ABSTRACT: There has been a cumulative interest across the globe in substituting the synthetic materials with sustainable, economical, and biobased green alternatives for utilization in diverse industrial applications. Galactomannans are among the most important sustainable, biodegradable macromolecules abundantly produced by plants, which can be exploited for a range of end-use applications. Functionalization of the galactomannans may improve the physicochemical properties for diverse industrial applications. In the present study, the cationic derivative of *Cassia tora* gum, a 1:5 galactomannan, was synthesized under heterogeneous alkaline conditions using CHPTAC. The effect of each reaction parameter on the degree of substitution (DS) was investigated using Taguchi L16 orthogonal array. The optimized cationic product with DS 0.28 showed promising results as a biopolymeric flocculant for wastewater treatment and as a wet strength additive for improving the physical strength of paper prepared from old corrugated carton for its recycling. The optimized product was characterized by advanced spectroscopic methods.

1. INTRODUCTION

Polysaccharides are the most abundant renewable organic biopolymeric materials in the biosphere, synthesized and consumed by nearly all plants, animals, and microorganisms of our planet. Plant seeds have been an ancient source of industrial hydrocolloids or polysaccharide gums. Intriguingly, despite being a major part of the biomass generated only a meagre quantity is utilized by humans. Galactomannans are the natural storage polysaccharides primarily present in the seeds of the Leguminosae plants and located in the endosperm of the ungerminated seeds having various physiological functions. The major sources of galactomannans are locust bean (*Ceratonia siliqua*), guar (*Cyamopsis tetragonoloba*), tara (*Caesalpinia spinosa* Kuntze), and fenugreek (*Trigonella foenum-graecum* L.) gums having different mannose to galactose ratios (M/G) as 4:1; 2:1; 3:1, and 1:1, respectively. The galactomannans obtained from different sources possesses characteristic functional properties, inherently determined by its molecular weight and the ratio of mannose to galactose. The inadequate availability of the industrial gums necessitate exploration of other novel galactomannans possessing comparable industrial potential and competence with currently demanded gums such as guar, locust bean, and so on.

A new promising galactomannan obtained from *Cassia tora*, a ruderal species, is a heteropolymer, composed of galactose and mannose units in a 1:5, Gal/Man ratio (1,5 galactomannan). The units are linked through a (1→4)-β-D-mannopyranose (Man) units attached to (1→6)-α-D-galactopyranose (Gal) units. Galactomannans exhibit unique and interesting physicochemical properties dependent on galactose: mannose ratio. These polymers are regarded as safe because of their biocompatibility, biodegradability, and nontoxicity. Consequently, numerous potential applications of galactomannans have been investigated: paper, textile, food, cosmetics, pharmaceuticals, paints, oil well drilling, mining, explosives, and water treatments. However, in many cases, natural gums are deficient in certain end-use properties viz. low solubility in cold water, uncontrolled hydration, changes in viscosity during storage, pH-dependent solubility, and fast biodegradability. To circumvent this issue, the gum is chemically modified to enhance the inherent properties of gums for specialty end-use industrial applications. Thus, the gum is altered by one or more reactions...
such as depolymerization, oxidation, hydroxyalkylation, carboxymethylation, cyanoethylation, cationization, and sulphation. Among all of these chemical modifications, cationization stands out because of diverse industrial applications of the modified derivative. It is a valuable method to impart properties having commercial significance. In the present study, the C. tora gum (CTG) was cationically modified to augment its demand for various applications. The cationic derivatives possess positively charged groups such as amino, imino, ammonium, and so on and are green substrates used to treat organic and inorganic matter in wastewater carrying a negative charge. Cationic (quaternized) derivatives of galactomannans are demanded in textile, cosmetic, personal care products, and food industries, modification of pulp and fiber to improve paper-making properties and dye uptakes. Cationic starches, cellulose, chitosan, and guar gum are commercially significant and industrially accepted polymers for their application as wet-end additives in the paper industry to improve retention, drainage and strength, natural bio flocculant, and as antimicrobial agents. Wastewater treatment by the coagulation–floculation process using cationic polymers is one of most important industrially accepted phenomenon for decades and generally used to reduce the turbidity of industrial and municipal waste water to reduce coagulant dosages, the volume of sludge and the ionic load of the wastewater and to save overall costs. Generally, the polymers used in coagulation–floculation treatments are synthetic poly-acrylamides, polyacrylic acids, and polystyrene sulfonic acids and their derivatives, which are not readily biodegradable along with neurotoxic and carcinogenic. Thus, there has been a cumulative interest in substituting the synthetic polymers with sustainable, inexpensive natural biobased alternatives.

In previous studies, CTG has been cationically etherified by quaternizing reagent. Intriguingly, in the first study systematic optimization and characterization has not been done, whereas in the other recent study lower degree of substitution (DS, 0.112) could be achieved. The cationic products having low DS do not exhibit sufficient activity as wet-strength additive and floculant. Therefore, it was envisaged to prepare a high DS product by using a different solvent system. In the present study desired cationic derivative (high DS) has been prepared by using aqueous isopropanol in heterogeneous alkaline conditions.
experimental results evidence the potential of the cationic derivative of C. tora galactomannan as a wet-strength additive in papermaking and a biopolymeric flocculant in wastewater treatment. A systematic statistical design was used to conduct the experiment for optimizing the reaction conditions for the synthesis of a cationic derivative of CTG with high DS (0.28) under heterogeneous alkaline conditions using 3-chloro-2-hydroxypropyl trimethylammonium chloride (CHPTAC). The cationic derivative was characterized by $^1$H NMR, HSQC, Fourier transform infrared spectroscopy (FTIR), field emission...
The cationization reaction of CTG proceeds through Williamson’s ether synthesis reaction (Scheme 1). The substitution of cationic moiety on to galactomannan takes place initially by etherification of hydroxyl functional groups where the generation of alkoxide ion occurs in presence of an alkaline medium. The reactive species 2,3-epoxypropyltrimethylammonium chloride (EPTAC) is formed in situ because of alkaline medium. The reactive species 2,3-epoxypropyltrimethylammonium chloride is also formed simultaneously as a byproduct in the reaction (Scheme 1).33

The five factors viz. reaction time, temperature (°C), concentration of alkali (pH), CHPTAC and the gum solvent ratio play an indispensable role in the cationization process. The effect of these parameters was assessed using Taguchi’s experimental design comprising a total of 32 experiments (Table 1). The response values in terms of DS and signal to noise (S/N) ratios of experimental design involving five factors were studied for optimization of the cationization process. The S/N ratio was used to assess the quality characteristics of the product.37–39 The highest S/N ratio signifies the optimum level for each factor and contributes to the maximum DS. The S/N ratio may be obtained by using eq 1.

$$S/N \text{ ratio } = -10 \log_{10}(MSD)$$  \hspace{1cm} (1)

where, MSD denotes the mean square deviation for output characteristic, and may be determined as (eq 2).

$$MSD = \frac{1}{n} \sum_{i=1}^{n} 1/\gamma_i^2$$  \hspace{1cm} (2)

Table 2. ANOVA for Testing the Effect of Factors on the Degree of Substitution

| factor                     | SS_T | df_p | MS_T | F    | p    |
|---------------------------|------|------|------|------|------|
| F_1 (time)                | 0.39671 | 3    | 0.13224 | 10.0953 | 0.00057 |
| F_2 (alkali)              | 0.86905 | 3    | 0.28968 | 22.1155 | 0.000006 |
| F_3 (CHPTAC)              | 0.22005 | 3    | 0.07335 | 5.59917 | 0.00805 |
| F_4 (temperature)         | 0.37721 | 3    | 0.12574 | 9.59917 | 0.00073 |
| F_5 (gum liquor ratio)    | 0.31692 | 3    | 0.10564 | 8.06484 | 0.00169 |

The ANOVA test at a 5% level of significance using STATISTICA release 7 was applied to determine the significance and percentage contribution of each reaction parameter in the experimental study. It represents the overall variance in the S/N ratio and indicates significant (<0.05) and nonsignificant (>0.05) p-values (Table 2). The results indicate that all of the factors are significantly affecting the substitution of the cationic moiety (Table 2).

The effect of reaction time (60–240 min) on the cationization of CTG was studied (Figure 1a). It was observed that the S/N ratio does not show a substantial change up to 180 min and thereafter increased up to the final level (240 min). The lower S/N ratio up to 180 min was due to the nondonisability of the gum in the aqueous isopropanol medium which prohibits the penetration of the alkaline solution into the gum molecules and also hinders the effective collision between molecules and consequently lowers the substitution reaction. However, an increase in reaction time increases the dispersibility of the gum leading to augmentation in the S/N ratio.

The effect of NaOH concentration (0.0125–0.05 mol) on the cationization of CTG was studied (Figure 1b). It was found that with an increase in the NaOH concentration, the S/N ratio started to decrease. Thus, 0.0125 mol of NaOH was adequate to generate the alkoxide ion on 0.0123 mol of gum and also to convert the CHPTAC into reactive species, EPTAC. On increasing the concentration of NaOH up to 0.05 mol the extent of side product (2,3-dihydroxy propyl trimethylammonium chloride) formation increases which leads to a decrease in the S/N ratio and affect negatively the cationization reaction.

The effect of CHPTAC concentration (0.00159–0.00638 mol) on the cationization of CTG was studied in terms of the effect on the S/N ratio (Figure 1c). The results illustrate the distinct pattern of the increase in the S/N ratio while increasing the CHPTAC concentration. The increase in the S/N ratio on increasing CHPTAC concentration was due to increased accessibility of the reacting groups with respect to available alkoxide ions generated on the galactomannan, thereby, facilitating the cationization reaction. Therefore, 0.00638 mol of CHPTAC was the optimized concentration within the varied reaction parameters taken under investigation.

The effect of reaction temperature on the cationization was studied in terms of the S/N ratio (Figure 1d). It illustrated a requisite role in maintaining the pH in aq. isopropanol medium. The results show an initial decrease in S/N ratio at a temperature ranging from 30 to 40 °C and thereafter a sudden increase was observed up to 50 °C and started to decrease again at 60 °C. This effect may be due to the variation in the pH of the reaction medium. At the lower temperature, the pH was observed as 13–14 in aq. isopropanol medium leading to the formation of 2,3-dihydroxypropyltrimethylammonium chloride as a by-product in a higher amount, whereas on increasing the temperature (up to 50 °C), the pH of the reaction medium decreases (pH, 10–11), consequently favors the cationization reaction.40 Further, on increasing the temperature up to 60 °C, the pH again started to drop down, thereby, resulting in a decrease in DS and the S/N ratio.

Cationization reaction in isopropl alcohol favors the reaction mechanism in obtaining high DS which is in...
agreement with other reports. The effect of the solvent system on the extent of the reaction is related to miscibility, the ability to solubilize the etherifying agents and to create an environment that favors cationization reaction. This effect may be due to the lesser extent of redox reaction occurred between isopropanol and NaOH which provide more hydroxide ions for the generation of alkoxide ions on the polymer chain, whereas in case of methanol, the generation of free hydroxide ions are lesser because of greater extent of redox reaction with NaOH. This effect may be due to the difference in the polarity of two solvents. The results reveal that by increasing the gum solvent ratio (01:05 to 01:20), the S/N ratio also increased. The increase in gum solvent ratio leads to the effective collision among the molecules and also provides enough water for the proper solubilization of alkali and gum molecules. Thus, it may be concluded that the solvent medium regulates the extent of reaction.

The experimental results suggest that these factors at the optimum levels strongly support the maximum DS. The optimized reaction conditions for cationization reaction are, time 240 min, alkali 0.0125 mol, CHPTAC 0.00638 mol, temperature 50 °C, and the gum–liquor ratio 1:20. A confirmatory experiment was conducted over the optimized reaction conditions and the result reveals a maximum DS that is 0.28, which validates the statistical design.

The TG curve for CTG and CCTG provides vital information about the thermal stability of polysaccharide (Figure 2a). The CTG and CCTG showed two-stage weight loss up to 800 °C, with the first one corresponding to the loss of moisture content up to 125 °C, and the second weight loss corresponding to the decomposition of polysaccharide. The TG curve of CTG showed that the main stage of degradation occurred in the range of 280–350 °C, corresponding to 65% of mass loss and the peak indicated by DTG (DTGmax) was at 320 °C (Figure 2b) which started to decompose after 350 °C. In the case of CCTG, maximum degradation occurs within the range of 250–350 °C having 45% of mass loss. The decomposition temperature of CCTG is 350 °C and the peak indicated by DTG (DTGmax) was at 325 °C. The final decomposition temperature for CTG and CCTG is almost similar but the percentage mass loss is dissimilar for both. The lesser percentage mass loss in case of CCTG (45%) in comparison to CTG (65%) indicated the better thermal stability of cationic derivative. The increased thermal stability of CCTG is due to increased electrostatic attraction between the polymer chain and consequent increases in columbic repulsion due to positive charge of the cationic moiety. Second, the substitution of hydroxyl groups with quaternary ammonium substituents leads to decrease in dehydration between CTG hydroxyls and increase in thermal stability due to the lesser number of hydroxyl groups.

XRD measurements were performed to investigate the change in the crystallinity of native and modified CTG. The XRD patterns of CTG and CCTG are shown in Figure 2c. The
results indicate that the crystalline peak appear in the 2θ range 10–30° in case of CTG. However, the derivatized product does not have any peak indicating crystallinity. Thus derivatization leads to disruption in the original crystalline structure.43,44 The X-ray diffraction showed that the amount of amorphous nature increased after quaternization of native
This loss in crystallinity can also be attributed because of the effect of alkaline environment during the modification, which consequently leads to utilization of cationic derivative as flocculants because amorphous granules would enhance their ability to form a colloidal or true solution in cold water.46

To determine the structure of CTG and CCTG, FTIR spectra were recorded. Spectra of CTG and CCTG are shown in the Figure 3. Broad band resulting from vibration of the hydroxyl groups (O–H) appears at 3400.7 cm⁻¹. The band at 2919.6 cm⁻¹ is assigned to characteristics symmetrical stretching vibration due to the –CH₂ groups.47 The band at 1622.8 cm⁻¹ in CTG is attributed to the scissoring of two O–H bonds of absorbed water molecules.48 The absorption band at 814.6 and 875.5 cm⁻¹ were assigned to α-D-galactopyranose and β-D-mannopyranose units of the anomeric region, respectively.15,47,49 CTG and CCTG have similar peaks in FTIR spectra, however, an additional adsorption band for the quaternary ammonium groups (C–N) appeared at 1477.7 cm⁻¹, which is absent in CTG. Another band at 900.9 cm⁻¹ was attributed to a new C–O–C linkage formed due to the quaternary ammonium groups. These two additional peaks are evident for the incorporation of a cationic moiety onto the backbone of the galactomannan.

¹H NMR spectra for CTG (Figure 4a) and CCTG (Figure 4b) was recorded to confirm the incorporation of quaternary ammonium substituents onto CTG. Corresponding to α- and β-anomeric confirmers of galactose and mannose units, four signals were identified in the CTG and CCTG. The chemical shift of the four anemic signals of ¹H NMR is consistent with the presence of a very small peak at 5.35 ppm which was assigned for α-D-galactopyranose and a larger peak at 4.98 ppm assigned for the anomeric proton of β-D-mannopyranose units. A peak at 5.14 ppm and another very small peak at 4.72 ppm were assigned to α-D-mannopyranose and β-D-galactopyranose units, respectively.19,50 The ¹H NMR spectrum of CTG and CCTG showed characteristic chemical shifts clustered between 3.52 and 4.08 ppm for ring protons of mannose/galactose units. A prominent peak at δ 3.19 ppm, corresponds to the nine hydrogens of quaternary methylammonium groups (+N(CH₃)₃), appeared in the spectrum of CCTG, which was found missing in the spectrum of CTG, clearly indicating the
incorporation of quaternary ammonium groups onto the galactomannan backbone.\textsuperscript{19,51}

HSQC spectra of CTG and CCTG (Figure 5) were also measured in D\textsubscript{2}O to assign the signals. Figure 5 shows the HSQC spectrum of CTG and CCTG. In the HSQC spectra of CCTG, the correlation peak for C-10 at 55 ppm is attributed to the $\text{-CH}_3$ carbon of the quaternary ammonium group that was observed at 3.19 ppm for the protons attached to C-10 position.\textsuperscript{43} These two peaks were absent in CTG spectra. The peaks at 99 and 96 ppm were assigned to C-1 in $^{13}$C spectra.

Figure 7. Rheological data of CTG and CCTG.

Figure 8. (a) Optical image of flocculation performance of CCTG, (b) Graphical representation of the flocculation performance of CCTG.
and peaks in the range 4.5–5 ppm for the protons at C-1, respectively. The new peak at 55 ppm and 3.19 ppm showing the correlation spectra for $^{13}$C and $^1$H NMR in the HSQC spectra confirms the incorporation of the quaternary ammonium group onto galactomannans backbone.

Field emission scanning microscopy was used to investigate the morphology of CTG (Figure 6a) and CCTG (Figure 6b). It can be observed from the photomicrographs that the surface morphology of native CTG shows a smooth and compact structure, whereas, CCTG structure is loose, coarse, wrinkled, and disruptive in comparison to the CTG structure. It could be concluded that during the cationization process, the incorporation of EPTAC onto the galactomannan chain interrupted the inter- and intra-molecular hydrogen bonding resulting in the disruption of the surface morphology and also destroyed the crystalline structure of native CTG as well.

The rheological behavior of CTG and CCTG was studied and the results are shown in Figure 7. The results reveal that the viscosity of CCTG (454.6 cps) solution (2% w/v) was higher than the CTG (164 cps). The stability of the solution was also observed up to 240 h. The results demonstrate increase in apparent viscosity of solution as the storage time was increased from 24 to 120 h; thereafter slender decline was noticed up to 240 h, which shows the stability of the cationic product in aqueous solutions. Both CTG and CCTG exhibited a typical non-Newtonian pseudoplastic behavior. The samples showed marked shear-thinning behavior, that is, on increasing the shear rate the viscosity starts decreasing. This shear-thinning behavior may be due to the breaking of entangled polysaccharide molecules network.52 Apparently, the viscosity thinning behavior may be due to the breaking of entangled polysaccharide molecules network.52 The samples showed marked shear-thinning behavior, that is, on increasing the shear rate the viscosity starts decreasing. This shear-thinning behavior may be due to the breaking of entangled polysaccharide molecules network.52

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2.1. Flocculation Characteristics. The flocculation performance was measured in terms of the absorbance of the supernatant liquid and the results are shown in Figure 8. The lower absorbance of the solution corresponds to better performance of the flocculating agent. The results demonstrate the comparative studies on the absorbance of supernatant liquid of kaolin suspensions (1%) by using different concentrations (10–50 ppm) of CCTG (DS 0.1, 0.28). It was observed that CCTG product with 0.28 DS is a better flocculant in kaolin suspension than the product with 0.1 DS. The effect is mainly due to the cationic CTG derivative neutralizing the negative surface of the kaolin suspension and settling of aggregates due to cationic and anionic electrostatic attraction.52 The adsorbed polysaccharide tends to form loops and extend some distance from the particle surface into the aqueous phase and the other end of polysaccharide also suspends and gets adsorbed on the surface of another particle forming a bridge between particles. For effective bridging, the length of polymer chains should be long enough to extend from one particle surface to another. Hence, the polymers with longer chains would be more effective than with the shorter chains.52 This bridging process between solid particles consequently forms large flocs with a three-dimensional network structure. Besides bridging, the addition of the flocculant increases charge neutralization leading to zeta potential value zero, at which maximum flocculation occurs. Figure 8 shows that on increasing the concentration from 10 ppm to 50 ppm the absorbance increases, which means the turbidity in the suspension increases and subsequent decrease in flocculation. This effect may also be due to the higher flocculant doses; the bridging becomes negligible and further an higher electric positive charge assist to suspend particles leading to mutual electrostatic repulsion.54 The high DS (0.28) product of CCTG at a minimum concentration (10 ppm) shows better flocculation results in comparison to 0.1 DS. It is clear from the aforementioned results that CCTG with DS 0.28 obtained during the optimization process, can be used potentially as an efficient flocculant in wastewater treatments.

2.2. Wet Strength Additive Characteristics. The efficiency of the cationic products of different DS 0.1 and 0.28 was also determined for the application of the products as a wet strength additive. Surface charge (zeta potential, mV) and the cationic demand of CCTG with DS 0.1 and 0.28 were measured and the results are summarized in Table 3.

| particulars                | DS 0.1 | DS 0.28 |
|----------------------------|--------|---------|
| pH                        | 7.37   | 7.58    |
| streaming potential (mV)   | −558   | +69     |
| charge demand (μeq/L)      | 482    | 288     |

The CCTG with DS 0.1 shows anionic nature having cationic demand of 482 μeq/L, whereas CCTG with DS 0.28 shows cationic nature with anionic demand of 288 μeq/L, which reveal that the CCTG with high DS (0.28) possess more cationic charge.

Bursting strength/Burst factor is an important property for the packaging of paper and paperboard and is an indicator of sheet bonding. It is predominantly an internal sheet property. Bursting strength is the maximum pressure that the paper can resist without breaking when an external force is applied in the perpendicular direction. Figure 9a shows that a dose level of 0.4% of CCTG (DS 0.28) demonstrate maximum improvement of 26% (4.7 units), whereas CCTG with lower DS (0.1) shows 17.6% (3.2 units) improvement in burst factor. On increasing the dose amount to 0.5%, both the products show a decrease in bursting strength. Hence, it can be concluded that the CCTG with 0.28 DS, being more cationic in nature has a better burst factor as compared to CCTG with DS 0.1 at an optimum dose of 0.4%.

Tensile strength/Breaking length (km) of the paper was evaluated by using CCTG with DS 0.1 and 0.28. It is the maximum force per unit width that a paper strip can resist before breaking when applied longitudinally. It primarily depends on the degree of bonding between areas and also on fiber strength. Both the products show a 21% improvement in tensile strength at a dose of 0.4%. The tensile strength was further increased to 30 and 31% for CCTG with DS 0.1 and 0.28, respectively, at 0.5% dose. Figure 9b shows that on increasing the dose amount of CCTG, the tensile strength of hand sheets formed from OCC also increased.

Tearing strength/tear factor was also evaluated for CCTG. It is the mean force required to continue the tearing of paper from an initial cut. Tearing strength is highly dependent on fiber orientation of sheet and affects run ability. Figure 9c shows the tearing strength performance of CCTG with 0.1 and 0.28 DS. Tearing strength for CCTG with DS 0.28 was found higher at the level of 0.1% dose as compared to the CCTG with DS 0.1. The tear factor for CCTG with DS 0.28 treated pulp has shown a 16% improvement at 0.5% dosage. Hence, it
can be concluded that the cationic product with higher DS (0.28) represent better tearing strength than the product with lower DS (0.1).

3. CONCLUSIONS
Cationic derivative of 1,5 galactomannan with a high DS 0.28 was successfully synthesized using aq. Isopropanol as a solvent medium and the reaction conditions were optimized by using the Taguchi L16 statistical design. The optimized product was systematically characterized by spectroscopic methods viz. FTIR, 1H NMR, HSQC, and FESEM, and the incorporation of cationic moiety on to the galactomannan chain was confirmed. A significant change was observed in the surface morphology by FESEM and loss of the crystalline structure by the XRD data. An improvement in the thermal stability of cationic derivative was observed. The aqueous solution of CCTG showed a non-Newtonian, pseudoplastic behavior, and was stable up to 240 h. Further, a preliminary study using CCTG (0.28) showed promising results as a biopolymeric flocculant and indicates its potential for utilization as an environment-friendly flocculating agent in wastewater treatments. The study also reveals the suitability of cationic gum with high DS as a wet strength additive in paper manufacturing for improving the physical strength properties viz. bursting, tensile, and tearing strength of the hand sheets made from OCC for its recycling.

4. EXPERIMENTAL SECTION
4.1. Materials. CTG was procured from M/s Goodrich Cereals, Haryana. 3-Chloro-2-hydroxypropyl trimethyl ammonium-chloride (CHPTAC) (60 wt % aqueous), sodium hydroxide, isopropanol, methanol, acetic acid (AR grade), and sulphuric acid (AR grade) were purchased from Merck India Ltd., Mumbai, India. Kaolin was procured from Loba Chemie, India. Deuterium oxide was procured from Sigma-Aldrich, St. Louis, Missouri, USA.

4.2. Methods. 4.2.1. Synthesis of CCTG. CTG (100 mesh, 0.0123 mol) was dispersed in aqueous alkaline (0.0125−0.05, mol NaOH) isopropanol solution (isopropanol: water, 80:20, v/v). The reaction flask was constantly stirred at temperature varying from 30 °C to 60 °C depending on the reaction conditions for a period of 10 min. At this stage, cationic reagent, CHPTAC (0.00159−0.00638 mol) was added dropwise with regular stirring for 60−240 min. The resulting mixture was cooled to ambient temperature and the product was filtered. The reaction product was washed with aqueous methanol (methanol/water: 80:20), filtered, dispersed in distilled water, and washed with aqueous acetic acid (5%, v/v) till neutral. The resultant product was precipitated with methanol and centrifuged (15,000 rpm for 12 min). It was followed by washing with 80% aqueous methanol (3 × 20 mL) and finally with pure methanol. The purified CCTG was dried at 60 °C for 6 h in oven.

4.2.2. Flocculation Test. Flocculation performance of the CCTG was carried out using coagulation–flocculation jar tests. A suspension of kaolin in water (1%, w/v) was dispersed in five 250 mL beakers. The CCTG (10−50 ppm) was added to the beakers. Immediately after the addition of the CCTG, all of the suspensions were stirred using magnetic bars at a constant speed of 100 rpm for 2 min followed by a slow agitation at 50 rpm for 5 min (flocculating time). The flocs were then allowed to settle down for 2 min (sedimentation time) and the supernatant was filtered and the turbidity measured. The experiments were repeated and the turbidity results were plotted.
time). At the end of the settling period, the absorbance of supernatant liquid at half the height of the clarified layer was measured using a UV—vis spectrophotometer (Chemito-2700) at 670 nm.

4.2.3. Evaluation of CCTG as Wet Strength Additive. The raw material for testing as a wet strength additive was obtained from old corrugated carton (OCC) for its recycled utilization. The OCC was manually torn into small pieces and slushed in a deionized water for 48 h, and lyophilized. The lyophilized sample (60 mg) was solubilized in D2O (1 mL) for recording of the NMR spectrum. The spectral recordings were performed using parameters: 1HN M R (300 MHz, 32 scans, 2.2282 s data acquisition time, 1 s relaxation delay time, temp, 30 °C; HSQC: 2 scans, 0.1946 s data acquisition time, and 1.500s relaxation delay time).

4.2.4. Experimental Design. The experiments were designed using an orthogonal L’16 array, a fractional factorial matrix. The output of designed experiments and the effect of various reaction parameters on characteristic properties (DS) were analyzed by the signal to noise ratio (S/N). The results were analyzed using STATISTICA release 7 software.

4.3. Characterization of CCTG. 4.3.1. Elemental Analysis. The nitrogen content of CCTG samples was determined by the Kjeldahl method.

4.3.2. Determination of DS. The DS of all of the CCTG samples was determined based on an increase in the nitrogen content using the following formula:

\[
DS = \frac{162.2 \times \% \text{ nitrogen}}{1401 - 151.6 \times \% \text{ nitrogen}}
\]

4.3.3. Rheological Properties of CCTG. The rheological properties were determined using a Brookfield DV-III Ultra Digital Viscometer. The spindle-21 was used at 10, 20, and 50 rpm maintaining 25 ± 1 °C of temperature.

4.3.4. Infrared Spectroscopy. FTIR spectra were recorded on a Fourier transform infrared spectrophotometer (PerkinElmer-Spectrum II). Samples were blended with KBr powder and pressed into pellets before measurements. The spectra were acquired by the accumulation of 16 scans, with a resolution of 4 cm−1 in mid IR (400–4000 cm−1) range.

4.3.5. 1H and 2D NMR (HSQC) Spectroscopy. The NMR spectra of CTG and CCTG were acquired using a 500 MHz Bruker spectrometer at 25 °C. The samples were hydrolyzed preceding to recording the spectra. CTG (0.5 g) was added to 20% aqueous sulfuric acid (50 mL) and stirred at 50 °C for 30 min. It was cooled and the resulting mixture was neutralized with saturated sodium bicarbonate solution. The solution was dialyzed in deionized water for 48 h, filtered, and lyophilized. The lyophilized sample (60 mg) was solubilized in D2O (1 mL) for recording of the NMR spectrum. The spectral recordings were performed using parameters: 1H NMR spectrum: 500.17 MHz, 32 scans, 2,22122 s data acquisition time, 1 s relaxation delay time, temp, 30 °C; HSQC: 2 scans, 0.1946 s data acquisition time, and 1.500s relaxation delay time.

4.3.6. Thermogravimetric and Differential Thermal Analysis. Thermogravimetric and differential thermal record-

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P.S.: conceptualization, supervision, visualization, investigation, writing—reviewing and editing. V.K.: supervision reviewing and editing, investigation. D.S.: investigation, data curation, writing, methodology.

**Notes**
The authors declare no competing financial interest.

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**REFERENCES**

1. Valetés, A.; Garrigós, M. C. Carbohydrate-based advanced biomaterials for food sustainability: a review. Mater. Sci. Forum. 2016, 842, 182–195.
(2) Dea, I. C.; Morrison, A. Chemistry and interactions of seed galactomannans. In Advances in Carbohydrate Chemistry and Biochemistry; Tipson, R. S., Horton, D., Eds.; Academic Press, 1975; Vol. 31, pp 241–312.

(3) Dey, P. M. Biochemistry of plant galactomannans. In Advances in Carbohydrate Chemistry and Biochemistry; Tipson, R. S., Horton, D., Eds.; Academic Press, 1978; Vol. 35, pp 341–376.

(4) Srivastava, M.; Kapoor, V. P. Seed galactomannans: a versatile biodegradable seed polysaccharide. Int. J. Biol. Macromol. 2013, 60, 83–92.

(6) Cerqueira, M. A.; Bourbon, A. I.; Pinheiro, A. C.; Martins, J. T.; Souza, B. W. S.; Teixeira, J. A.; Vicente, A. A. Galactomannans use in the development of edible films/coatings for food applications. Trends Food Sci. Technol. 2011, 22, 662–671.

(7) Hallagan, J. B.; La Du, B. N.; Pariza, M. W.; Putnam, J. M.; Borzelleca, J. F. Assessment of cassia gum. Food Chem. Toxicol. 1997, 35, 625–632.

(8) Hoffman, A. S. Hydrogels for biomedical applications. Adv. Drug Deliv. Rev. 2012, 64, 18–23.

(9) Gil, E. S.; Li, J.; Xiao, H.; Lowe, T. L. Quaternary Ammonium Cyclodextrin Nanoparticles for Enhancing Doxorubicin Permeability across the In Vitro Blood–Brain Barrier. Biomacromolecules 2009, 10, 505–516.

(16) Geres, S.; Dawadi, R. P.; Arad, S. Chemical modifications of biopolymers: quaternization of the extracellular polysaccharide of the red microalgae Porphyridium sp. Carbohydr. Polym. 2000, 43, 75–80.

(17) Heinze, T.; Haack, V.; Rensing, S. Starch derivatives of high degree of functionalization. 8. Synthesis and flocculation behavior of cationic starch polyelectrolytes. Macromol. Mater. Eng. 2002, 287(8), 495-502.

(18) Krentz, D.-O.; Lohmann, C.; Schwarz, S.; Bratskaya, S.; Liebert, T.; Laube, J.; Heinze, T.; Kulicke, W.-M. Properties and flocculation efficiency of highly cationized starch derivatives. Starch-Stärke 2006, 58, 161–169.

(19) Bigand, V.; Pinel, C.; Da Silva Perez, D.; Rataboul, F.; Huber, P.; Petit-Conil, M. Cationisation of galactomannan and xylan hemicelluloses. Carbohydr. Polym. 2011, 85, 138–148.

(20) Song, Y.; Sun, Y.; Zhang, X.; Zhou, J.; Zhang, L. Homogeneous quaternization of cellulose in NaOH/urea aqueous solutions as gene carriers. Biomacromolecules 2008, 9, 2259–2264.

(21) Granö, H.; Yli-Kauhaluoma, J.; Suortti, T.; Käki, J.; Nurmi, K. Preparation of starch betainate: a novel cationic starch derivative. Carbohydr. Polym. 2000, 41, 277–283.

(22) Nachtergaele, W. The benefits of cationic starches for the paper industry. Starch-Stärke. 1989, 41, 27–31.

(23) Banerjee, C.; Ghosh, S.; Sen, G.; Mishra, S.; Shukla, P.; Bandopadhyay, R. Study of algal biomass harvesting using cationic guar gum fractions from the natural plant source as flocculant. Carbohydr. Polym. 2013, 92, 675–681.

(24) Radosta, S.; Vorwerk, W.; Ebert, A.; Begli, A. H.; Grülc, D.; Wastyn, M. Properties of low-substituted cationic starch derivatives prepared by different derivatization processes. Starch-Stärke 2004, 56, 277–287.

(25) Rodríguez, R.; Alvarez-Lorenzo, C.; Concheiro, A. Rheological evaluation of the interactions between cationic celluloses and carbopol 974P in water. Biomacromolecules 2001, 2, 886–893.

(26) Venkataraman, S.; Lee, A. L. Z.; Tan, J. P. K.; Ng, Y. C.; Lin, A. L. Y.; Yong, J. Y. K.; Yi, G.; Zhang, Y.; Lim, I. J.; Phan, T. T.; Yang, Y. Y. Functional cationic derivatives of starch as antimicrobial agents. Polym. Chem. 2019, 10, 412–423.

(27) Yan, L.; Tao, H.; Bangal, P. R. Synthesis and flocculation behavior of cationic cellulose prepared in a NaOH/urea aqueous solution. Clean: Soil, Air, Water 2009, 37, 39–44.

(28) Larsson, A.; Wall, S. Flocculation of cationic amylpectin starch and colloidal silicon. The effect of various kinds of salt. Colloids Surf., A 1998, 139, 259–270.

(29) Ovenden, C.; Xiao, H. Flocculation behaviour and mechanisms of cationic inorganic microparticle/polymer systems. Colloids Surf., A 2002, 197, 225–234.

(30) Sirviö, J.; Honka, A.; Liimatainen, H.; Niinimäki, J.; Hormi, O. Synthesis of highly cationic water-soluble cellulose derivative and its potential as novel biopolymeric flocculation agent. Carbohydr. Polym. 2011, 86, 266–270.

(31) Suopaivari, T.; Liimatainen, H.; Hormi, O.; Niinimäki, J. Coagulation–flocculation treatment of municipal wastewater based on anionized nanocelluloses. Chem. Eng. J. 2013, 231, 59–67.

(32) Li, S. Y.; Niu, C. M.; Zhong, H. Y. Preparation and characterization of cationic Casia tora gum. Adv. Mater. Res. 2013, 781–784, 526–530.

(33) Sharma, D.; Kumar, V.; Nautiyal, R.; Sharma, P. Synthesis and characterization of quaternized C. tora gum using Taguchi L16 approach. Carbohydr. Polym. 2020, 232, 115731.

(34) El-Sheikhl, M. A. Carboxymethylation of maize starch at mild conditions. Carbohydr. Polym. 2010, 79, 875–881.

(35) Houng, J.-Y.; Hsu, H.-F.; Liu, Y.-H.; Wu, J.-Y. Applying the Taguchi robust design to the optimization of the asymmetric reduction of ethyl 4-chloroacetoacetate by baker's yeast. J. Biotechnol. 2003, 100, 239–250.

(36) Steinberg, D. M. Robust Design: Experiments for Improving Quality. In Handbook of Statistics; Ghosh, S., Rao, C. R., Eds.; Elsevier, 1996; Vol. 13, pp 199–240.

(37) Li, L.; Lv, J.; Chen, W.; Wang, W.; Zhang, X.; Xie, G. Application of Taguchi method in the optimization of swimming capability for robotic fish. Int. J. Adv. Robot. Syst. 2016, 13, 102.

(38) Tutar, M.; Aydin, H.; Yuce, C.; Yavuz, N.; Bayram, A. The optimisation of process parameters for friction stir spot-welded AA3003-H12 aluminium alloy using a Taguchi orthogonal array. Mater. Des. 2014, 63, 789–797.

(39) Yang, W. H.; Tarrg, Y. S. Design optimization of cutting parameters for turning operations based on the Taguchi method. J. Mater. Process. Technol. 1998, 84, 122–129.

(40) Gu, Q.; Zhou, K.; Liu, J.; Liu, P.; Cao, Y.; Qiu, J. Rod-like NaN03: mechanisms for stable solvothermal synthesis, temperature-mediated phase transitions and morphological evolution. RSC Adv. 2014, 4, 15104–15110.

(41) Pal, S.; Mal, D.; Singh, R. P. Cationic starch: an effective flocculating agent. Carbohydr. Polym. 2005, 59, 417–423.

(42) Li, X.; Gao, W.-y.; Huang, L.-j.; Wang, Y.-l.; Huang, L.-q.; Liu, C.-x. Preparation and physicochemical properties of carboxymethyl
Fritillaria ussuriensis Maxim. starches. *Carbohydr. Polym.* 2010, 80, 768–773.

(43) Aggarwal, P.; Dollimore, D. The effect of chemical modification on starch studied using thermal analysis. *Thermochim. Acta* 1998, 324, 1–8.

(44) Kuo, W.-Y.; Lai, H.-M. Changes of property and morphology of cationic corn starches. *Carbohydr. Polym.* 2007, 69, 544–553.

(45) Zhang, M.; Ju, B.-Z.; Zhang, S.-F.; Ma, W.; Yang, J.-Z. Synthesis of cationic hydrolyzed starch with high DS by dry process and use in salt-free dying. *Carbohydr. Polym.* 2007, 69, 123–129.

(46) Pi-Xin, W.; Xiu-Li, W.; Xue, D.-h.; Xu, K.; Tan, Y.; Du, X.-b.; Li, W.-b. Preparation and characterization of cationic corn starch with a high degree of substitution in dioxane-THF-water media. *Carbohydr. Res.* 2009, 344, 851–855.

(47) Yuen, S.-N.; Choi, S.-M.; Phillips, D. L.; Ma, C.-Y. Raman and FTIR spectroscopic study of carboxymethylated non-starch polysaccharides. *Food Chem.* 2009, 114, 1091–1098.

(48) Mudgil, D.; Barak, S.; Khatkar, B. S. X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *Int. J. Biol. Macromol.* 2012, 50, 1035–1039.

(49) Figueiro, S.; Góes, J.; Moreira, R.; Sombra, A. On the physicochemical and dielectric properties of glutaraldehyde crosslinked galactomannan-collagen films. *Carbohydr. Polym.* 2004, 56, 313–320.

(50) Tako, M.; Tamaki, Y.; Teruya, T. Discovery of Unusual Highly Branched Galactomannan from Seeds of *J. Biomater. Nanobiotechnol.* 2018, 9, 101–116.

(51) Amar-Lewis, E.; Azagury, A.; Chintakunta, R.; Goldbart, R.; Traitel, T.; Prestwood, J.; Kost, J. Quaternized starch-based carrier for siRNA delivery: from cellular uptake to gene silencing. *J. Controlled Release.* 2014, 185, 109–120.

(52) Bhandari, P. N.; Singhal, R. S.; Kale, D. D. Effect of succinylation on the rheological profile of starch pastes. *Carbohydr. Polym.* 2002, 47, 365–371.

(53) Wei, Y.; Cheng, F.; Zheng, H. Synthesis and flocculating properties of cationic starch derivatives. *Carbohydr. Polym.* 2008, 74, 673–679.

(54) Khalil, M. I.; Aly, A. A. Preparation and evaluation of some cationic starch derivatives as flocculants. *Starch-Stärke* 2001, 53, 84–89.

(55) Bratby, J. *Coagulation and Flocculation*; Uplands: Croydon, England, 1980.

(56) Tsai, J.-T.; Liu, T. K.; Chou, J. H. Hybrid Taguchi-genetic algorithm for global numerical optimization. *IEEE Trans. Evol. Comput.* 2004, 8, 365–377.

(57) Sohrabi, M. R.; Khavaran, A.; Shariati, S.; Shariati, S. Removal of Carmoisine edible dye by Fenton and photo Fenton processes using Taguchi orthogonal array design. *Arabian J. Chem.* 2017, 10, S3523–S3531.