

GUM KARAYA-G-POLY (ACRYLAMIDE): MICROWAVE ASSISTED SYNTHESIS, OPTIMISATION AND CHARACTERISATION

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ABSTRACT

Objective: The objective of the present research is to formulate acrylamide grafted Karaya gum by using microwave-assisted grafting method and optimisation is performed by using Box behnken design.

Methods: The extracted mucilage of gum Karaya was modified into grafted gum Karaya by using a microwave-assisted method. Acrylamide was used as monomer and ceric ammonium nitrate (CAN) is used as redox initiator. The experimental design for optimisation include three independent variables gum concentration (X1), ceric ammonium nitrate (CAN) amount (X2) and irradiation time (X3) while the dependent variables were % yield (Y1), % grafting (Y2) and % grafting efficiency (Y3). The optimised formulation was characterized by fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), x-ray diffraction (XRD) and scanning electron microscopy (SEM) analysis.

Results: After optimisation the formulation with acrylamide amount 5 g, ceric ammonium nitrate amount 200 mg and irradiation time of 2 min was selected as optimized formulation. The optimised formulation has percentage grafting of 853.5%, with grafting efficiency of 77.59%.

Conclusion: The application of box behnken design for optimisation was performed successfully in microwave assisted grafting of acrylamide on karaya gum.

Keywords: Gum karaya, Acrylamide, Microwave-assisted grafting, Box behnken design

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INTRODUCTION

The karaya gum, also known as sterculia, kadaya, katilo, kullo and kuterra [1]. The Joint Expert Committee for Food Additives (JECFA/FAO) defines gum karaya as the dried exudates obtained from *Sterculia urens* Roxd and other related species of *Sterculia* (family Sterculiaceae) or *Cochlospermum gossypium* AP De Candolle or other species of *C kunth* (family Bixaceae). The gum consists mainly of high-molecular-weight acetylated polysaccharides which, on hydrolysis, yield galactose, rhamnose and galacturonic acid together with a small amount of glucuronic acid [2].

Dispersion of karaya gum is thermolabile; during heating, polymer conformation changes, resulting in an increased solubility and a permanently decreased viscosity. In cold water, dispersions can be as concentrated as 5% while in hot water and low-pressure concentrations of about 18 and 20% can be reached. Karaya gum has a strong capacity to fix with water molecules; gum particles are not fully solubilized, instead it presents a phenomenon called swelling that consist in an increment in total volume with relation to dry mass that can be about the 60 times the original volume [3].

Natural polysaccharides and their derivatives are always preferred over synthetic products in the formulation of pharmaceutical products due to safety, free availability and low cost [4]. The modification of natural gums reduces their uncontrolled hydration and microbial contamination [5]. Microwave assisted grafting is the best method for modification of natural polymers to obtain the hybrid materials with better properties [6]. In grafting method, the monomers are covalently bonded onto the polymer chain backbone and the product is described as copolymer [7]. Earlier studies reported good sustained release behaviour of gum kondagogu [8], xanthan-g-poly(acrylamide) [5] and guar-g-poly(acrylamide) [9].

In the present research, extracted karaya gum was grafted by using acrylamide as the monomer and ceric ammonium nitrate as redox initiator. Various monomers have been reported earlier for the process of grafting such as acrylamide [10], methyl acrylate [11], acrylonitrile [12] and acrylic acid [13]. The microwave-assisted

graft-copolymerization of acrylamide on karaya gum was optimised by using box behnken design.

MATERIALS AND METHODS

Materials

Karaya gum was purchased from the sigma-aldrich. Acrylamide and ceric ammonium nitrate were procured from LOBA Chemicals, India. All other chemicals used were of analytical grade and distilled water was used throughout the experiments.

Extraction of mucilage from karaya gum

Step 1: Karaya gum was used for the extraction of mucilage. Gum was dried hot air oven at 40 °C for 2 h. Fried karaya gum was boiled for 2 h for the release of mucilage into water. Now with the help of muslin cloth the material was squeezed to separate marc from filtrate.

Step 2: Filtrate was centrifuged for 20 min at 5000 rpm. The supernatant was collected and kept in the refrigerator for 30 min. Equal volume of acetone was added; precipitate was separated and treated with petroleum ether. The precipitate was separated from petroleum ether and kept for drying in hot air oven at 40 °C [14].

Grafting of extracted mucilages

Grafting of extracted mucilage with acrylamide was done by employing microwave-assisted grafting. Briefly, appropriate amount of extracted mucilage was dissolved in distilled water by stirring 1 h. To this, acrylamide was added and stirred to dissolve. For grafting with redox-initiator, ceric ammonium nitrate was added to this above solution and irradiates to microwave (NN-CT654M, Panasaonic Japan) at different temperatures and time intervals. After that, to the grafted mucilage 3 times the volume of acetone was added and kept overnight to allow the formation of precipitates. The solution was filtered using whatmann filter paper and washed the filtrate with water: methanol to remove unreacted monomer and initiator. The grafted mucilage was then dried in an oven at 40 °C for 24 h followed by its weighing [5, 15].

Experimental design

Box behnken response surface designs are used to require three levels, coded as -1, 0, and +1. Box behnken design (BBD) was used for the optimization of the grafting process, having three independent variables and three dependent variables using design expert (version 7.0 state-ease Inc., Minneapolis, MN). Independent variables were percentages of gum concentration (X1), ceric ammonium nitrate amount (X2) and irradiation (X3) while the dependent variables were % yield (Y1), % grafting (Y2) and %

grafting efficiency (Y3) as shown in table 1. The nonlinear quadratic model for this design is given as

$$Y = \beta_0 - \beta_1 \cdot X_1 + \beta_2 \cdot X_2 + \beta_3 \cdot X_3 - \beta_4 \cdot X_1 \cdot X_2 - \beta_5 \cdot X_1 \cdot X_3 - \beta_6 \cdot X_2 \cdot X_3 - X_1^2 - \beta_8 \cdot X_2^2 + \beta_9 \cdot X_3^2$$

Where Y is the measured response of the dependent variables, β_0 is the intercept, β_1 - β_9 are the regression coefficients computed from the observed experimental values of Y. X1, X2 and X3 are the coded value of the independent variables. $X_1 X_2 X_3$ (a, b = 1, 2, 3) and X_i^2 (i = 1, 2, 3) represent the interaction and quadratic terms, respectively [16, 17].

Table 1: Levels of independent variables in box behnken design

Factor	Name	Units	Minimum	Maximum
X1	Acrylamide amount	g	3	5
X2	CAN amount	mg	100	300
X3	Irradiation time	min	2	4

The graft copolymer of karaya with acrylamide was characterized by FTIR, DSC, XRD and SEM and % grafting (%G) and % grafting efficiency (%GE) were calculated by using following equations [18]:

$$\% \text{ Grafting (\%G)} = \left(\frac{W_1 - W_0}{W_0} \right) * 100$$

$$\% \text{ Grafting Efficiency (\%GE)} = \left(\frac{W_1 - W_0}{W_2} \right) * 100$$

Where W_0 , W_1 and W_2 denote the weight of original karaya, the weight of grafted karaya and weight of monomer used respectively.

Table 2: Box-behnken design matrix

Independent variables				Dependent variables			
S. No.	Formulation code	Acrylamide amount (g) (X1)	Ceric ammonium nitrate amount (mg) (X2)	Irradiation time (min) (X3)	Yield (Y1)	% Grafting (Y2)	% Grafting efficiency (Y3)
1	F1	4	200	3	0.6385	27.7	3.077777778
2	F2	4	200	3	0.6823	36.46	4.051111111
3	F3	3	200	2	2.0053	301.06	43.00857143
4	F4	5	100	3	2.3714	374.284	34.02581818
5	F5	4	200	3	1.7101	242.02	26.89111111
6	F6	4	300	4	1.7313	246.26	27.36222222
7	F7	4	200	3	0.7112	42.24	4.693333333
8	F8	3	300	3	1.7096	241.92	34.56
9	F9	4	200	3	0.7001	40.02	4.446666667
10	F10	5	200	4	2.9469	489.38	44.48909091
11	F11	4	100	4	1.2939	15.78	17.64222222
12	F12	5	200	2	4.7675	853.5	77.59090909
13	F13	4	100	2	0.5914	18.28	2.031111111
14	F14	4	300	2	1.5019	200.38	22.26444444
15	F15	5	300	3	3.5614	612.28	55.66181818
16	F16	3	100	3	1.2134	142.68	20.38285714
17	F17	3	200	4	1.4379	187.58	26.79714286

Fourier transform infrared spectroscopy

The samples were subjected to FTIR spectroscopy by using KBr pellet in the Fourier Transform Infrared spectrophotometer [19].

Differential scanning calorimetry

Differential Scanning Calorimetric thermogram of karaya and grafted karaya was recorded by using differential scanning calorimeter in the temperature range of 40 °C-250 °C at a heating rate of 10 °C per minute in nitrogen atmosphere [20].

XRD analysis

X-ray diffractogram of pure gum and grafted gum was recorded by employing Ultima-4, Rigaku company, Japan) using K-beta filter, $\text{Cu-K}\alpha$ radiation generated at 40 kv and 30 ma in the differential angle range of 10-60 ° 2 θ [21].

Scanning electron microscopy

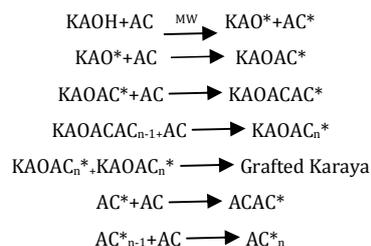
Scanning electron micrograph of grafted karaya particles was taken using a SEM (Model: JSM 5200, Japan). The samples were prepared by absorbing the particles on double side adhesive tape, which stuck to aluminium stab and gold-coated under vacuum using a sputter

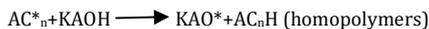
coater. Samples were exposed to vacuum for 5-10 min at 40 mA and investigated at an accelerating voltage of 15 kV and 10 kV [22].

RESULTS AND DISCUSSION

Preparation and optimization of karaya-g-poly(acrylamide)

The extracted and dried mucilage of gum karaya was used for the purpose of grafting by the microwave-assisted grafting method. Microwave-assisted grafting is always found to be the better method as compared to conventional grafting technique. Gum aegle marmilos [7], gum ghatti [23] and gum agar [24] have been grafted successfully by the researchers. The proposed mechanism for grafting of acrylamide on the surface of karaya gum is given below:





Where KAOH stands for karaya gum, AC stands for Acrylamide and MW stands for Microwave irradiation.

It is proposed that -OH groups of gum will absorb the MW energy and will cleave generating acrylamide radicals and karaya radicals. These free radicals will then further react with other molecules of gum to formulate the grafted gum till all the gum molecules get consumed. After that, acrylamide radicals react with each other to formulate the homopolymers in solution [23].

Microwave-assisted grafting of gum karaya was performed and explored by the box-behnken design matrix.

Independent variables were percentages of gum concentration (X1), ceric ammonium nitrate amount (X2) and irradiation time (X3) while the dependent variables were yield (Y1), % grafting (Y2) and % grafting efficiency (Y3). The experimental design and results of experimental data are given in table 2. Polynomial equations with this design for yield, % grafting and % grafting efficiency are as following:

$$\text{Yield} = 0.88844 + 0.91101275.X_1 + 0.37926.X_2 - 0.1820125.X_3 + 0.173445.X_1.X_2 - 0.3133.X_1.X_3 - 0.118275.X_2.X_3 + 1.417645.X_1^2 - 0.09213.X_2^2 + 0.483315.X_3^2$$

$$\% \text{Grafting} = 77.688 + 182.0255.X_1 + 75.852.X_2 - 36.4025.X_3 + 34.689.X_1.X_2 - 62.66.X_1.X_3 - 23.655.X_2.X_3 + 283.529.X_1^2 - 18.426.X_2^2 + 96.663.X_3^2$$

$$\% \text{Efficiency} = 0.632 + 10.87738312.X_1 + 8.220809524.X_2 - 3.575544733.X_3 + 1.864714286.X_1.X_2 - 4.222597403.X_1.X_3 - 2.628333333.X_2.X_3 + 29.08602597.X_1^2 - 1.560402597.X_2^2 + 10.2534026.X_3^2$$

Where X₁ is acrylamide Amount, X₂ is the amount of CAN and X₃ is irradiation time.

In ANOVA, model F Values implies that the model is significant. Values of "Prob>F" less than 0.05 indicate model terms are significant. The Pred R-square and Adeq R-square are significant. The noise ratio greater than 4 is desirable. The ratio of 7.41, 7.41

and 6.157 indicates that the signal is adequate and this model can be used in navigation of design space [7].

The result of polynomial equations for Yield, % grafting and % grafting efficiency revealed that variable irradiation time (X₃) showed a negative effect on yield than acrylamide amount (X₁) and amount of ceric ammonium nitrate (X₂) which showed the positive effect. The interaction effect X₁.X₂ was found to be synergistic while the interaction effect of X₁.X₃ and X₂.X₃ showed a negative effect. Furthermore the quadratic effect of X₁² is more significant as compared to X₂² and X₃². The magnitude of coefficients of polynomial equations showed that concentration of acrylamide (X₁) had a more pronounced effect on yield, % grafting and % grafting efficiency.

The effect of independent variables on yield, % grafting and % grafting efficiency had been shown by the response surface plots.

Fig. 1 showed the interaction effect of acrylamide amount and ceric ammonium nitrate (CAN) amount on yield. It was observed that as the concentration of acrylamide increased yield also increased significantly. However the concentration of ceric ammonium nitrate (CAN) had no significant effect on the yield of grafted gum. Fig. 2 showed the interaction effect of acrylamide amount and irradiation time on yield. It was observed that as the concentration of acrylamide, increased yield also increased significantly. However, irradiation time had no significant effect on the yield of grafted gum [7, 8].

Fig. 3 showed the interaction effect of acrylamide amount and ceric ammonium nitrate (CAN) amount on % grafting. It was observed that as the concentration of acrylamide increased % grafting also increased significantly. However, ceric ammonium nitrate (CAN) amount had no significant effect on the % grafting. Fig. 4 showed the interaction effect of acrylamide amount and irradiation time on % grafting. It was observed that as the concentration of acrylamide increased % grafting also increased significantly. However, irradiation time had no significant effect on % grafting. Fig. 5 showed the interaction effect of ceric ammonium nitrate (CAN) amount and irradiation time on % grafting. It was observed that as the concentration of ceric ammonium nitrate (CAN) amount and irradiation time had no significant effect on the % grafting [7, 8].

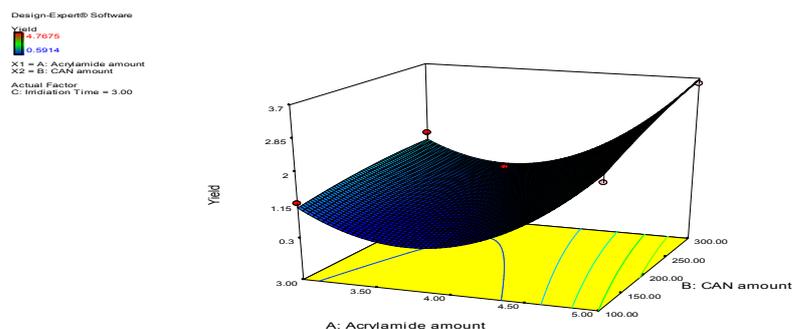


Fig. 1: 3D surface response graph between acrylamide amount (A₁), ceric ammonium nitrate (CAN) amount (B₂) with yield (Y₁)

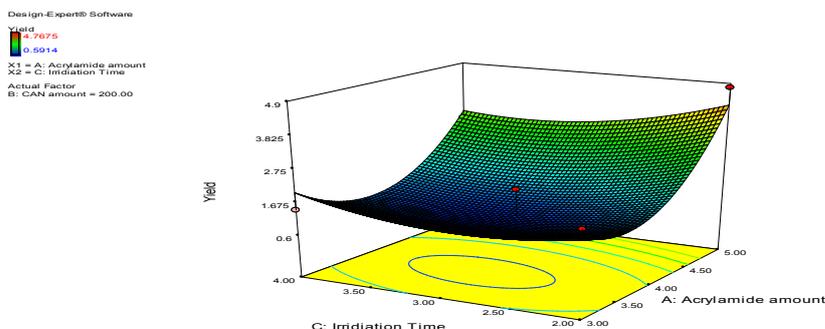


Fig. 2: 3D surface response graph between acrylamide amount (X₁), irradiation time (C₁) with yield (Y₁)

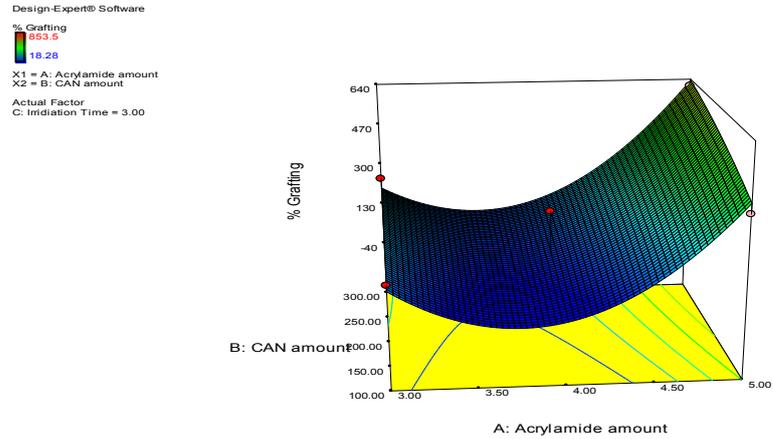


Fig. 3: 3D surface response graph between acrylamide amount (A), ceric ammonium nitrate (CAN) amount (B) with % grafting (Y2)

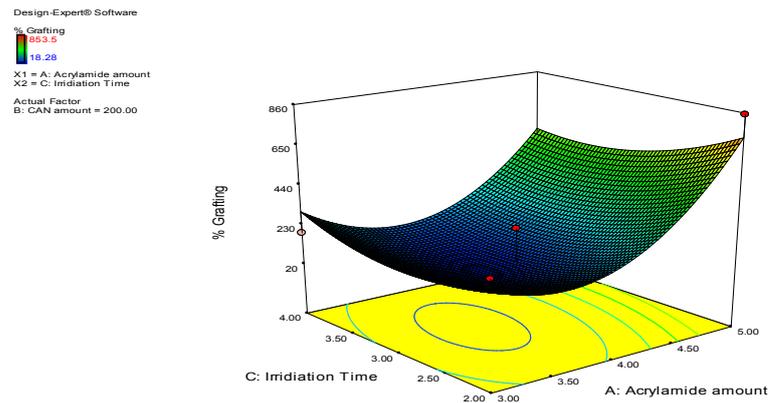


Fig. 4: 3D surface response graph between acrylamide amounts (A), irradiation time (C) with % grafting (Y2)

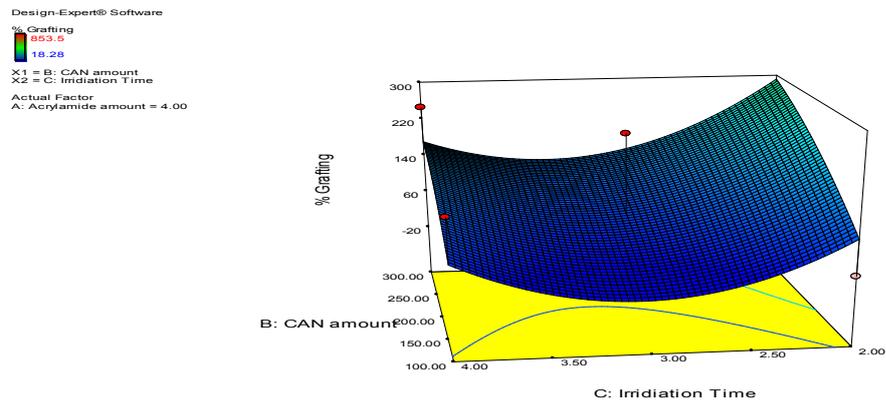


Fig. 5: 3D surface response graph between ceric ammonium nitrate (CAN) amount (B), irradiation time (C) with % grafting (Y2)

Fig. 6 showed the interaction effect of acrylamide amount and ceric ammonium nitrate (CAN) amount on % grafting efficiency. It was observed that as the concentration of acrylamide increased % grafting also increased significantly. However, ceric ammonium nitrate (CAN) amount has no significant effect on % grafting efficiency. Fig. 7 showed the interaction effect of acrylamide amount and irradiation time on % grafting efficiency. It was observed that as

the concentration of acrylamide increased % grafting also increased significantly. However, Irradiation time had no significant effect on the % grafting efficiency. Fig. 8 showed the interaction effect of ceric ammonium nitrate (CAN) amount and irradiation time on % grafting efficiency. It was observed that ceric ammonium nitrate (CAN) amount and Irradiation time have no significant effect on the % grafting efficiency [7, 8].

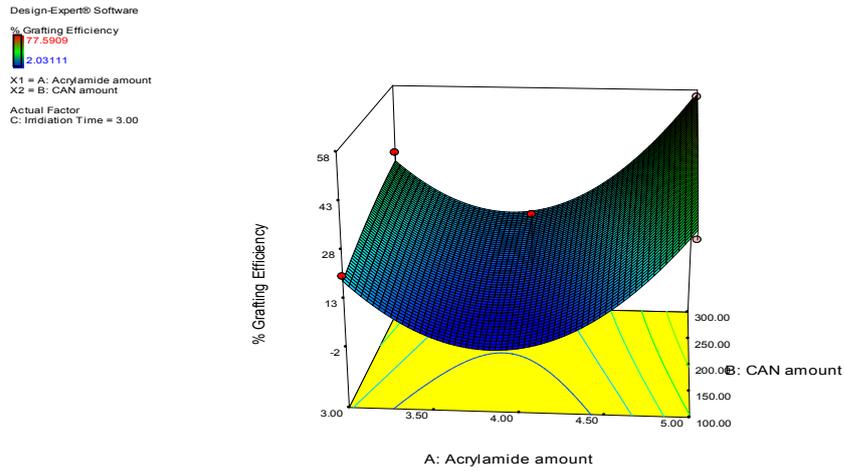


Fig. 6: 3D surface response graph between acrylamide amount (A_1), ceric ammonium nitrate (CAN) amount (B_2) with % Grafting efficiency (Y_3)

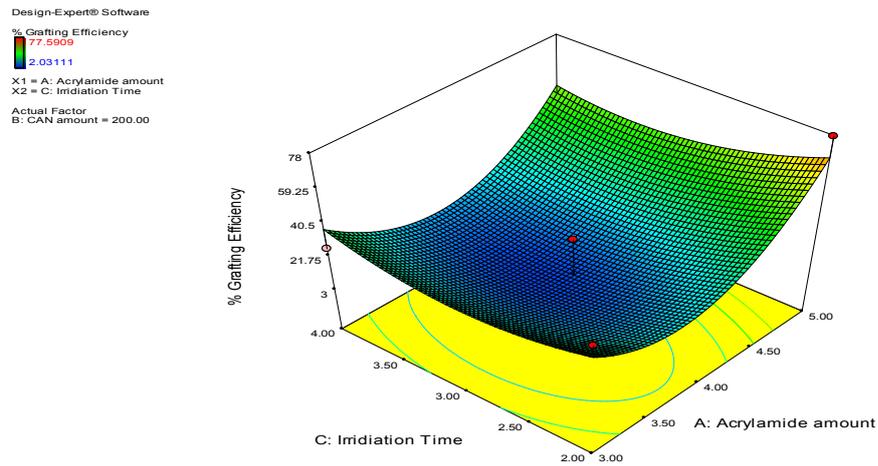


Fig. 7: 3D surface response graph between acrylamide amounts (A), irradiation time (C) with % grafting efficiency (Y_3)

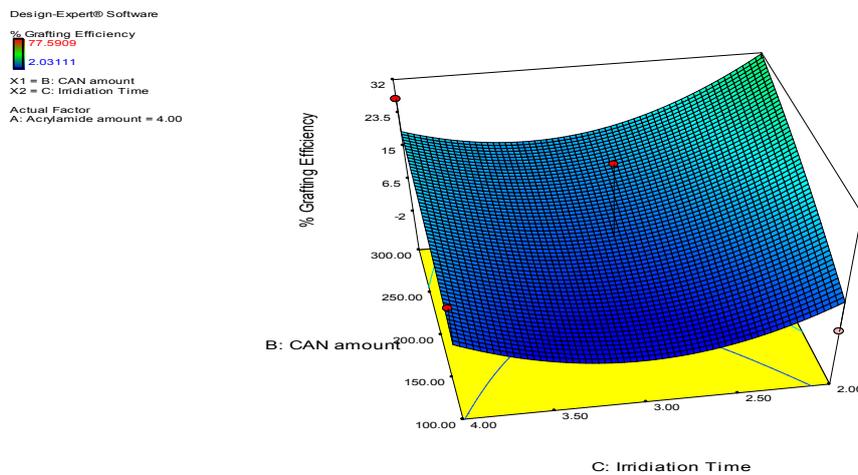


Fig. 8: 3D surface response graph between ceric ammonium nitrate (CAN) amount (B), irradiation time (C) with % grafting efficiency (Y_3)

Selection of optimized batch

The design expert software was used to optimize the factors by using the approach of desirability.

The formulation with maximum desirability was selected. Thus the formulation with an acrylamide amount of 5 g, irradiation time of 2 min and CAN amount of 200 mg was selected as an optimized formulation.

Characterization of optimized batch

The optimized batch of acrylamide grafted karaya gum was subjected to fourier transform infrared spectroscopy, differential scanning calorimetry, x-ray diffraction and scanning electron microscopic analysis.

Fourier transform infrared spectroscopy

The fourier transform infrared spectra of karaya gum (fig. 9) showed the characteristic peak. The spectrum showed a C=O stretching

vibration at 1722.46 cm⁻¹, O-H stretching vibration at 3282.75 cm⁻¹, C≡C-H: C-H stretching at 2939.18 cm⁻¹. The FTIR spectra of acrylamide (fig. 10) showed C=O Stretching at 1740.82 cm⁻¹, N-H stretching at 1604.40 cm⁻¹, C-N Stretching in aliphatic amine at 1130.79 cm⁻¹.

The FTIR spectra of grafted karaya gum (fig. 11) showed a very broad spectrum band at 3337.75, which confirms the grafting by overlapping of the OH group of karaya with the NH group of acrylamide. This FTIR studies have been supported by the literature [21, 25].

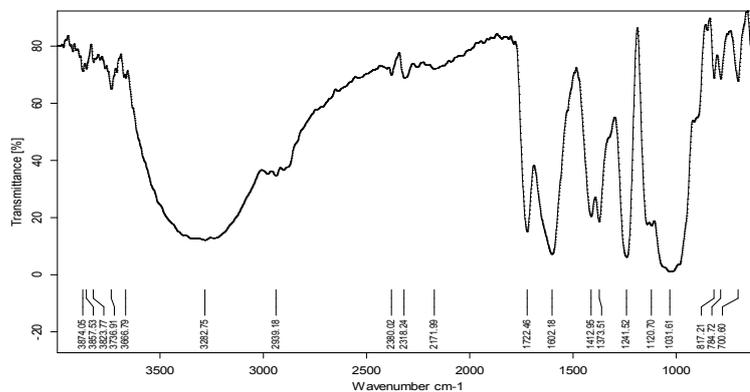


Fig. 9: FTIR spectrum of extracted karaya gum

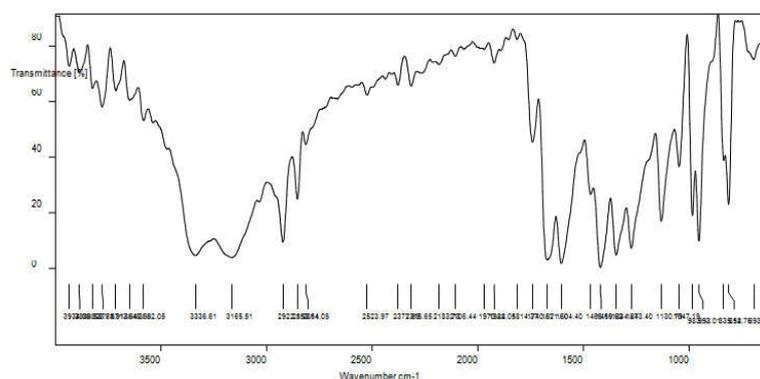


Fig. 10: FTIR spectrum of acrylamide

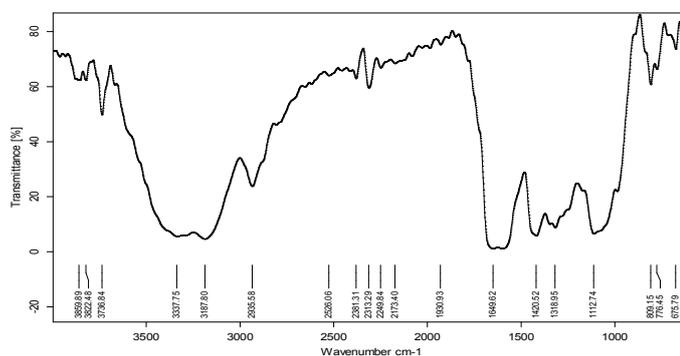


Fig. 11: FTIR spectrum of grafted karaya gum

X-ray diffraction

The X-ray diffraction spectra showed the amorphous nature of gum karaya (fig. 12) as no characteristic peak was observed. The XRD spectra

of acrylamide (fig. 13) confirmed its crystalline nature. However, the XRD spectra of grafted gum (fig. 14) showed less intense peaks but crystalline, which confirmed the grafting of acrylamide onto the surface of gum and which have also been confirmed by literature [26, 27].

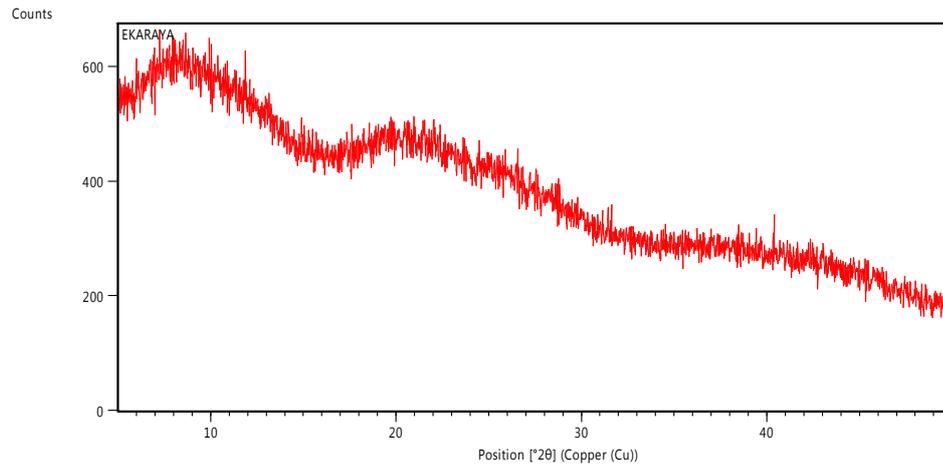


Fig. 12: XRD analysis of extracted karaya

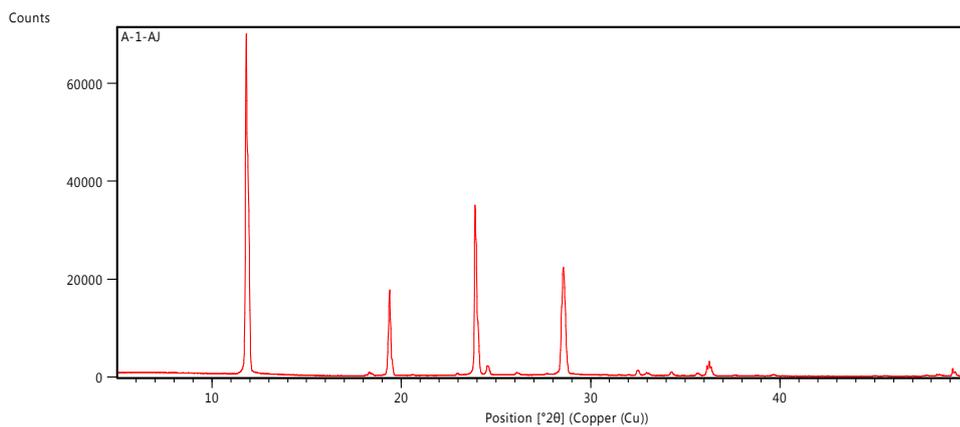


Fig. 13: XRD analysis of acrylamide

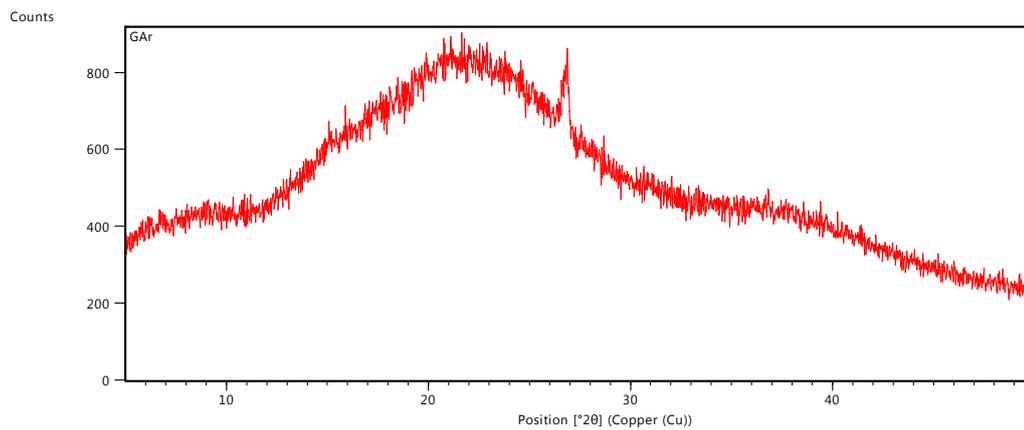


Fig. 14: XRD spectra of grafted Karaya

Differential scanning calorimetry

The glass transition temperature of extracted karaya was found to be 79.1 °C as showed in fig. 15. It indicates the amorphous nature of gum due to broad endothermic peak. The DSC curve for acrylamide

showed the sharp peak at 92.7 °C, which confirmed its crystalline nature. The DSC curve for grafted karaya gum showed the shift in glass transition temperature from 79.1 °C to 42.1 °C. This shift in endothermic peak to lower side of temperature may be due to the formation of loose polymer matrix [28-31].

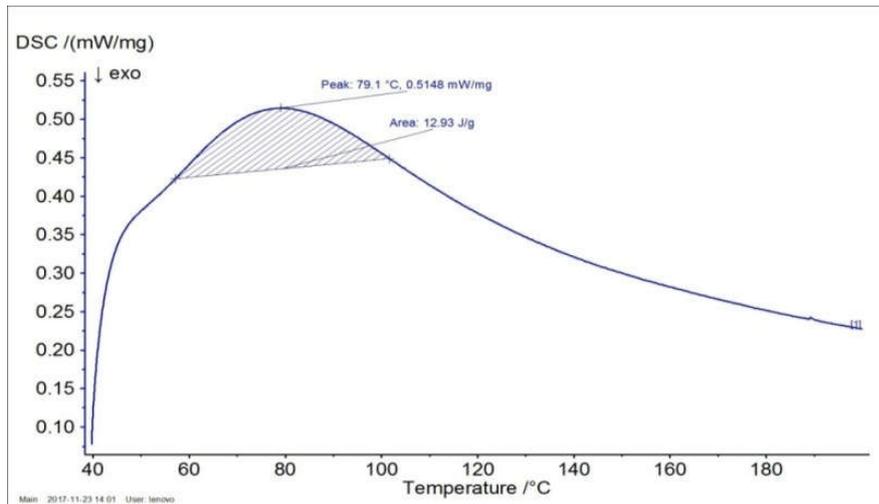


Fig. 15: DSC of extracted karaya

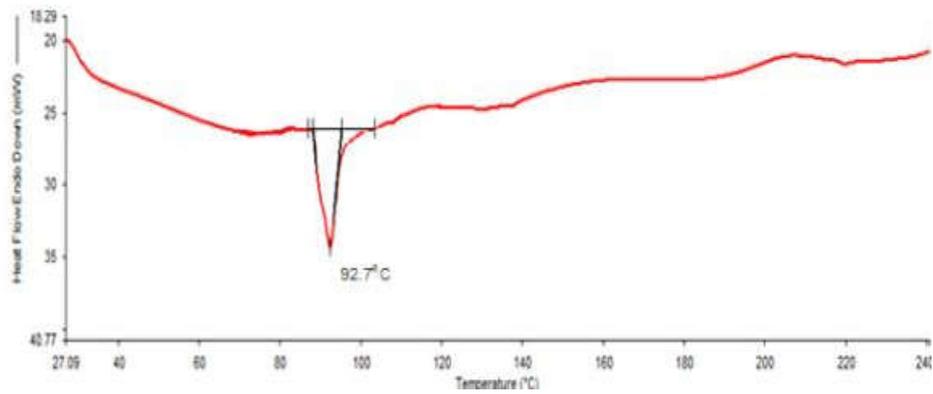


Fig. 16: DSC of acrylamide

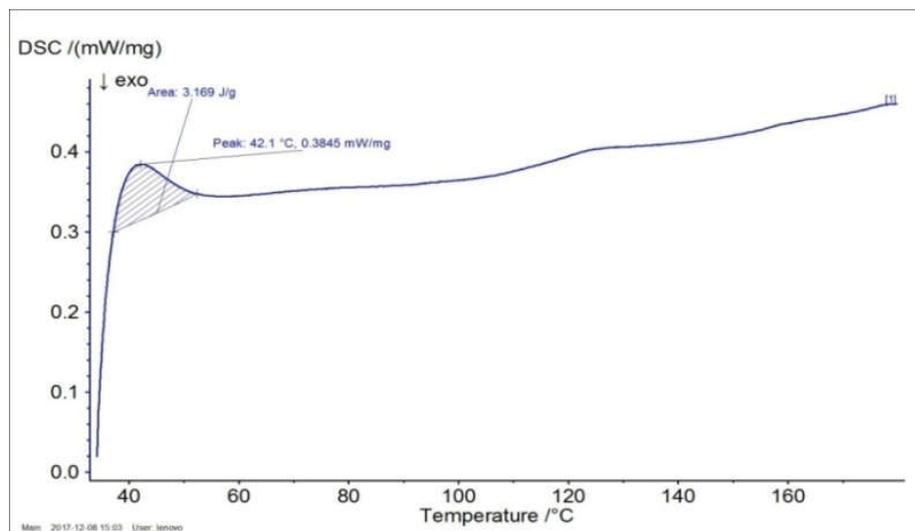


Fig. 17: DSC of grafted karaya gum

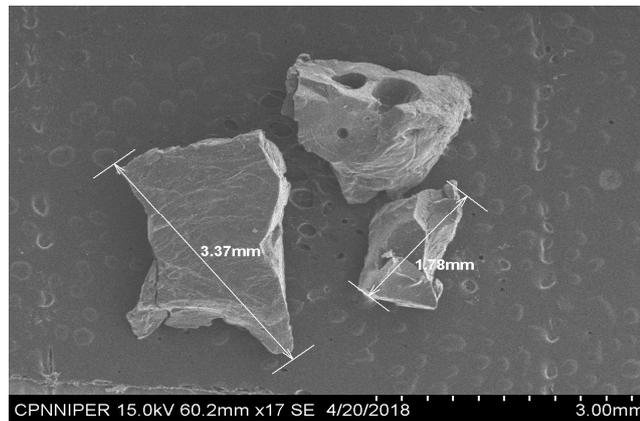


Fig. 18a: Scanning electron microscopy of karaya gum grafted

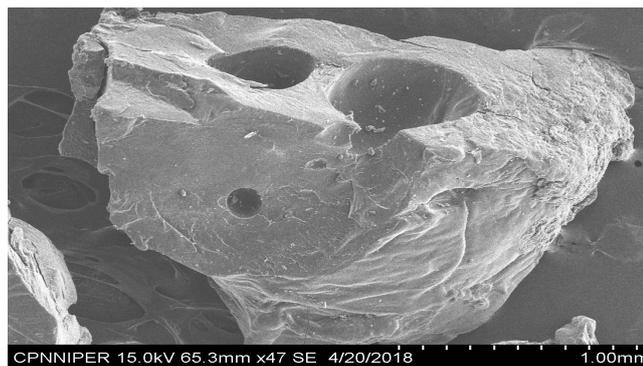


Fig. 18b: Scanning electron microscopy of karaya gum grafted

Morphology

Morphological studies of grafted gum karaya were performed by scanning electron microscopy studies (fig. 18a and fig. 18b). The change in surface morphology was clearly revealed by scanning electron micrographs, which showed that more unevenness and roughness due to the deposition of acrylamide on the surface of karaya gum [32-35].

CONCLUSION

The acrylamide grafted karaya gum was successfully prepared and optimized by using design expert software by applying the box behnken design. The optimized batch has been further used for confirmation of grafting by means of characterization. Analytical studies of the optimized batch confirmed the grafting of acrylamide on the surface of karaya gum. This grafted gum can further prove to be successful and better polymer as compared to ungrafted karaya gum in the formulation of sustained-release formulations.

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Nil

AUTHORS CONTRIBUTIONS

Authors declare that the work done by the names mentioned in the article and all the liabilities and claims related to the content of the article will be borne by the authors.

CONFLICT OF INTERESTS

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

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