to branched polymer network. During grafting, acrylamide side chains attach with the polymer backbone and form a branched polymer network which leads to increase the viscosity. Grafting efficiency is the percentage determination of acrylamide attachment per unit amount of acrylamide given during the synthesis. For the other batches i.e. F6, F7 and F8, the viscosity is also linearly related with the respective grafting efficiency.

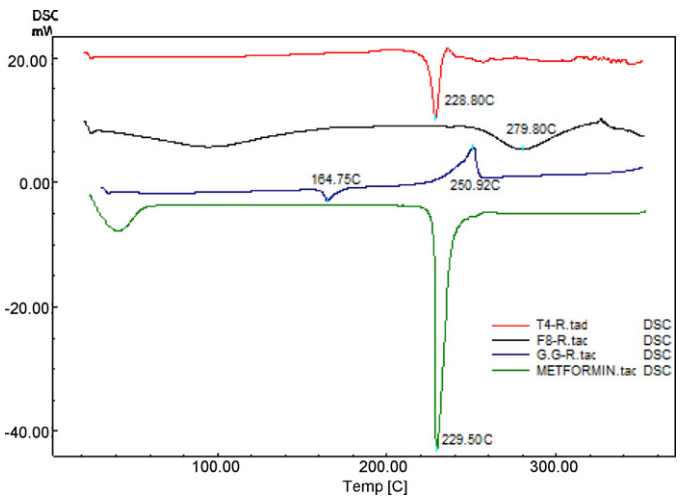

Fig. 4. DSC thermogram of gellan gum (G.G-R.tac), acrylamide grafted gellan gum (F8-R.tac), metformin hydrochloride (METFORMIN.tac) and tablet formulation (T4-R.tac).

3.6. Differential scanning calorimetry

DSC curves for the 2nd run of different batches of GG-g-AAm are shown in Fig. 4. Peak at around 70 °C, due to loss of absorbed moisture in sample, usually observed in GG is absent. The moisture was eliminated during 1st heating of the sample. An endothermic peak at 164 °C and one exothermic peak at 250 °C are recorded in DSC curve of GG. The exothermic peak is probably due to depolymerization with formation of water, carbon monoxide and methane (Zohuriaan & Shokrolahi, 2004). Graft polymer shows a distinct feature in the DSC curve (Fig. 4) having three endothermic peaks at 279 °C, 328 °C and 330 °C. The transition midpoint temperature (T_m) varies with the different process parameters, which were taken for the formation of the GG-g-AAm. It can be seen in Table 3 that the T_m value for a group of formulations (F5, F6, F7 and F8) (279.2–282.32 °C) was greater than that of another group (F2, F3 and F4) which were from 262 °C to 269 °C. T_m is an indicator of thermostability and generally the higher the value of T_m, the more thermodynamically stable is the macromolecule. So, the formulations F5, F6, F7 and F8 are more thermodynamically stable than F2, F3 and F4 which complies with higher GE% of formulations F5, F6, F7 and F8 (Table 3). The differences in values of Gibb's free energy or standard free energy (ΔG^0), enthalpy and entropy of depolymerized grafted gum have been shown in Table 3. The maximum negative value of ΔG^0 indicates the higher stability of the grafted gum. Higher negative value of ΔG^0 of the formulations F5, F6, F7 and F8 are in between -33.22 and $-54.25\,J\,g^{-1}$ in comparison to the value of F2, F3 and F4 which lies between -5.85 and $-6.39\,J\,g^{-1}$, indicating higher thermodynamic stability of formulations F5, F6, F7 and F8 thus, complying with the results shown in Table 3.

As shown in Fig. 4, MTF shows a very sharp peak at 228.8 °C indicating crystalline nature of MTF. Similarly, thermogram of tablet formulation also shows unaltered sharp peak of MTF thus proving that no sign of incompatibility exists between the drug and excipients.

3.7. Scanning electron microscopy

Fig. 5 shows the scanning electron micrographs of gellan gum and grafted gellan gum. The acrylamide particles are polyhedral in shape (figure not shown), while the gellan gum particles are fibrous in nature. The SEM images of grafted copolymer show that the grafting of acrylamide onto gellan gum brings about the change in the shape and size of the gellan gum particles. It is noticed that

Table 3
Thermodynamic parameters derived from DSC of various batches of acrylamide grafted gellam gum (AAm-g-GG).

Batch	Peak point T_m (°C)	Heat of fusion ΔH_s (J g^{-1})	Heat capacity ΔC_p (mW)	Free energy ΔG^0 (J g^{-1})	Enthalpy ΔH^0 (J g^{-1})	Entropy ΔS^0 (J g^{-1} K^{-1})
F2	262.83	−45.635	−0.22	−6.40	6.69	−0.01
F3	268.00	−65.36	−0.36	−5.85	22.12	−0.03
F4	269.42	−42.48	−0.20	−5.96	6.40	−0.01
F5	280.54	−226.72	−0.71	−54.25	−45.29	−0.18
F6	281.38	−230.73	−0.96	−38.17	15.40	−0.25
F7	282.32	−186.40	−0.74	−33.22	4.02	−0.16
F8	279.80	−202.98	−0.72	−42.68	−19.52	−0.16

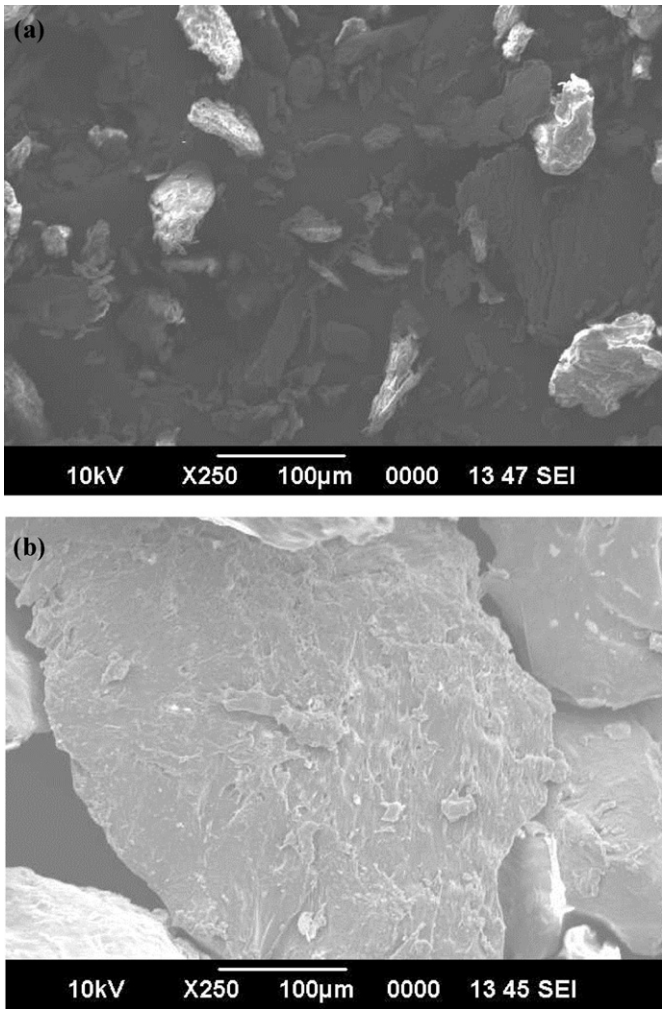

Fig. 5. SEM images of (a) gellan gum (GG) and (b) acrylamide grafted gellan gum (AAm-g-GG).

AAm-g-GG particles are of larger dimensions that the native gum particles. The grafting introduces changes on the surface and size of the carbohydrate particles. The irregular morphology as a lobule aggregate with higher heterogeneity is shown in case of grafted polymer.

3.8. Acute toxicity study

The results are shown in Table 4. It is clear that there was no mortality found within the observation period of 14 days after dosing. As per the "Organization of Economic Co-operation and Development (OECD) guideline for the test of chemicals" 425, adopted "17 December 2001" Annexure – 4, the LD_{50} value is greater than 2000 mg/kg dose of AAm-g-GG, so recognizing the need to protect animal welfare, testing in animal is discouraged. As per the globally harmonized system (GHS) if LD_{50} value is greater than the 2000 mg/kg dose then the test product will be fallen under the "Category 5" and toxicity rating will be "zero". So, AAm-g-GG is under the "category 5" as well as toxicity rating is "zero".

3.9. Biodegradability study of grafted gellan gum

Fig. 6(a)–(e) shows the fungal growth for various batches of films of acrylamide grafted gellan gum. After 21 days, fungi growth was clearly visible under microscope for all the batches of acrylamide grafted gellan gum. It is clearly visible in the figures which shows apparent fungi growth for 0, 3, 7, 14 and 21 days in all the Petri dishes. The apparent growth of fungi in mineral salts agar medium (no carbon contains) proves that the carbon present in the grafted polymer had been utilized by the fungi for its growth. Thus it can be easily concluded that acrylamide grafted gellan gum is biodegradable in nature.

3.10. Powder X-ray diffraction

X-ray difractrogram of pure metformin hydrochloride and drug loaded tablet formulation are represented in Fig. 7. From the difractogram it is possible to understand the drug is in crystaline state in its native form as well as in tablet. It is clear from Fig. 7 that there is no incompatibility between the drug and AAm-g-GG.

Table 4
Mortality rate of animals after a single dose of 2000 mg/kg body weight.

Observation time period	Mortality				
	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5
30 min	O	O	O	O	O
4 h	O	O	O	O	O
1st day	O	O	O	O	O
3rd day	O	O	O	O	O
7th day	O	O	O	O	O
14th day	O	O	O	O	O

'O', survival and 'X', death.

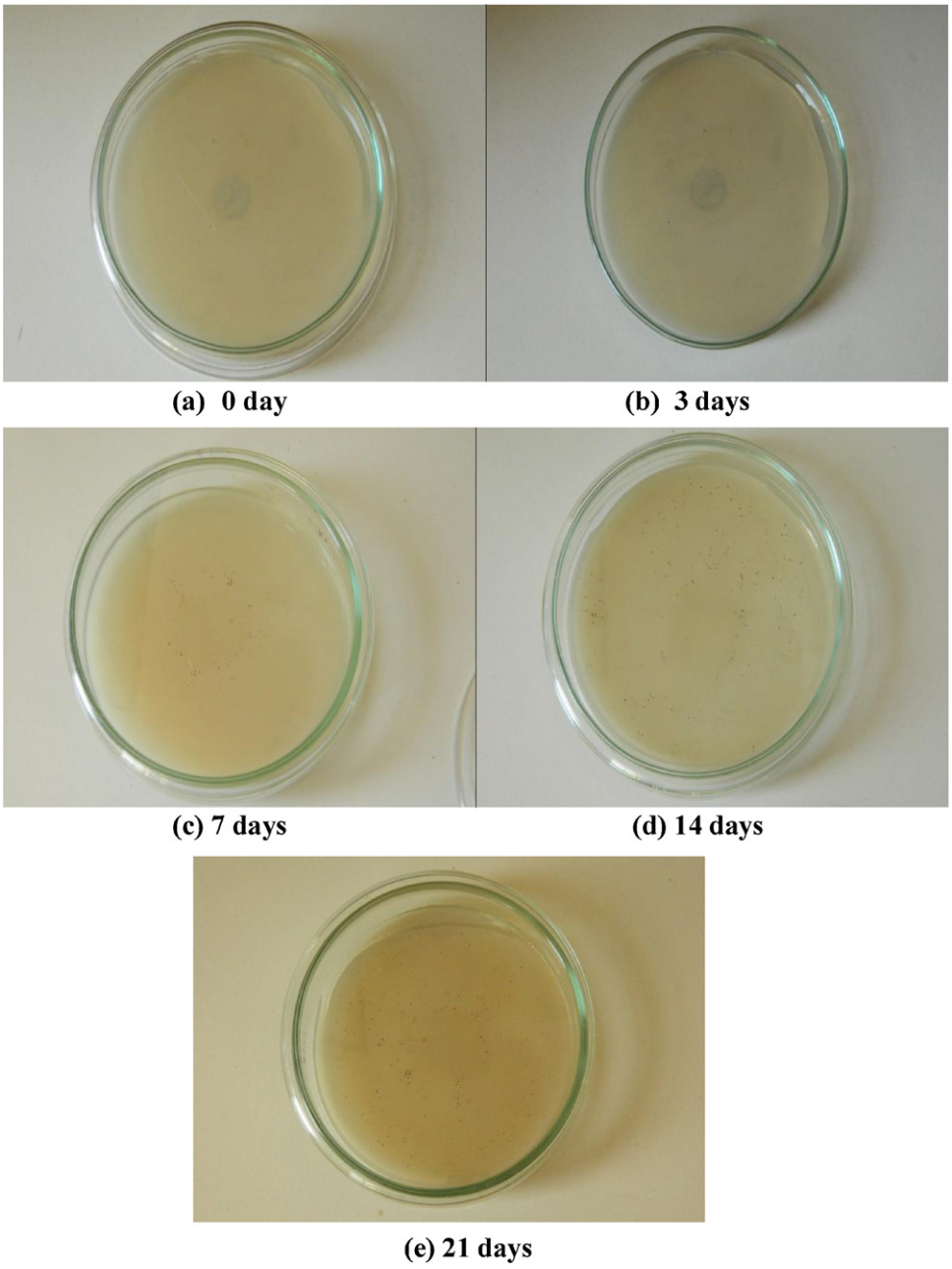

Fig. 6. Evidence of fungi growth (*A. niger*) on surface of acrylamide grafted gellan gum films.

3.11. In vitro drug release studies and release mechanism

The results of in vitro drug release study from different tablet formulations are presented in Fig. 8. The cumulative percentage of drug release (CPR) from various formulations verses time plot showed that the release of drug from the matrix tablet is more rapid (2–4 h) in case of T2, T3 and T4 than that of other tablet formulations (T5–T8). This occurred due to the fact that in case of

Table 5
Kinetic modeling of release data from different tablet formulations containing different batches of AAm-g-GG and $t_{50\%}$.

Formulation code	Zero order	First order	Higuchi kinetic	Hixson Crowell	Korsemeyer Peppas		$t_{50\%}$ (h)
					n	R^2	
T1	–	–	–	–	–	–	–
T2	0.903	0.826	0.966	0.955	0.363	0.979	0.49
T3	0.924	0.871	0.965	0.954	0.483	0.969	0.42
T4	0.948	0.895	0.981	0.97	0.524	0.981	0.49
T5	0.969	0.86	0.991	0.984	0.556	0.992	2.44
T6	0.961	0.86	0.988	0.98	0.517	0.989	2.17
T7	0.957	0.858	0.988	0.98	0.483	0.989	1.94
T8	0.96	0.848	0.988	0.978	0.533	0.988	2.27

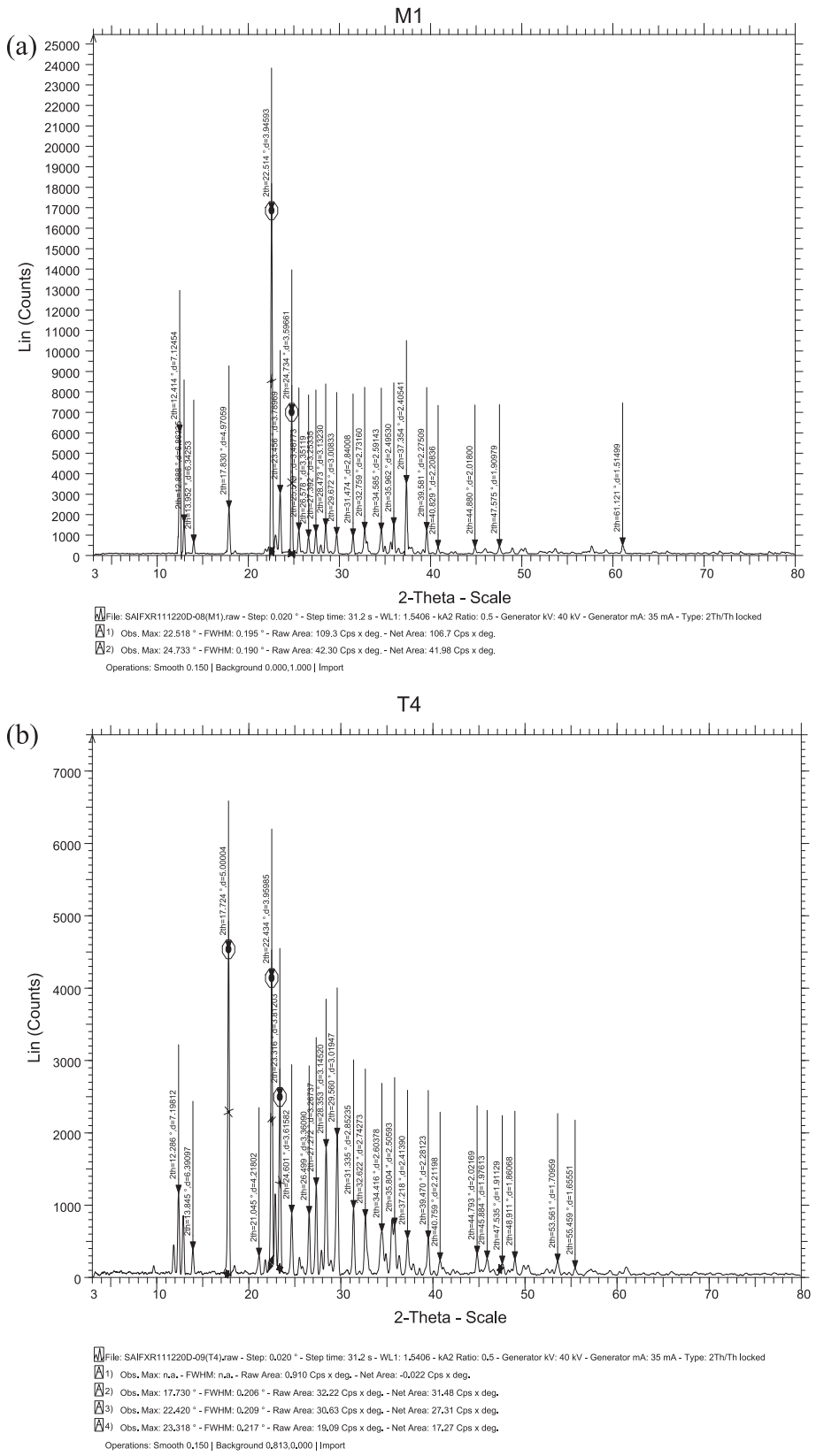

Fig. 7. X-ray diffractogram of (a) metformin hydrochloride (M1) and (b) tablet formulation (T4).

those three formulations the matrix is made up of grafted gums having lower grafting efficiency, thus these less branched network of the polymer leads to rapid swelling of the matrix. In case of tablet T5, T6, T7 and T8 drug release continued up to 8 h as the matrix is formed of comparatively greater branched grafted gum, which leads to retarded swelling. The regression coefficient (R^2) values from different kinetic models, diffusion coefficient (n) and $t_{50\%}$ values are represented in Table 5. This result also resembles with the

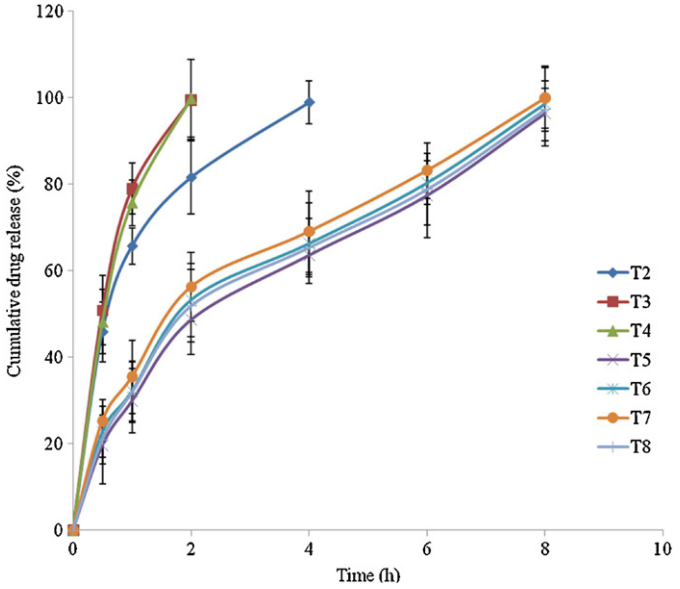

Fig. 8. Release profile (percent release ± SD, $n = 3$) of MTF from the tablet formulation containing different batches (F2–F8) of AAm-g-GG.

$t_{50\%}$ values (Table 5). All the formulations follow the Higuchi release kinetics (R^2 = 0.965 to 0.991). The diffusion coefficient (n) values for all the formulations are within the range of 0.363–0.556 thus signifying the release mechanism is Case-I (Fickian) diffusion or square root of time kinetics.

4. Conclusion

Acrylamide grafted gellan gum (AAm-g-GG) was synthesized using microwave assisted cerric (IV) ion induced grafting method. The synthetic parameters were optimized by using three independent process variables, amount of CAN, amount of acrylamide and microwave irradiation time. Higher level of all these three variables had given the higher grafting efficiency (GE%) of grafted gum. The grafting of acrylamide onto gellan gum was confirmed by FTIR and ^{13}C NMR. The spontaneity of the grafting process and the thermodynamic stability of grafted gum were successfully evaluated from DSC. The morphology of the modified gum was lobule shape and more heterogenic than the native gum which was revealed by SEM. The monomer (acrylamide) is toxic in nature but from the toxicity study in mice it is revealed that there is no morbidity or mortality case during the LD_{50} study at a dose of 2000 mg/kg body weight of AAm-g-GG. Sustained release tablet of metformin hydrochloride was developed by using this grafted gum as a rate controlling polymer. It retarded the release up to 8 h and the release profile followed Higuchi square root kinetic model. The release mechanism was governed by Fickian diffusion. FTIR and XRD studies of drug and grafted polymer were done and it shows no sign of incompatibility. Thus, microwave assisted cerric (IV) induced graft co-polymerization is an easy, efficient, less time consuming and reproducible (due to the supply of fixed energy form microwave) method for the development of graft co-polymer which can be used as an rate controlling polymer for the development of sustained release dosage from.

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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