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Synthesis of acrylate guar-gum for delivery of bio-active molecules

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Abstract. **Modification of natural polymers by graft copolymerization is a promising technique as it functionalizes these biopolymer to their potential, imparting desirable properties onto them. Grafting with vinyl monomers is the route for modifying the properties of the naturally occurring guar-gum for their better industrial exploitation and development of various commercial products. Acrylated guar-gum chain is synthesized and analysed using Fourier transform infrared, differential scanning calorimetry and X-ray diffraction techniques to gain an insight into the particle size and structural features. Chlorpyrifos is then entrapped into the polymer, and its release is studied under various conditions. Critical factors influencing the size, entrapment efficiency and release behaviour of entrapped chlorpyrifos have been studied.**

Keywords. **Polymers; graft copolymerization; guar-gum; FT-IR; DSC; XRD.**

1. Introduction

The development of controlled-release formulations (CRFs) is highly desirable both from a convenience and compliance perspective.[1] These formulations are capable of releasing the desired molecules at a prescribed rate, leading to relatively constant dosing to the target area. Yet another benefit is the ability to administer target molecules in infrequent regimens.[2] Various reports have been published for the novel application of CRFs for delivery of bio-active molecules in the field of drug delivery, imaging, diagnosis and pesticide delivery. Focusing on pesticide delivery CRFs have the potential to reduce the environmental burdens associated with the use of pesticides. CRFs have proven a promising boon for farmers and pesticide applicators such that they now need to apply the active ingredients in lesser quantity for covering a much larger area. Additionally, reducing the pesticide quantity, decreases risk to the environment. CRFs have also been tailored to selectively kill target insects leaving the useful ones alive.[3]

Guar-gum is a long-chain polysaccharide biopolymer[4] extracted from *Cyamopsis tetragonolobus* seeds and is widely used as a stabilizing, cogelling and thickening agent in soups, puddings, sauces, mayonnaise, ice creams and juices.[5] This is mainly due to the fact that these hydrophilic macromolecules produce high viscous solution, suspensions and gels at relatively low concentrations (<1 wt%). Besides the mentioned food applications, unmodified guar-gums are used in a number of industries (pharmaceutics, personal care, enhanced oil recovery) in which their high content of insoluble residues, weak and unstable solution transparency and slow dissolution speeds are desirable. Guar-gum is non-ionic and has a semi-flexible random coil conformation.[4] The chemical structure of guar-gum consists of D-mannose monomer units linked to each other by β-(1→4) linkage, and in order to form connection with the main chain, the D-galactose branch is joined by an α-(1→6) bond. On the average, the galactose branches occur on every other mannose unit. The exact ratio of galactose to mannose varies with the growing season. The average degree of substitution (D.S.) of galactose units is ∼0.61–0.69 and this molecular parameter is believed to be a constant for the species. At a lower degree of substitution galactomannans are not water soluble or have marginal solubility.[6] However for improved solubility, guar-gum is generally chemically modified (depolymerization, oxidation, hydroxyalkylation, carboxymethylation, cyanoethylation, quaternization and sulphonation) to produce guar-gum propyl and methyl ethers, esters and phosphates. De-polymerized and oxidized galactomannans show enhanced solubility, excellent resistance to shear degradation, salt tolerance, faster hydration rate, enzyme resistance, dispersibility, improved dry flow and better temperature stability. The main characteristics of guar-gum includes its solubility in hot and cold water (but insoluble in most organic solvents), strong hydrogen bonding properties, excellent thickening, emulsion stabilizing and film-forming properties. At very low concentration, guar-gum has excellent settling (flocculation) properties and it can act as a filter aid.[7] It is non-ionic and maintains a constant high viscosity over a broad range of pH. It is compatible with a variety of inorganic and organic substances including certain dyes and various constituents of food. The viscosity of guar-gum solution increases gradually with the increase in the concentration of guar-gum in water. The viscosity of guar-gum is influenced

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by temperature, pH, the presence of salts and other solids. It has excellent ability to control rheology by economic water phase management. It forms highly viscous colloidal dispersions when hydrated in cold water. The time required for complete hydration in water and to achieve maximum viscosities depends on various factors such as pH, temperature, grade of powder used, equipment. Among various strategies adopted to regulate the application of insecticide, encapsulation/entrapment is a very popular technique to develop controlled/sustained release device. In the process of encapsulation/entrapment an active ingredient is added to the biopolymer, which can then act as a slow or safe release carrier of the pesticide/insecticide.

Chlorpyrifos is a moderately toxic crystalline chlorinated organophosphate insecticide that inhibits acetyl cholinesterase and is used to control insect pests. However, it is a neurotoxin and is a suspected endocrine disruptor. It has also been associated with asthma, reproductive and developmental toxicity. Recent research indicates that foetus exposed to chlorpyrifos have an increased risk of delays in mental and motor development after birth and an increased occurrence of pervasive developmental disorders such as attention-deficit hyperactivity disorder (ADHD).[8] Yet another study has demonstrated a correlation between pre-natal chlorpyrifos exposure and lower weight and smaller head circumference at birth.[9] Chlorpyrifos has been found to be highly toxic to amphibians and a recent study by the United States Geological Survey found that chlorpyrifos oxon, a main breakdown metabolite product of chlorpyrifos is even more toxic to these animals. Thus over-application of chlorpyrifos needs to be controlled.

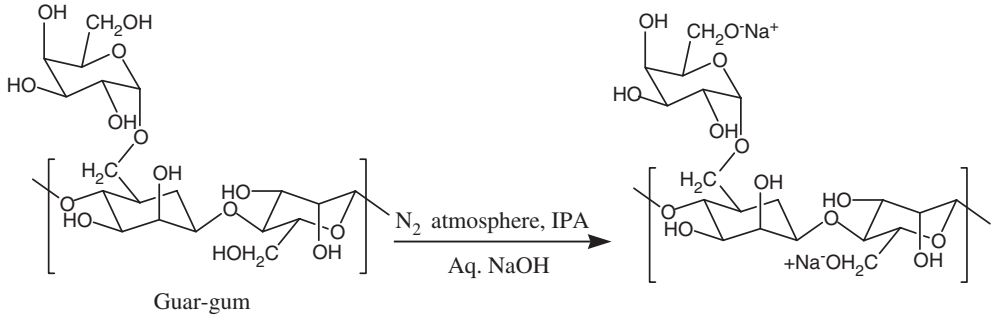
2. Experimental

2.1 *Synthesis of sodium salt of guar-gum (Na-GG)*

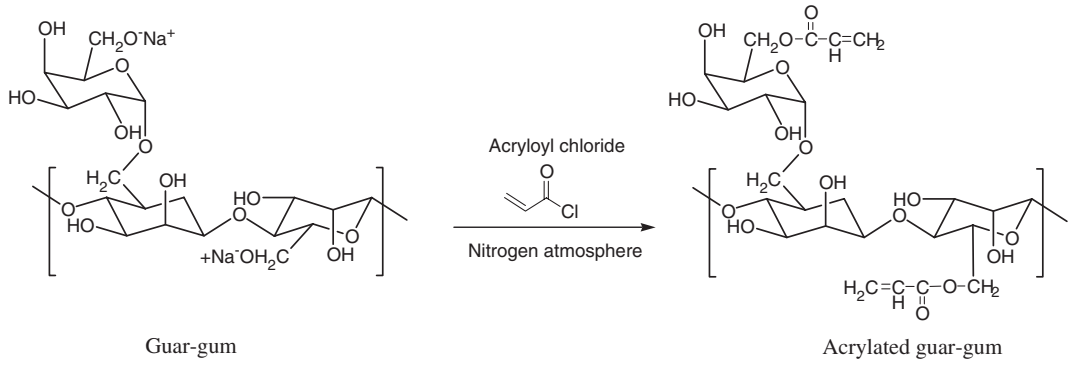
Na-GG has been prepared as per the reported method proposed by Pandya *et al*[10] (Scheme 1). A round bottom flask of 250 ml equipped with r.p.m.- and temperature-controlled stirrer and a nitrogen passing tube has been taken. Mixture of guar-gum (5 g) in 150 ml isopropyl alcohol (IPA) has been stirred vigorously under nitrogen blanket for 15 min. After this, 20 ml of 30% w/v aqueous sodium hydroxide has been added drop wise during next 15 min at room temperature. Stirring has been continued for 1 h to activate guar-gum. Sodium salt of guar-gum so obtained has been yellowish in appearance. This reaction mixture has been used as it is for acrylation reaction.

2.2 *Acrylation of guar-gum (GG-AC)*

Acrylation of guar-gum has been conducted under nitrogen blanket (Scheme 2). A round bottom flask of 250 ml equipped with r.p.m.- and temperature-controlled stirrer and a nitrogen passing tube has been used as reaction kettle.



Scheme 1. Synthesis of sodium salt of guar-gum.



Scheme 2. Acrylation of guar-gum.

In a typical acrylation reaction, 5 g of Na-GG (obtained earlier in IPA) has been stirred and purged with a slow stream of nitrogen for 1 h at room temperature. Inhibitor-free acryloyl chloride monomer (10 ml) has been then added drop wise through addition funnel. The acrylation reaction has been carried out for varying time intervals (1–4.5 h) and at varying temperatures (25–300°C). After completion of acrylation reaction, the mixture has been filtered and acidified with 1 N HCl to remove the unreacted hydroxides and sodium salt. The crude reaction mixture has been washed with distilled water and finally lyophilized to obtain pure acrylated powdered product of guar-gum.

2.3 *Grafting of acrylic acid onto the acrylated guar-gum chain (AA-GG-AC)*

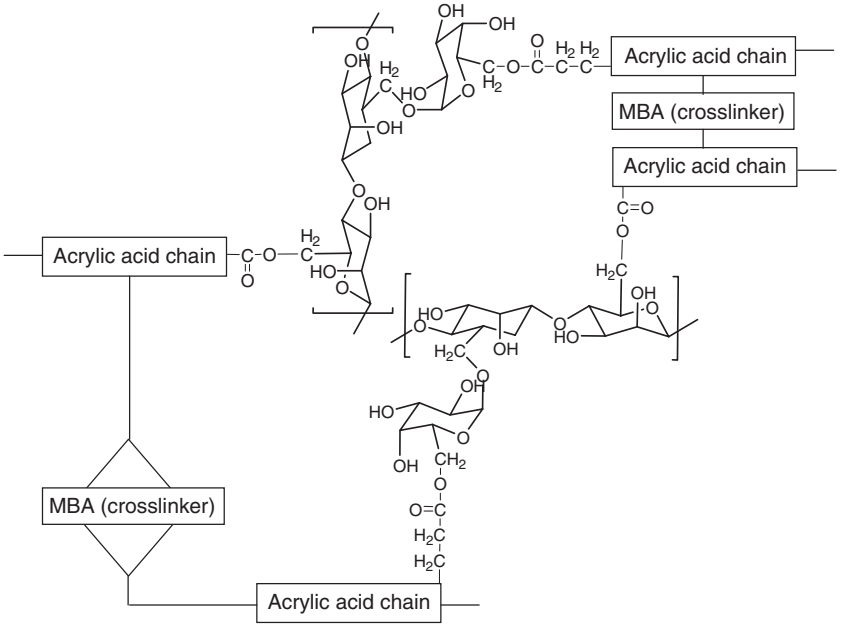
A weighed amount of acrylated guar-gum powder and acrylic acid has been fed slowly into a rapidly stirred mixture of 35 ml doubled-distilled water and 5 ml ethanol then allowed to hydrate for at least 2 h under constant stirring in a 50 ml round-bottomed flask at 60°C (Scheme 3). The solution has been bubbled with nitrogen for 15 min and then weighed amount of MBA as crosslinker has been added prior to the addition of the initiator APS to initiate the radical solution polymerization. After polymerization has been carried out for 30 min under nitrogen atmosphere, the supply of nitrogen gas has been stopped and the mixtures polymerization has been allowed to proceed at 60°C for 24 h. The grafted polymer obtained have been poured into dialysis bag and immersed in deionized water at room temperature for 3 days refreshing the water every day. The polymers obtained have been lyophilized to obtain moisture-free powdered material.

3. Results and discussion

Polymer modifications represent a valuable synthetic approach to unique polymer compositions, structure and properties not readily available by the direct polymerization of monomers. Modified polymeric products already exist in the commercial world (modified celluloses, for example) so the approach is not new. However, it is an interesting and challenging opportunity to develop new materials for a variety of specialty applications using the 'chemistry on polymers' approach. Guar-gum as mentioned earlier is a hydrophilic polymer; the acrylation of guar-gum would bring two effects. The first would be to, increase the hydrophobic nature of the resultant acrylated guar-gum derivatives by the consumption of hydroxyl groups and the other would be the formation of derivatives with free terminal double bonds which would help in reactive compatibilization of the other monomer. Acrylic acid grafting of guar-gum has been done with an objective of making the polymer pH responsive towards the alkaline pH in order to open a wide application in the area of controlled release of pesticide/insecticides. Although guar-gum has wide industrial applications, it suffers from some drawbacks like biodegradability, which limits its uses considerably. These drawbacks can be improved through the grafting of vinyl monomer, which imparts new properties to the polymeric backbone.

3.1 *FT-IR analyses*

The infrared spectra of guar-gum, sodium salt of guar-gum, acrylated guar-gum and their crosslinked product with acrylic acid have been recorded using a model Perkin Elmer spectrum BX2 FT-IR system. Spectra have been recorded with



Scheme 3. Grafting of acrylic acid onto the acrylated guar-gum chain.

Spectrum V5.3.1 software in the range 4000–400 cm^{-1}. The KBr pellet technique has been adopted for recording the spectra. Specifically speaking, approximately 2 mg of the desired powder sample has been thoroughly mixed with 200 mg of spectroscopic grade KBr and pressed into pellets for recording the spectra. Figure 1 shows the spectra of guar-gum, sodium salt of guar-gum, acrylated guar-gum and their crosslinked product with acrylic acid. In the case of pure guar-gum (figure 1a) the band at 3402 cm^{-1} represents O–H stretching vibration. The band at 2925 cm^{-1} is due to C–H stretching of the $–CH_2$ groups. The band at 1654 cm^{-1} is due to ring stretching of galactose and mannose. The bands due to C–H bending and O–H bending appear at 1435 and 1026 cm^{-1}, respectively. The band at 870 cm^{-1} can be attributed to the C–H deformation. In addition, the band in the region 1155 and 1015 cm^{-1} are due to C–O–C stretching and C–O stretching, respectively. The weak bands around 770 cm^{-1} are due to ring stretching and ring deformation of α-D-(1–4) and α-D-(1–6) linkages.

FT-IR spectrum of the sodium salt of guar-gum is depicted in figure 1b. The major difference between pure guar-gum and its sodium derivative is that the intensity of the peaks related to O–H group is diminished. In figure 1b, the band at 3401 cm^{-1} represents O–H stretching vibration with much diminished in intensity as compared with pure guar-gum. This indicates that some of the O–H group present in guar-gum is converted to its sodium derivative. This is also clear from the appearance of much diminished band at 1026 cm^{-1} due to O–H bending. The FT-IR spectrum of acryloyl chloride interaction with guar-gum is depicted in figure 1d. The evidence of the formation of acrylated guar-gum has been obtained from its spectrum that has additional peak at 1735 cm^{-1} which has been caused due to the C=O stretching vibrations, indicating the presence of ester group in acrylated guar-gum. Apart from these peaks, the change in the intensity and the position of the peaks of guar-gum are shifted. The peak due to the O–H stretching is sharper as compared to that seen in its precursor as on derivatization, the polymeric association is broken and also some hydroxy groups are also consumed in the acrylation of guar-gum. The spectra of GG-AC-AA have broad and diminished peaks at 1735 cm^{-1} (due to C=O stretching of ester) with a medium intensity peak at 1402 cm^{-1} in the spectrum. Additionally, the product formation has been confirmed by the disappearance of O–H bending vibration, indicating the mechanism of O–H side grafted reaction.

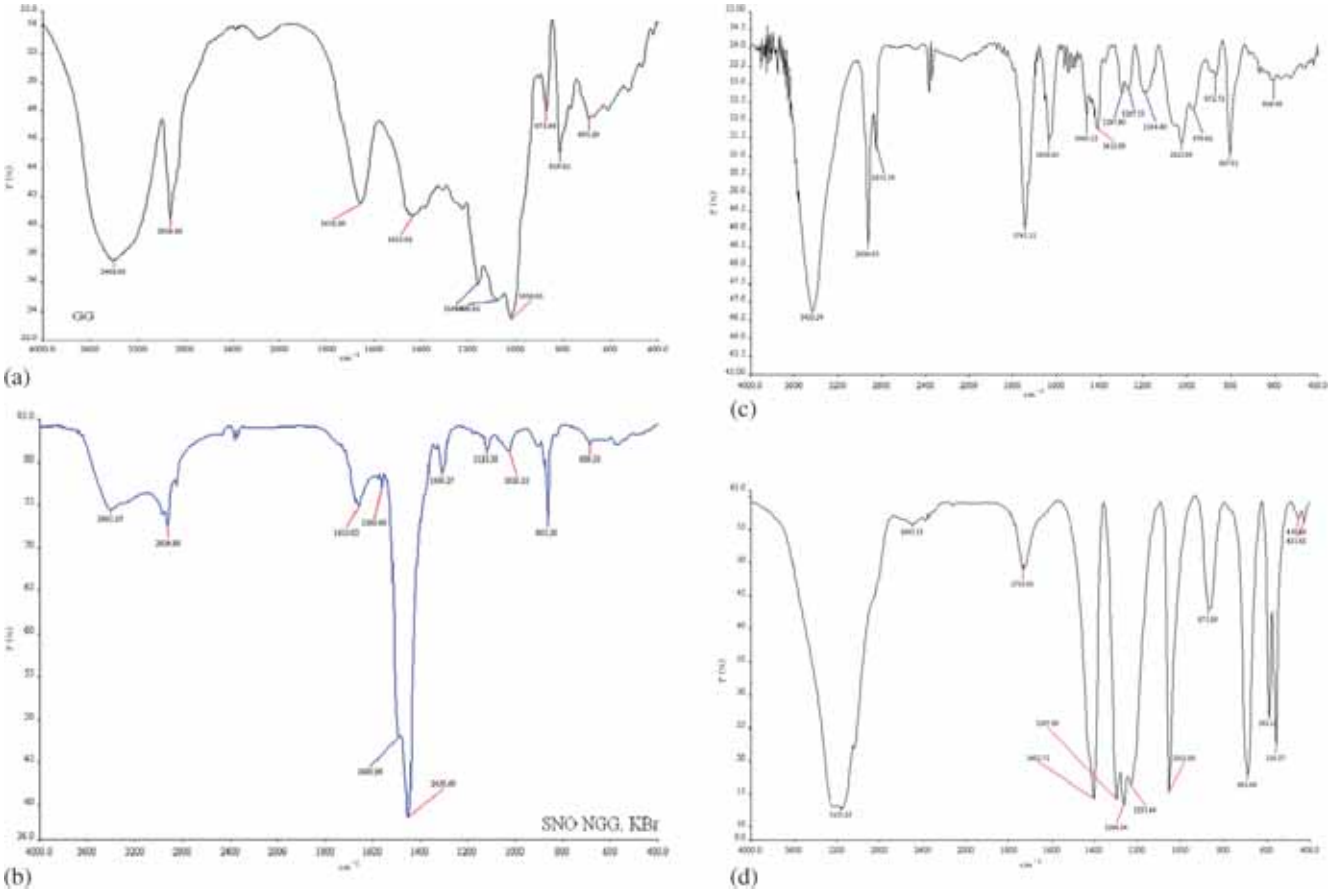


Figure 1. FT-IR spectra of **(a)** guar-gum (GG), **(b)** sodium salt of guar-gum (NaGG), (**c**) acrylated guar-gum (GG-AC) and (**d**) acrylic acid grafted on acrylated guar-gum (AA-GG-AC).

3.2 *Differential scanning calorimetry (DSC) study*

DSC thermograms have been obtained for pure guar-gum, sodium salt of guar-gum, acrylated guar-gum and their crosslinking with acrylic acid by using Perkin Elmer DSC Q200 V23.10 build 79 Version 2.0 instrument. Samples have been heated at a rate of 20°C min^{-1} under dry nitrogen at a rate of 50 ml min^{-1} in aluminium pans for a range of −90 to 450°C.

Figure 2a displays the DSC thermogram of pure guar-gum. Two endotherms correspond to T_g and T_c and one exotherm for T_m of pure guar-gum is displayed in the thermogram. Glass transition temperature (T_g) of guar-gum appears at 108.25°C. The dip temperatures 240.58 and 308.70°C correspond to the crystalline temperature (T_c) and melting temperature (T_m) of guar-gum, respectively. The broad humps of T_g, T_c and T_m are due to polymeric nature of the sample. Figure 2b exhibits DSC thermogram of sodium salt of guar-gum. The characteristic temperatures corresponding to T_g, T_c and T_m appear at 104.39, 129.90 and 286.51°C, respectively. This shows that due to the formation of sodium salt of the pure guar-gum the characteristics temperature of the polymer is shifted. In other words, crystallinity is achieved at a lower temperature (<110°C) as compared with the pure guar-gum. Figure 2c which displays the thermogram of acrylated guar-gum, T_c appears at 80.55°C. Here it is 27.70°C lesser than the pure guar-gum. T_g and T_m appear at 211.41 and 296.07°C, respectively. After 363.09°C the polymer starts decomposing. Figure 2d shows the spectrum of acrylic acid grafted guar-gum. T_g of the sample appears at 119.86°C and T_m at 254.52°C. It does not show any peak or hump related to T_c (temperature of crystallinity). This indicates that the sample is amorphous in nature and hence confirms the grafting of acrylic acid on the chain of acrylated guar-gum successfully.

3.3 *Wide angle X-ray diffraction*

Wide angle XRD pattern have been obtained for pure guar-gum, sodium salt of guar-gum, acrylated guar-gum and their crosslinking with acrylic acid by using Philips analytica PW 1830 X-ray VB equipped with a 2θ compensating slit, CuKα radiation (1.54 Å) at 40 kV, 40 mA passing through Ni filter with a wavelength of 0.154 nm at 20 mA and 35 kV. Data collection has been made in a continuous scan mode with a step size of 0.01° and step time of 1 s over a of 2θ range of 0–120°. Data analysis has been performed with PC-APD diffraction Software. Figure 3a displays the XRD pattern of guar-gum. Peaks obtained shows broad hump in the range of 3–30° 2θ which confirms its amorphous structure. Figure 3b displays the XRD of sodium salt of guar–gum. There is no noteworthy change observed in its diffraction pattern except that the broad hump appearing in the range of 20–60° 2θ, in this case has shifted.

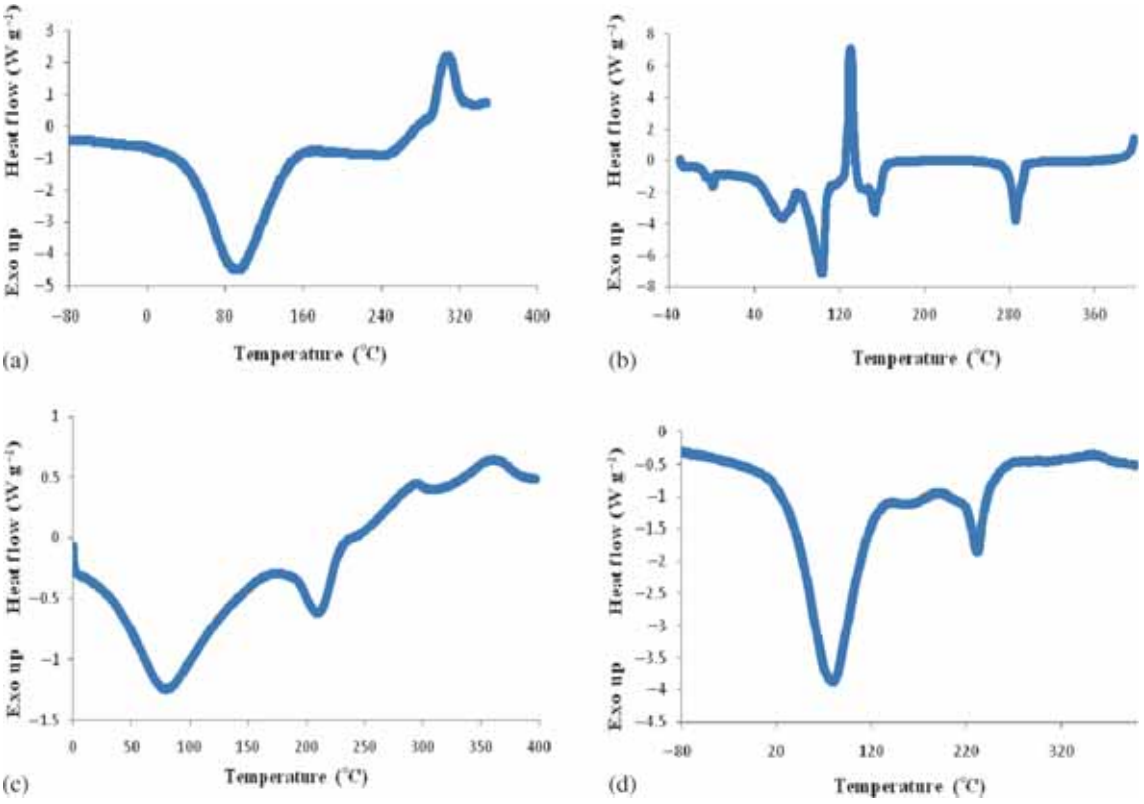


Figure 2. DSC thermogram of (**a**) pure guar-gum, (**b**) sodium salt of guar-gum, (**c**) acrylated guar-gum and (**d**) acrylic acid grafted guar-gum.

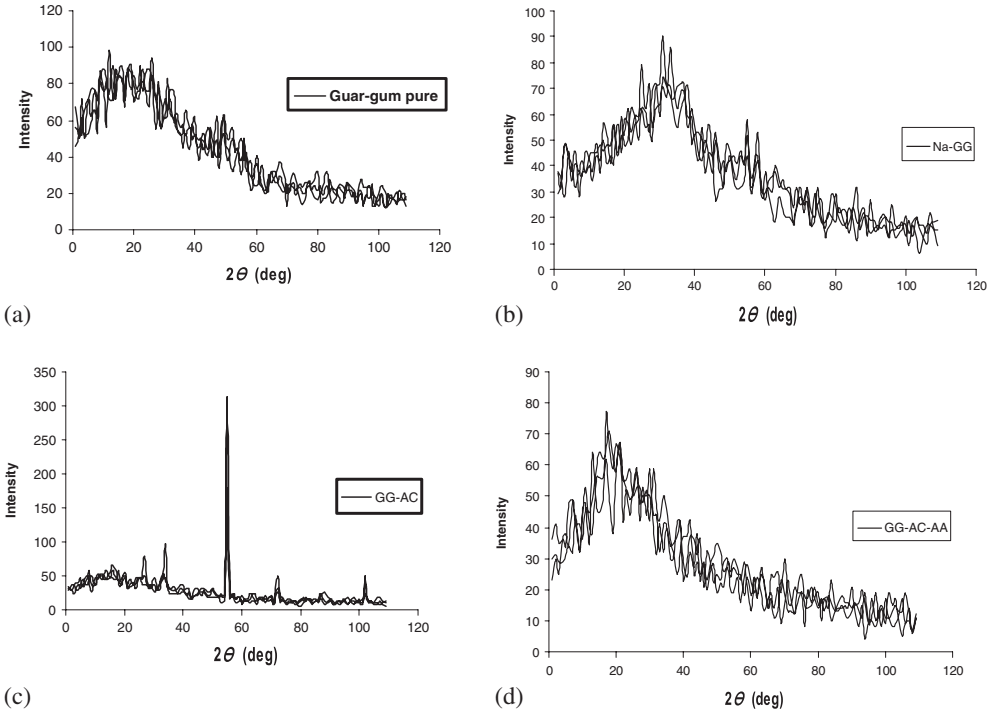


Figure 3. X-ray diffraction of (**a**) pure guar-gum, (**b**) sodium salt of guar-gum, (**c**) acrylated guar-gum and (**d**) acrylic acid grafted guar-gum product.

Figure 3c exhibits diffraction pattern of acrylated guar-gum. Interestingly, it is noteworthy to mention here that after acrylation crystallinity appears in the diffraction pattern of guar-gum. At $2\theta = 55°$ sharp peak is obtained along with broad hump at 3–30° 2θ (for guar-gum) with diminished intensity confirms the successful acrylation of guar-gum. Figure 3d shows diffraction pattern of acrylic acid grafted acrylated guar-gum. Here again broad hump is obtained in the range of 3–45° 2θ shows the amorphous nature of the polymers. The better definition of the diffraction band in figure 3d suggests that the overall amorphous nature, even being low increases after crosslinking. The increment, in this case in a high degree, approaches the polymer chain and could weaken the chain organization and decrease the material crystallinity.

The pesticide content of each sample has been determined in triplicate and the mean values have been obtained. The entrapment efficiency ($E\%$) has been calculated by using following equation:

$$\text{Entrapment efficiency } (E\%) = \frac{(P/M)\, W_{\mathrm{T}}}{W_{\mathrm{A}}} \times 100,$$

where W_{A} is the amount of pesticide used in the formulation, W_{T} the total weight of the synthesized polymer after lyophilization and P the amount of pesticide estimated in M amount of polymer taken.

The entrapment efficiency ($E\%$) of the modified acrylated guar-gum formulation entrapping pesticide chlorpyrifos has been found to be about 56 w/w %.

3.4 *Release kinetics study of chlorpyrifos*

3.4a *Chlorpyrifos entrapment efficiency*: An accurately weighed quantity of 100 mg of lyophilized formulation containing chlorpyrifos has been dispersed in 100 ml of dichloromethane. The extraction of entrapped chlorpyrifos in the lyophilized product has been done by sonicating the samples for 1 h with intermitted shaking. The sample has been left for equilibration under normal refrigerated condition for 5 h. Further centrifuged at 10,000 r.p.m. for 10 min. The supernatant has been diluted appropriately with the dichloromethane. The entrapment efficiency has been calculated by recording the absorbance of chlorpyifos at 228 nm by using UV–vis spectrophotometer.

3.4b *In vitro release kinetic studies*: A modified acrylated guar-gum material has been synthesized in the current chapter. An experiment has been conducted to assess its applicability to be used for bio-active molecule delivery. The agro-economic importance of chlorpyrifos is well established; hence it has been selected for the delivery of bio-active molecule by using modified acrylated guar-gum material.

Initially various release mediums have been tried, *viz.*, double distilled water, pH 6.0, 7.0, 7.4 and 9.2 buffers, but the release of chlorpyrifos form the modified guar-gum formulation in these media were non-significant. A very slow diffusion of the entrapped molecule form the formulation to the bulk dissolution medium has been observed. Thus it becomes essential to use some organic solvent to enhance this slow

diffusion process. A significant release of chlorpyrifos has been observed, when a methanolic buffer pH 7.4 (25:75 v/v) was used. This emphasized to use the methanolic buffer pH 7.4 (25:75 v/v) as a dissolution medium for further release studies.

The release behaviour of chlorpyrifos form the modified acrylated guar-gum has been assessed by taking 15 mg of lyophilized power formulation in 50 ml in methanolic buffer pH 7.4 (25:75 v/v %) as a dissolution medium. The release experiment has been performed under static conditions.

A series of standard dilutions have been prepared in methanolic buffer pH 7.4 (25:75 v/v). All the standard dilutions, viz., 3.20, 4.00, 6.40, 8.00 and 10.67 μg ml^{-1} were scanned in the UV region of 200–400 nm. The UV scan showed the wavelength maxima peaks at 288 and 228 nm for all the standard dilutions. The λ_{max} 228 nm is more intense than λ_{max} 288 nm, thus λ_{max} 228 nm has been selected to record the absorbance values for further analysis. A calibration curve has been plotted from recorded absorbance values at λ_{max} 228 nm against known standard dilutions. The correlation coefficient (r^2) value for calibration curve has been calculated in figure 4a. The r^2-value has been found to be 0.9983 which approach nearly 1, confirming the validity of Beer–Lambart law.

The release study of entrapped chlorpyrifos pesticide from modified acrylated guar-gum formulation has been performed by suspending the lyophilized sample in methanolic buffer pH 7.4 (25:75 v/v). All the experiments have been performed in triplicate. The results have been expressed as mean ± standard deviation. The error bar has been used to indicate

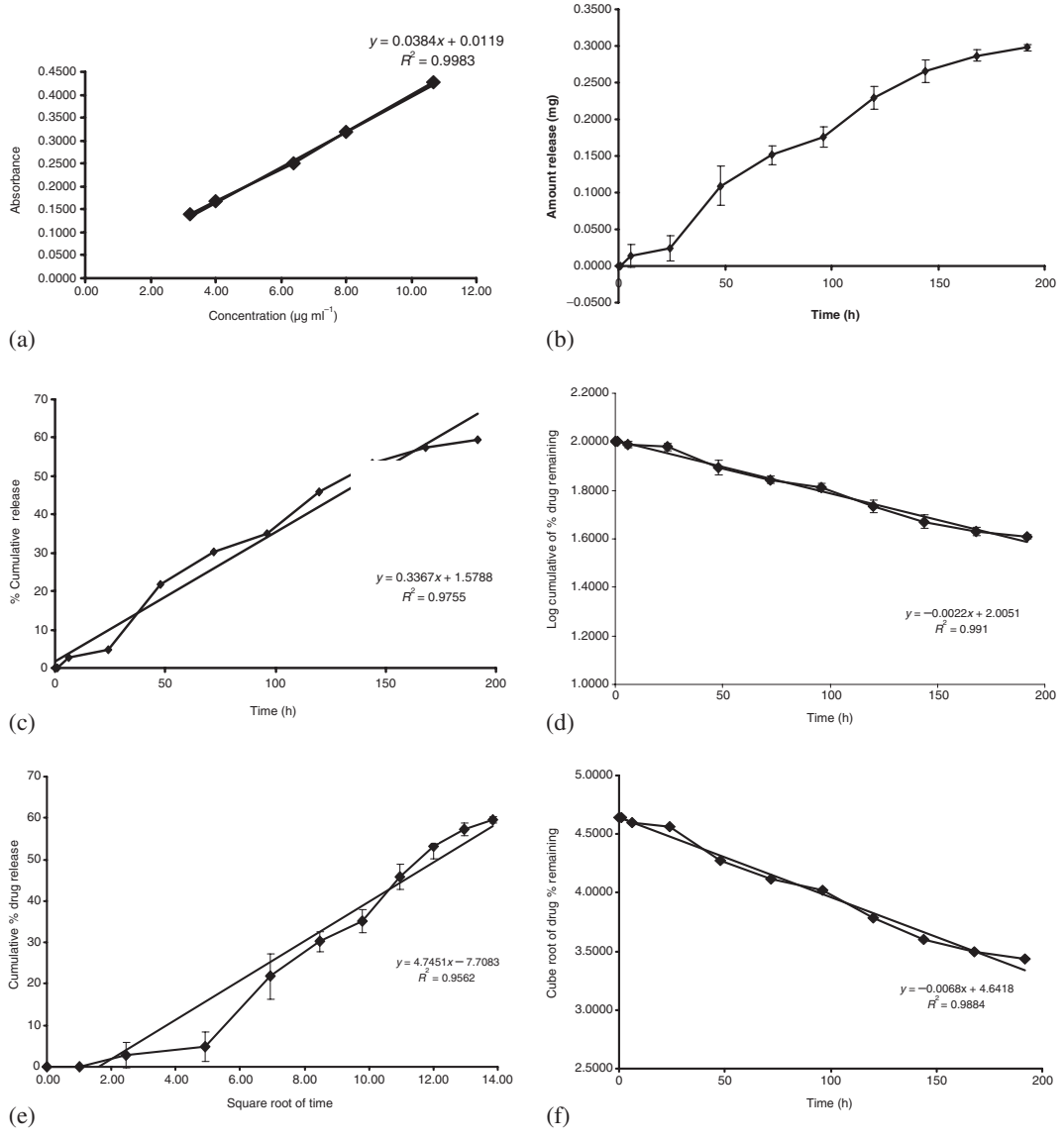


Figure 4. Standard curve of chlorpyrifos at λ_{228} nm in (**a**) methanolic buffer pH 7.4 (25:75 v/v%), (**b**) cumulative % chlorpyrifos release in various media, (**c**) zero-order release in methanolic buffer pH 7.4, (**d**) first-order release in methanolic buffer pH 7.4, (**e**) Higuchi release in methanolic buffer pH 7.4 and (**f**) Hixson–Crowell release methanolic buffer pH 7.4.

Table 1. Compiled data of regression coefficient and rate constant for various release models in methanolic buffer pH 7.4.

Release model	Zero order		First order		Higuchi		Hixson–Crowell	
Dissolution medium	r^2	k_o (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	k_{HC} ($h^{-1/3}$)
Methanolic buffer pH 7.4	0.9755	0.3367	0.9910	−0.0022	0.9562	4.745	0.9884	−0.0068

experimental deviations in all the data points of the graph. The absorbance of withdrawn samples is recorded at λ_{max} 228 for 1, 6, 24, 48, 72, 96, 120, 144, 168 and 192 h. The slope values obtained from calibration curve (figure 4b) has been used to calculate the amount of percentage chlorpyrifos released from the formulation. The data interpretation has been done by goodness-of-fit-model-dependent approach. The *in vitro* data obtained from the dissolution experiment has been attempted to fit in various kinetic models, *viz.,* zero-order, first-order, Higuchi and Hixson-Crowell. The regression analysis has been performed for the selected models. The regression co-efficient value obtained after linearization has been used to predict the possible mechanism of chlorpyrifos release. The kinetic models have been plotted (figure 4c–f) and data have been summarized in table 1.

The various kinetic models for release study produced r^2-value for zero-order plot (figure 4c) 0.9755, first order (figure 4d) gave $r^2 = 0.9910$, Higuchi model (figure 4e) gave 0.9562 and Hixson-Crowell (figure 4f) gave 0.9884, describing the chlorpyrifos release rate relationship with concentration of chlorpyrifos. The best linearity has been found in first-order equation plot where r^2 was observed as 0.9910. This indicates the release of chlorpyrifos from formulation is concentration-dependent process. Although it is worth mentioning to state that, it took almost 8 days to release 60% of the entrapped chlorpyrifos (figure 4b) from the modified acrylated guar-gum formulation. Although first-order release is conventional one but in the present case, the formulation controlled chlorpyrifos release slowly and extended the same for elongated period of time.

4. Conclusions

Guar-gum has been successfully acrylated using acryloyl chloride and modified by grafting with acrylic acid to form graft co-polymer acrylate, guar-gum-grafted-polyacrylic acid. Grafted guar-gum shows better thermal stability, low viscosity and increase stimuli responsiveness. The grafted guar-gum was characterized using FT-IR, DSC, TG-DTA and XRD. Pesticide chlorpyrifos was entrapped as a model bioactive molecule during grafting to study the entrapment efficiency and release behaviour. A very slow diffusion of the entrapped molecule form the formulation to the bulk dissolution medium has been observed when different buffer media were used for the release study. A significant release of chlorpyrifos has been observed, when a methanolic buffer pH 7.4 (25:75 v/v) was used. By studying the calibration curve the entrapment efficiency of the formulation was found to be 60%. Goodness-of-fit-model-dependent approach has been used to interpret the data obtained and attempts have been taken to fit the same in various kinetic model. From the study it is revealed that the release of chlorpyrifos from formulation is concentration dependent and follows conventional first-order kinetics for slow and extended period of time.

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