

Investigations on Microwave-Assisted Synthesis of Carboxymethyl *Cassia* Gum

Manisha Sharma ¹ , Rimpay ¹ , Shakuntla Verma ¹ , Tarun Kumar ¹ , Munish Ahuja ^{1,*} 

¹ Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India

* Correspondence: munishahuja17@yahoo.co.in;

Scopus Author ID 7101943061

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Abstract: The objective of this study was to chemically alter *Cassia* gum by means of carboxymethylation reaction with the aid of microwave radiations, producing highly substituted carboxymethylated *Cassia* gum. The carboxymethyl substitution of *Cassia* gum was optimized using response surface methodology. The optimal parameters were found to be microwave power of 50 W and microwave exposure time of 7.36 minute which provided carboxymethylated gum with a degree of substitution of 2.5 (Predicted 2.3). Structural changes after carboxymethylation reaction were confirmed using Fourier-transform infrared and X-ray diffraction. Further, diclofenac sodium-loaded Ca²⁺- gelled carboxymethylated *Cassia* gum beads were prepared. The effect of microwave exposure on beads was studied at different powers. However, no significant influence of microwave exposure on the release of diclofenac from beads was observed. The beads were observed to release diclofenac by zero-order kinetics with anomalous release mechanism. In conclusion, the microwave-assisted carboxymethyl modification of *Cassia* gum can be carried out to prepare modified gum with potential for use as matrix material for pharmaceutical applications.

Keywords: *Cassia* gum; Carboxymethylation; Microwave; Beads; Degree of substitution.

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1. Introduction

The endosperm flour i.e. *Cassia* gum can be obtained from herbaceous perennial weed *C. tora* L. and *C. obtusifolia* L. after removing the germ (41%) and hull (27%) present in the seeds [1-3]. It is one of the lowest accessible polymers in India and research has shown that the backbone polysaccharide comprises 1-4 linked D-manopyranose and D-glucopyranose units. The gum comprising 75% of galactomannans have numeral properties such as thickening and gelling agent [2], good adsorbent, and coagulating/flocculating agent [4] and binding agent [5]. The applications of *Cassia* gum are limited because of its small water solubility, the dull colour of the polymer product, and its fast biodegradability [6]. Numerous findings have explored the alteration of *Cassia* gum in order to improve acceptability and gum quality [7]. These techniques include cyanoethylation [8-10], grafting [11], and carbamoylethylation [8-10, 12]. From various physico-chemical approaches, carboxymethylation modification is a widely used technique because of its low cost, easy processing and enhancing functional properties [13]. Basically, carboxymethylation is an etherification process in which hydroxyl group present in glucose get replaced with carboxymethyl group (-CH₂COO⁻) [14]. Modified carboxymethyl polysaccharide is typically more dissolvable, low viscous and anionic than native polysaccharide. Furthermore, the anionic property imparted by carboxymethylation to

polysaccharides paves the way for its development in the production of ionic polymeric materials [15-16]. So, carboxymethylation technique was utilized for enhancing the polysaccharide functionality.

On reviewing the literature, it was found that microwave assisted technology has been employed to modify the release behaviour of natural gum matrices by cross-linking. This high valued technique is used for the synthesis of polymers, organic, inorganic compounds and nanomaterials [17-18]. Considering the same, in the present work MW-assisted carboxymethyl modification of *Cassia* gum was attempted. The modification of *Cassia* gum was optimized using systematic Design of Experiment (DoE) approach, employing two-factor, three-level central composite experimental design. The modified gum was characterized by determining the degree of substitution, Fourier-transform infrared, X-ray diffraction studies. The modified gum was utilized to prepare ionically gelled beads with calcium chloride as ionic cross-linker and diclofenac as the model drug. Further, the effect of microwave exposure on beads was also studied to modify the release characteristics of *Cassia* gum by cross-linking.

2. Materials and Methods

2.1. Materials.

Cassia gum powder and diclofenac sodium were obtained as gift samples from Mahesh Argo Food Industries (Rajasthan, India) and Coax Bioremedies, (Hisar, India) respectively. Monochloroacetic acid was procured from Thomas Baker Chemicals Private Limited (Mumbai, India). Sodium hydroxide, glacial acetic acid and methanol were obtained from Sisco Research Laboratories Private Limited (Mumbai, India). Calcium chloride-dihydrate AR and hydrochloric acid were procured from S.D. fine chem. Ltd. (Mumbai, India).

2.2 Methods.

2.2.1. Preparation of microwave assisted carboxymethyl *Cassia* gum.

Microwave aided method was utilized to carboxymethylate *Cassia* gum. About 0.5 g of *Cassia* gum powder was mixed with 20 ml of chilled sodium hydroxide (45% w/v). The same amount of aqueous monochloroacetic acid (75% w/v) was added up to the prepared solution of *Cassia* gum in the same vial by vortex shaker. The prepared blend was heated at 70°C with the aid of stirring at 1200 rpm at different microwave power (50-150 W) with different time periods (2.5-7.5 minutes) (Monowave 200, Anton Paar, Austria). After microwave synthesis, the mixture was suspended in 20 ml of methanol (80% v/v). The suspended precipitate, so collected, was filtered and washed with glacial acetic acid up to neutralization. Then, carboxymethyl *Cassia* gum was washed with aqueous methanol (80% v/v) several times and dried at 80°C for 24 h in hot air oven [19].

2.2.2. Experimental design.

Microwave assisted carboxymethylation of *Cassia* gum was optimized using two-factor, three-level central composite design. To study the optimum combination of variables on carboxymethylated *Cassia* gum, the effect of factors i.e. microwave power (X_1) and microwave irradiation time (X_2) were used as independent variables and studied the degree of substitution which was chosen as a dependent variable. The impact of the independent variables was explored at three concentrations, i.e. small (-1), medium (0) and high (+ 1). The

experimental design and statistical analysis of results were carried out using Design Expert® software (version 11.0.0, Stat-ease Inc. Minneapolis, MN).

2.3. Characterization of carboxymethylated *Cassia* gum.

2.3.1. Detection of degree of substitution.

The degree of substitution (DS) was detected by acid wash method [20]. Accurately weighed carboxymethylated *Cassia* gum (0.5 gm) was spread in 20ml of HCl reagent and sonication (Powersonic 405. Hwashin Technology Seoul. Korea) was done for 3-4 h. Separation of large particles was done through filtration, followed by washing with methanol (70% v/v) up to neutralization and dried at 80 °C in hot air oven. A weighed amount of carboxymethylated *Cassia* gum (0.3 gm) was dispersed in 10 ml of methanol (70% v/v) and stirred for 30 minutes by adding a fraction of distilled water (30 ml) and 0.5 N NaOH (7.5 ml). Further, sonication was carried out for 4 h to solubilize the carboxymethylated *Cassia* gum completely. The excess of NaOH was back titrated with 0.5N HCl. The degree of carboxymethylated *Cassia* gum was calculated as per the following equation given by Eyer et al. 1947 [21]:

$$DS = \frac{0.162A}{1-0.058A}$$

Where, A is milliequivalents of NaOH required per gram of the sample and the value of A was calculated using the following equation:

$$A = \frac{(V_{NaOH} \times N_{base}) - (V_{acid} \times N_{acid})}{W}$$

Where, V_{base} and N_{base} are volume and the normality of sodium hydroxide used while V_{acid} and N_{acid} are the volume and normality of hydrochloric acid used.

2.3.2. Fourier Transform-Infrared spectroscopy (FT-IR).

The Fourier transform infrared spectra of *Cassia* gum and carboxymethylated *Cassia* gum (IR-affinity-I, Shimadzu, Japan) were recorded using potassium bromide (KBr) disc method in the spectrum range between 4000 cm^{-1} – 400 cm^{-1} . Individually, Small amounts of native and modified *Cassia* gum with KBr was compressed in pellets using IR hydraulic press (CAP-15 T, PCI Analytics, Mumbai, India).

2.3.3. X-Ray diffraction analysis (XRD).

Cassia gum powder and carboxymethylated *Cassia* gum powder were also characterized by the diffraction patterns with nickel filters obtained by XRD (Miniflex 2, Rigaku, Japan). Both samples were fixed on glass holders and scanned at an approximate spectrum of 10°-80° diffraction angle (2θ) with a processing rate of 0.05/min, where, the voltage was 30 kV and the current was 15 mA.

2.4. Preparation of microwave cross-linked carboxymethylated *Cassia* gum beads.

The interaction between carboxymethylated *Cassia* gum and calcium ion was explored by preparing ionically gelled carboxymethylated *Cassia* gum beads in which diclofenac sodium was used as a model drug. An aqueous solution of carboxymethylated *Cassia* gum (0.5 g) containing diclofenac (100 mg) was added drop by drop into the calcium chloride (CaCl_2) solution (10% w/v) using sterile syringe. The prepared beads were kept in CaCl_2 solution for 5

minute to complete the gel process and dried in the oven. After drying, prepared beads (200 mg) were modified in household microwave (MH-6348BS, LG Electronics India Pvt. Ltd., India) at variable power (160, 320, 480 and 640 W) where the optimized power (480 W) was selected and beads were modified [22]. After successful microwave cross-linking of carboxymethylated *Cassia* gum beads, the comparison of the release rate of carboxymethylated *Cassia* gum beads with microwave cross-linked beads was carried out to confirm the cross linking.

2.5. Evaluation of carboxymethylated *Cassia* gum beads with microwave cross-linked carboxymethylated *Cassia* gum beads.

2.5.1. Fourier Transform-Infrared spectral analysis (FT-IR).

KBr pellet technique was employed to record the FT-IR spectra of diclofenac sodium drug loaded carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads. Required amount of both beads (2 mg) were precisely weighed and triturated in pestle and mortar with KBr (100 mg) individually. In IR hydraulic presses (80 kg/cm²) (CAP-15 T, PCI Analytics, Mumbai, India), the mixture was pressed into pellets for 30 sec. Scans between 4000 cm⁻¹ and 400 cm⁻¹ were recorded for spectral analysis (IR Affinity-I, Shimadzu, Japan).

2.5.2. Determination of Entrapment efficiency.

The total entrapment of drug, diclofenac sodium was determined spectrophotometrically at wavelength 276 nm, which is present in weighed quantities of dried beads (Cary 5000. Varian, Australia). In the phosphate buffer (pH 6.8) solution, 100 mg beads were smashed and digested under the probe sonicator (Q55, Q Sonica, USA) to an intensity of 55% over five minute. Then, filter out the dispersed particles and analyze the filtrate for drug content. Further, the efficiency of trapping (EE) was calculated using equation:-

$$\% \text{ Entrapment Efficiency} = \frac{\text{amount of diclofenac found in beads}}{\text{Total diclofenac used in the preparation of beads}} * 100$$

2.5.3. In vitro release behaviour.

By utilizing USP Type-II dissolution apparatus (TDL-08L. Electrolab. India), a comparative *in vitro* release analysis of carboxymethylated *Cassia* gum beads with microwave cross-linked carboxymethylated *Cassia* gum beads were conducted. Beads (equal to 100 mg of drug) were put into muslin cloth and tied on paddle in a dissolution apparatus filled with phosphate buffer (1000 ml, pH 6.8). The temperature was maintained of (37±0.5)°C at 50 rpm for 24 h. 5 ml of sample aliquots were removed at periodic intervals and substituted by equivalent new medium quantity. Using UV-spectrophotometer (Cary 5000, Varian, Australia), the obtained samples were evaluated at 274 nm for the content of diclofenac sodium [13].

3. Results and Discussion

Carboxymethylated *Cassia* gum, so obtained, was found to be off-white color and odourless. The results of degree of substitution (Y₁) of various batches of prepared carboxymethylated *Cassia* gum as per the design protocol are presented in Table 1.

Table 1. Evaluation of various batches of carboxymethylated *Cassia* gum prepared as per central composite design.

Run	Microwave Power (W) (X_1)	Microwave Time (minute) (X_2)	Degree of substitution (Y_1)	Percent yield (%)
1	50	2.5	0.986	96.1
2	100	2.5	0.871	98.7
3	150	2.5	0.711	99.5
4	50	5	0.387	91.4
5	100	5	0.429	89.9
6	150	5	0.049	97.9
7	50	7.5	2.592	79.5
8	100	7.5	2.250	79.1
9	150	7.5	2.592	78.2
10	100	5	0.227	91
11	100	5	0.429	89.4
12	100	5	0.430	88.8
13	100	5	0.387	89

The results were fitted in various polynomial models and it was observed that the response (Y_1) i.e. degree of substitution fitted best into the quadratic response surface model. The polynomial model for the response Y_1 can be expressed by following equation.

$$Y_1 = 4.50826 - 0.004243X_1 - 1.86759X_2 + 0.000550X_1X_2 - 2.75 \times 10^{-6}X_1^2 + 0.213699X_2^2$$

On subjecting the polynomial model to ANOVA analysis, the model was found to be significant ($P < 0.05$) with non-significant lack of fit ($P > 0.05$) as shown in table 2. The R^2 value for response Y_1 is 0.9839. Adequate precision measures the signal to noise ratio while the ratio of 22.218 indicates an adequate signal. Thus, the model can be used to navigate the design space.

Table 2. The coefficient estimate and the P-values of different variables of carboxymethylated *Cassia* gum.

Factor	Y_1	
	Estimate	P-value
X_1	-0.1022	0.1419
X_2	0.8110	0.0001
X_1X_2	0.0688	0.3934
X_1^2	-0.0069	0.9418
X_2^2	1.34	0.0001

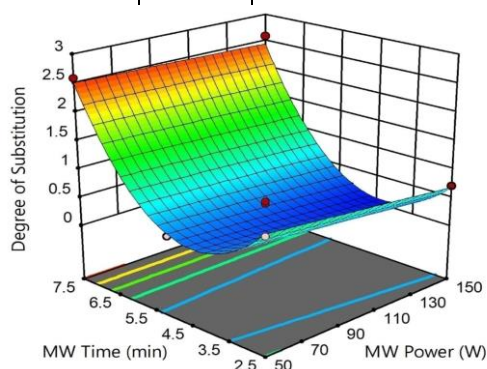


Figure 1. Response surface plot indicating combined response of microwave power and time of exposure on degree of substitution.

It can be observed that the response Y_1 is affected significantly by the significant synergistic linear and quadratic contributions of X_2 . Figure 1 illustrates the 3-dimensional response surface plot showing the combined effect of microwave power (X_1) and microwave time (X_2).

It was also observed that the influence of microwave time on degree of substitution is much greater than the influence of microwave power. Further, there is a curvilinear relationship between microwave exposure time and degree of substitution. As, microwave exposure time gets increased from 2.5 to 7.5 minutes, there was a slight decrease in the degree of substitution but further increase in microwave exposure time increased the degree of substitution. Thus, greater the microwave exposure, greater was the degree of substitution. Further, numerical optimization was done through Design Expert Software, which gives three solutions. So, the optimal parameters were found to be microwave power of 50W and microwave exposure time of 7.36 minutes which provided carboxymethylated gum with a degree of substitution of 2.5 (predicted 2.3). Thus, the proposed model is suitable for synthesizing products with desired degree of substitution.

3.1. Characterization of carboxymethylated *Cassia* gum.

3.1.1. FT-IR spectroscopy.

Figure 2 depicts the FT-IR spectra of crude *Cassia* gum with carboxymethylated *Cassia* gum. In IR spectra of *Cassia* gum, broad absorption band at 3424.67 cm^{-1} is due to O-H stretching. Absorption peak originating at 2878.80 cm^{-1} is due to C-H stretching vibrations of alkanes. The peaks appearing at 1667 cm^{-1} and 1164 cm^{-1} owing to C=O stretching vibration (asymmetric) and C-O-C stretching respectively. On the other hand, the IR spectra of carboxymethylated *Cassia* gum showed absorptive pattern with characteristics absorption peak at 3441.06 cm^{-1} is due to O-H stretching. Peaks at 2920.27 cm^{-1} and 2853.73 cm^{-1} are due to C-H stretching of alkanes. The peak appearing at 1735.96 cm^{-1} is due to C=O stretching while the extra peak at 1616.37 cm^{-1} and 1418.31 cm^{-1} is attributed to C=O stretching and carboxylate anions confirm the addition of carboxymethyl group on *Cassia* gum. Also, Peak at 1026.14 cm^{-1} can be due to C-O stretching of -C-O-C- [23].

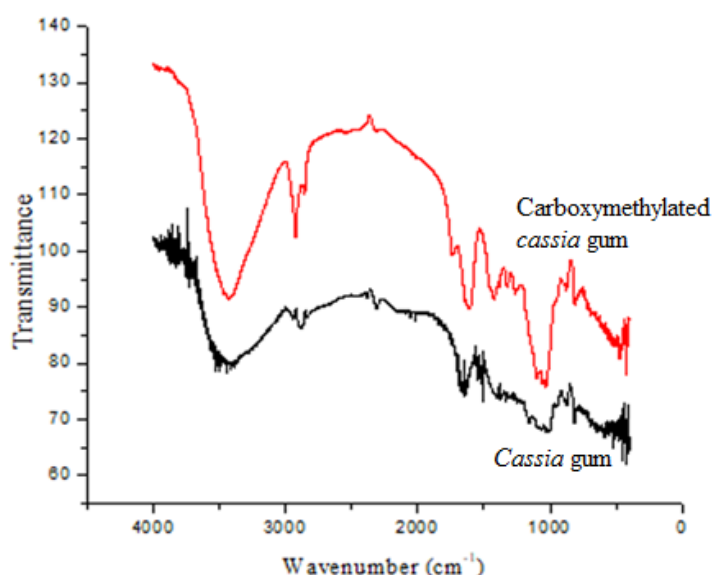


Figure 2. FT-IR spectra of *Cassia* gum and carboxymethylated *Cassia* gum.

3.1.2. X-Ray diffraction analysis (XRD).

Figure 3 displays the X-Ray diffractogram of *Cassia* gum and carboxymethylated *Cassia* gum. X-Ray diffraction spectra of *Cassia* gum are typical of amorphous material. On the other hand, microwave assisted carboxymethylated *Cassia* gum displays sharp peak at

16.74°, 24.3° (2θ) which indicates a slight increase in crystallinity of *Cassia* gum on carboxymethylation.

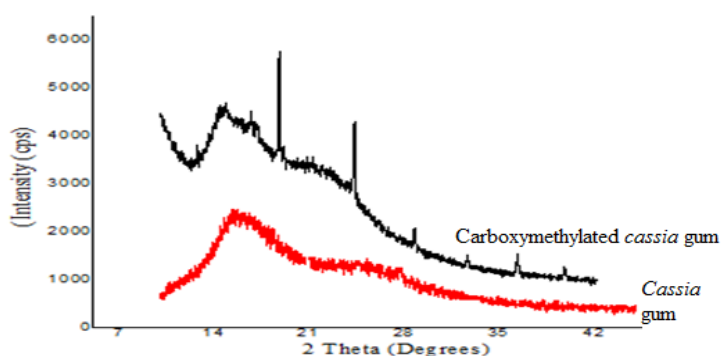


Figure 3. XRD spectra of *Cassia* gum and carboxymethylated *Cassia* gum

3.1.3. Pharmaceutical evaluation of beads.

Carboxymethylation of *Cassia* gum imparts anionic character on the gum; as a result, it interacts with calcium ions to form gels. This property was used to prepare ionically gelled beads of diclofenac which was used as the model drug. Further, it had already been reported that microwaves can be used to tailor the release of drugs from beads by inducing cross-linking [22]. In the present study, the beads prepared were cross-linked by treatment with microwave exposure at different powers. The beads cross-linked at less than 480W were found to erode at a faster pace while beads cross-linked on more than or equal to 480W power were found to be porous, so cross-linking at 480W was considered satisfactory.

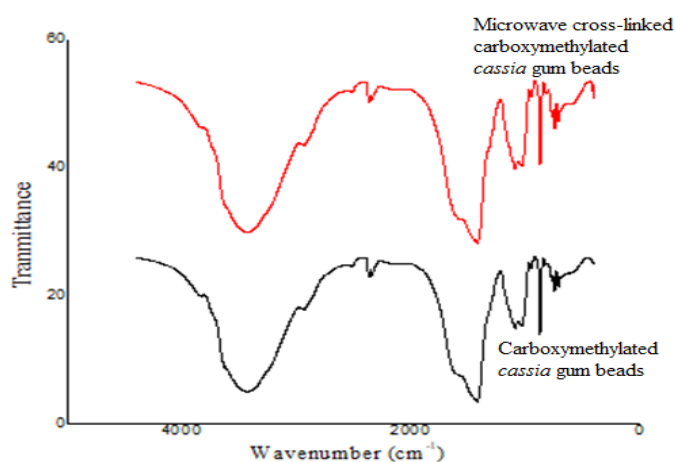


Figure 4. FT-IR spectra of carboxymethylated *Cassia* gum beads and microwave cross-linked *Cassia* gum beads.

3.1.4. FT-IR spectra of beads.

The FT-IR spectra of carboxymethylated *Cassia* gum beads (Figure 4) showed the broad absorption band at 3455.43 cm^{-1} can be due to OH stretching and absorption peak originated at 2628.64 cm^{-1} due to C-H stretching vibrations of alkanes. Peaks at 1629.37 cm^{-1} and 1420.05 cm^{-1} are due to C=O asymmetric stretching vibrations of carboxylic acid of glucuronic acid while at 1061.61 cm^{-1} , peak was seen which can be due to C-O-C stretching. The FT-IR spectra of microwave cross-linked carboxymethylated *Cassia* gum beads showed absorptive pattern with a characteristic absorption peak at 3422.45 cm^{-1} and 2628.94 cm^{-1} is due to OH stretching and C-H stretching of alkanes respectively. The peaks appearing at

1614.31 cm^{-1} is attributed to C=O stretching while the peak at 1420.02 cm^{-1} and 1063.02 cm^{-1} corresponds to carboxylate anions and C-O stretching of -C-O-C respectively [24].

3.1.5. Entrapment efficiency.

The entrapment efficiency of diclofenac sodium in carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads were found to be 64.4% and 61.7% respectively. Thus, it was concluded that there was no significant change of microwave exposure on the drug content of beads.

3.1.6. *In vitro* drug release.

The *in vitro* drug release of diclofenac sodium loaded microwave assisted carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads are shown in Figure 5. It was observed that almost 100% of the drug got release within 60 minutes for both carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads. Thus, no significant difference in *in vitro* drug release was observed on treatment with microwave radiation. During an earlier study, microwave exposure of chitosan beads was also reported to modify the release of drug due to cross-linking induced by microwaves [22]. However, in the present study not such effect was observed.

To determine the kinetics and release mechanism, the data was analysed to various models i.e. zero order, first order, Higuchi and Korsmeyer-Peppas. The R^2 values for zero order, first order, Higuchi and Korsmeyer-Peppas release kinetics were found to be 0.919, 0.268, 0.457 and 0.726 respectively for carboxymethylated *Cassia* gum beads, while for the carboxymethylated *Cassia* gum beads cross-linked using microwave showed respective R^2 value of 0.923, 0.241, 0.419 and 0.690 respectively. The results thus indicate that the release of diclofenac from the beads follows zero order kinetics. Further, the value of release exponent (n) for carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads were found to be 0.82 and 0.78 respectively, which points to the anomalous release mechanism i.e. combination of erosion and diffusion.

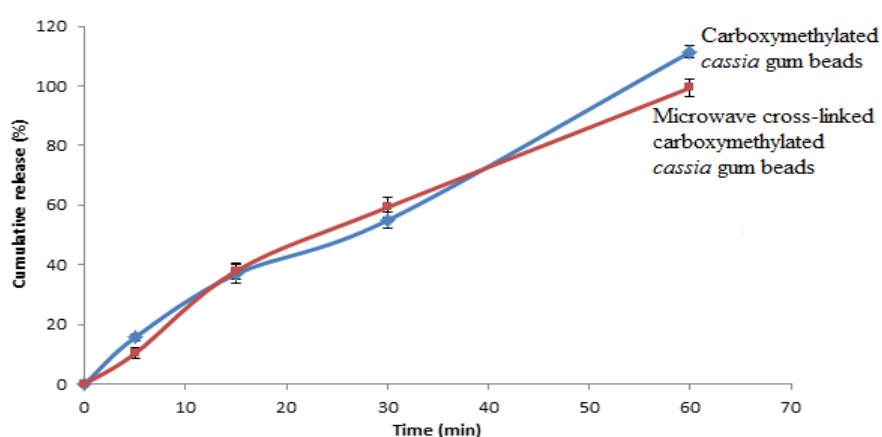


Figure 5. *In vitro* release profiles of carboxymethylated *Cassia* gum beads and microwave cross-linked carboxymethylated *Cassia* gum beads.

4. Conclusions

Carboxymethylation of *Cassia* gum was achieved by employing microwave assisted technique. The response surface methodology was used to optimize the effect of microwave power and microwave exposure time on the degree of substitution. FT-IR and XRD

investigations confirmed the carboxymethylation of *Cassia* gum where XRD results indicated that carboxymethylation of *Cassia* gum imparts crystallinity to the inherent amorphous *cassia* gum. The obtained carboxymethylated *Cassia* gum was finally formulated into ionically gelled beads and further evaluated for pharmaceutical applications. The prepared beads were also treated with microwave exposure to modify the release behavior. However, no significant influence of microwave exposure on the release behavior of Ca²⁺-gelled carboxymethyl *Cassia* gum was observed. On the basis of the results of our study, it can be concluded from that microwave assisted carboxymethyl modification of *Cassia* gum can be utilized to derive a polymeric material with potential pharmaceutical applications.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Wu, D.T.; Liu, W.; Han, Q.H.; Wang, P.; Xiang, X.R.; Ding, Y.; Zhao, L.; Zhang, Q.; Li, S.Q.; Qin, W. Extraction Optimization, Structural Characterization, and Antioxidant Activities of Polysaccharides from Cassia Seed (*Cassia obtusifolia*). *Molecules* **2019**, *24*, 1-16, <https://doi.org/10.3390/molecules24152817>.
2. Paudel, P.; Seong, S.H.; Shrestha, S.; Jung, H.A.; Choi, J.S. In Vitro and in Silico Human Monoamine Oxidase Inhibitory Potential of Anthraquinones, Naphthopyrones, and Naphthalenic Lactones from Cassia obtusifolia Linn Seeds. *ACS Omega* **2019**, *4*, 16139-16152, <https://doi.org/10.1021/acsomega.9b02328>.
3. Meng, Y.; Liu, Y.; Fang, N.; Guo, Y. Hepatoprotective effects of Cassia semen ethanol extract on non-alcoholic fatty liver disease in experimental rat. *Pharmaceutical Biology* **2019**, *57*, 98-104, <https://doi.org/10.1080/13880209.2019.1568509>.
4. Hou, J.; Gu, Y.; Zhao, S.; Huo, M.; Wang, S.; Zhang, Y.; Qiao, Y.; Li, X. Anti-Inflammatory Effects of Aurantio-Obtusin from Seed of Cassia obtusifolia L. through Modulation of the NF-κB Pathway. *Molecules* **2018**, *23*, 1-15, <https://doi.org/10.3390/molecules23123093>.
5. Prajapati, V.D.; Jani, G.K.; Moradiya, N.G.; Randeria, N.P. Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydrate Polymers* **2013**, *92*, 1685-1699, <https://doi.org/10.1016/j.carbpol.2012.11.021>.
6. Huanbutta, K.; Sittikijyothin, W. Use of seed gums from Tamarindus indica and Cassia fistula as controlled-release agents. *Asian Journal of Pharmaceutical Sciences* **2018**, *13*, 398-408, <https://doi.org/10.1016/j.ajps.2018.02.006>.
7. Jang, S.H.; Yang, D.K. The combination of Cassia obtusifolia L. and Foeniculum vulgare M. exhibits a laxative effect on loperamide-induced constipation of rats. *PLOS ONE* **2018**, *13*, 1-13, <https://doi.org/10.1371/journal.pone.0195624>.
8. Sharma, B.R.; Kumar, V.; Soni, P.L.; Sharma, P. Carboxymethylation of *Cassia tora* gum. *Journal of Applied Polymer Science* **2003C**, *89*, 3216-3219, <https://doi.org/10.1002/app.12430>.
9. Sharma, B.R.; Kumar, V.; Soni, P.L. Cyanoethylation of *Cassia tora* gum. *Starch/Starke* **2003b**, *55*, 38-42, <https://doi.org/10.1002/star.200390004>.
10. Sharma, B.R.; Kumar, V.; Soni, P.L. Carbamoylethylation of *Cassia tora* gum. *Carbohydrate Polymers* **2003a**, *54*, 143-147, [https://doi.org/10.1016/S0144-8617\(03\)00132-2](https://doi.org/10.1016/S0144-8617(03)00132-2).
11. Sharma, B.R.; Kumar, V.; Soni, P.L. Graft copolymerization of acrylamide onto *Cassia tora* gum. *Journal of Applied Polymer Science* **2002**, *86*, 3250-3255.
12. Liu, M.; Niu, C.; Shaoying, L.I. Synthesis and characterization of carboxymethyl *Cassia tora* gum. *Journal of Hebei University of Science & Technology* **2014**, *35*, 548-554.

13. Rani, D.; Ahuja, M. Carboxymethylation of *Lepidium sativum* polyuronide, its characterization and evaluation as a nanometric carrier. *International Journal of Biological Macromolecules* **2017**, *99*, 233-240, <https://doi.org/10.1016/j.ijbiomac.2017.02.036>.
14. Theis, V.T.; Santos, Q.A.V.; Appelt, P.; Barbosa-Dekker, M.A.; Vetvicka, V.; Dekker, H.F.R.; Cunha, A.A.M. Fungal exocellular(1-6)- β -D-glucan: Carboxymethylation, characterization, and antioxidant activity. *International Journal of Molecular Sciences* **2019**, *20*, 1-15, <https://doi.org/10.3390/ijms20092337>.
15. Rimpay; Abhishek, R.; Ahuja, M. Evaluation of carboxymethyl Moringa gum as nanometric carrier. *Carbohydrate Polymers* **2017**, *174*, 896-903, <https://doi.org/10.1016/j.carbpol.2017.07.022>.
16. Brhane Y. Evaluation of carboxymethylated *Plectranthus edulis* starch as a suspending agent in metronidazole benzoate suspension formulations. *PLOS One* **2020**, *15*(3), 1-17, <https://doi.org/10.1371/journal.pone.0228547>
17. Malik, S.; Ahuja, M. Gum kondagogu-g-poly (acrylamide): Microwave-assisted synthesis, characterization and release behavior. *Carbohydrate Polymers* **2011**, *86*, 177-184, <https://doi.org/10.1016/j.carbpol.2011.04.027>.
18. Sun, H.; Shao, X.; Jiang, R.; Ma, Z.; Wang, H. Effects of ultrasonic/microwave-assisted treatment on the properties of corn distarch phosphate/corn straw cellulose films and structure characterization. *Journal of Food Science & Technology* **2018**, *55*, 1467-1477, <https://doi.org/10.1007/s13197-018-3063-8>.
19. Abhishek; Rimpay; Ahuja, M. Moringa gum-g-poly(*N*-vinyl-2-pyrrolidone) – a potential buccoadhesive polymer. *International Journal of Biological Macromolecules* **2018**, *109*, 732-739, <https://doi.org/10.1016/j.ijbiomac.2017.12.112>.
20. Meenakshi; Ahuja, M.; Verma, P. MW-assisted synthesis of carboxymethyl tamarind kernel polysaccharide-g-polyacrylonitrile, optimization and characterization. *Carbohydrate Polymers* **2014**, *113*, 532-538, <https://doi.org/10.1016/j.carbpol.2014.07.041>.
21. Eyster, R.W.; Klug, E.D.; Diephuis, F. Determination of degree of substitution of sodium carboxymethylcellulose. *Analytical Chemistry* **1947**, *19*, 24-27, <https://doi.org/10.1021/ac60001a007>.
22. Nurjaya, S.; Wong, T.W. Effects of microwave on drug release properties of matrices of pectin. *Carbohydrate Polymers* **2005**, *62*, 245-257, <https://doi.org/10.1016/j.carbpol.2005.07.029>.
23. Kumar, D.; Mundlia, J.; Kumar, T.; Ahuja, M. Silica coating of carboxymethyl tamarind kernel polysaccharide beads to modify the release characteristics. *International Journal of Biological Macromolecules* **2019**, *146*, 1040-1049, <https://doi.org/10.1016/j.ijbiomac.2019.09.229>.
24. Minkal; Ahuja, M.; Bhatt, C.D. Polyelectrolyte complex of carboxymethyl gum katira-chitosan: preparation and characterization. *International Journal of Biological Macromolecules* **2018**, *106*, 1184-1191, <https://doi.org/10.1016/j.ijbiomac.2017.08.128>.