International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.4, pp 1713-1719, 2014-2015

Synthesis and Characterization of Carboxyethyl *Delonix regia* Gum.

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Abstract: The recent studies focus on derivatization of natural polysaccharides for development of various drug delivery systems. *Delonix regia* gum is a natural polysaccharide obtained from the endosperm of *Delonix regia* seeds, consist of the main chain of mannose united linked through β (1→4) and side chain of single galactose unit linked through α (1→6). The purpose of the present study was to synthesize carboxyethyl *Delonix regia* gum from *Delonix regia* gum. The reaction conditions were optimized with respect to amount of sodium hydroxide, 2-chloropropionic acid and reaction temperature. Derivatives were evaluated for viscosity and sodium content. Viscosity of all batches of derivatives was found between 16.31±0.21 to 31.19±0.14 cp. Derivatization of gum was confirmed by FTIR analysis. The optimized derivative was characterized by X-ray diffraction study and scanning electron microscopy.
Key Words: Carboxyethyl *Delonix regia* gum, 2- chloropropionic acid, viscosity.

Introduction

Now a days, plant derived polymers have attracted tremendous interests due to their diverse applications such as tablet binders, emulgents, thickeners in cosmetics and suspensions, as film forming agents and transitional colloids.[1] Most of these polysaccharides share basic structural similarities, known as galactomannans. The mannose-elements form a linear chain consisting of (1→4)-β-D-mannopyranosyl residues, with (1→6) linked α-D-galactopyranosyl residues as side chain at varying distances.[2, 3] The ratio of galactose to mannose residues depends on the plant source, and it can range from 1.0/1.0 to 1.0/5.6.[4]

These natural polymers are modified to alter their physicochemical properties. Natural gums can also be modified to have tailor-made products for drug delivery systems and thus can compete with the synthetic controlled release excipients available in the market[1]. Modification of polymers can be achieved through derivatization of functional groups, grafting with polymers, cross-linking with ions etc.[5] Derivatization of functional groups can be carried out by carboxymethylation[6-8], carbomoylethylation[1], carboxyethylation.[9, 10] Chemical modifications of polysaccharides are also carried out by esterification, oxidation and hydroxypropylation. Carboxymethylation generally increases the hydrophilicity and solution clarity of the polysaccharides and makes it better soluble in aqueous system.[11] Alkali metal carboxyethyl groups can be introduced into starch by reaction under strongly alkaline conditions with reagents such as acrylamine, acrylonitrile or 3-chloropropionic acid.[12]

Delonix regia plant widely grown in tropical and sub-tropical regions. It is an ornamental tree due to its beautiful red flowers.[13] *Delonix regia* gum (DRG) is a natural polysaccharide obtained from the endosperm of *Delonix regia* seeds, consist of the main chain of mannose united linked through β (1→4) and side chain of single galactose units linked through α (1→6).[14] The molar ratio of D-mannose to D-galactose in DRG is 4:1 and hence they are known as galactomannans.[13] Natural gums have certain drawbacks, like uncontrolled rate of hydration, fall in viscosity on storage, high susceptibility to microbial contamination, solution clarity and require some modifications to overcome these problems. The main objective of the present work was to synthesize and characterize carboxyethyl derivative of DRG. Reaction conditions were optimized by 2^3 factorial design using Design Expert trial version 8.0.7.1.

Materials and Methods

Materials

Dried seed of *Delonix regia,* Sodium hydroxide (Qualigens, Fischer Scientific), 2-chloropropionic acid (94%) (Alfa Aesar, England) and all other chemicals and solvents used were of analytical grade.

Methods

Isolation and Purification of Seed Gum:

Dried seed pods of *Delonix regia* were collected from the local area of Baramati, Maharashtra and authenticated from Biological Survey of India, Pune. *Delonix regia* gum was isolated and purified using our proposed method.[6]

Synthesis of carboxyethyl *Delonix regia* gum:

2^3 factorial design was used for the synthesis of carboxyethyl *Delonix regia* gum[6, 10]. Derivatives were prepared by varying the reaction parameters such as amount of Sodium hydroxide (NaOH), 2-chloropropionic acid and reaction temperature (Table No.2). Thus amount of NaOH, 2-chloropropionic acid and temperature were selected as independent factors. The viscosity was selected as dependent variable. Actual and coded values of independent factors were given in Table No.1.

Table No. 1: Factorial Design in Terms of Actual and Coded Values of Factors.

Factor	Actual values		Coded values	
	Low level	High level	Low level	High level
Temperature (deg C)	60	70	-1	+1
Sodium hydroxide (gm)	4.8	8	-1	+1
2-chloropropionic acid (ml)	20	40	-1	+1

Table No. 2: 2^3Factorial Design for Carboxyethylation of *Delonix regia* Gum.

Batch Code	Factor A	Factor B	Factor C	Response
	Temperature (deg C)	Sodium hydroxide (gm)	2-chloropropionic acid (ml)	Viscosity (cp)
CEDRG 1	70	4.8	40	16.47
CEDRG 2	60	8	40	30.24
CEDRG 3	70	8	40	20.1
CEDRG 4	60	4.8	20	22.9
CEDRG 5	60	8	20	31.19
CEDRG 6	70	4.8	20	16.31
CEDRG 7	70	8	20	27.05
CEDRG 8	60	4.8	40	27.11

The method is based on previous procedures, involving derivatization of gum.[10, 15] 20 gm of purified DRG was dispersed in 115 ml of 2-propanol, in a round bottom flask fitted with thermometer, dropping funnel and mechanical stirrer. After the DRG was well dispersed, required amount of sodium hydroxide was dissolved in 20 ml water and this ice cold solution was added to gum slurry. It was stirred for 20-30 min. Then the temperature was raised slowly to 15^o C. 2-chloropropionic acid was added drop wise with continuous stirring.

The reaction mixture was heated to a specified temperature (Table No. 2) for 2 h, then it was cooled and the pH was adjusted to 7 with glacial acetic acid. Finally the product was washed using methanol: water (80:20), subsequently with methanol and dried. Viscosity of all derivatives was determined and subjected to further analysis.

Optimization and data analysis:

All the batches were analyzed using design expert software 8.0.7.1 version. Polynomial equation was generated for the response variables. The desirability function was used for the optimization. The optimized batch of CEDRG was prepared (Table No. 3) and subjected to further characterization.

Table No. 3: Formula for Optimized CEDRG batch.

Factor A	Factor B	Factor C	Response	Desirability
Temperature (deg C)	Sodium hydroxide (g)	2-chloropropionic acid (ml)	Viscosity (cp)	
60	7.996	20	31.178	0.999

Characterization of Carboxyethyl *Delonix regia* Gum:

Viscosity:

The viscosities of 1% w/v solution of DRG and CEDRG were measured at 25°C using Brookfield viscometer (Cap 1000+visco).[16]

FTIR Spectroscopy:

Fourier transform infrared (FTIR) spectra of DRG and CEDRG were recorded on BRUKER FTIR spectrophotometer over the wave number range from 4000-400 cm^{-1} as shown in Figure1.

Sodium Content:

Sodium content was determined using non-aqueous titration method.[17] 0.5 gm of carboxyethyl *Delonix regia* gum was dispersed in glacial acetic acid; 2 ml of acetic anhydride was added and heated on the water bath for 2 hours. The contents were cooled to room temperature and titrated against 0.1 N perchloric acid using crystal violet as the indicator. Each ml of 0.1 N perchloric acid to ≈ 0.003 gm of sodium.

X-ray Diffraction Studies:

XRD diffraction studies were carried out (BRUKER D8 Advanced) for DRG and CEDRG, shown in Figure 3. Powder samples were scanned in the range 5-55^0 of 2

Scanning Electron Microscopy:

The morphological features of DRG and optimized CEDRG were studied with a scanning electron microscopy (JEOL and Tokyo, Japan JSM-6360).

Results and Discussion

Synthesis of carboxyethyl *Delonix regia* gum:

Carboxyethyl derivatives of *Delonix regia* gum were synthesized by preparing slurry of gum in 2-propanol. Using 2-propanol, non homogenous system occurs forming a concentrated layer of NaOH around the gum. This is because of low solubility of NaOH in non polar solvent. It was observed that, NaOH deprotonates the free hydroxyl groups of gum to form alkaoxides which increase their nucleophilicity.[7] Then the etherifying agent (2-chloropropionic acid) was added to form its reaction with gum alkoxide.

Optimization and data analysis:

All the batches were analyzed using design expert software 8.0.7.1 trial version. The desirability function was used for the optimization. The optimized batch of CEDRG was prepared and characterized (Table No. 3). Desirability for optimized batch of CEDRG was 0.999. The significance of the parameters on the variables was assessed by analysis of variance (ANOVA). The effect on viscosity was observed to be significant by ANOVA (Table No. 4). Polynomial terms were utilized to evaluate response. The polynomial equation in terms of coded factors was found as follows:

$$Y_{Viscosity} = 23.82 – 3.84\ A + 3.32\ B – 0.34\ C + 0.27\ AB – 1.36\ AC – 1.64\ BC – 0.14\ ABC.$$

From the regression equation of viscosity, negative sign for A (Temperature), C (2-chloropropionic acid) and the interaction terms A-C, B-C and ABC represents, the decrease in response with increase in the value of factors. However, positive sign for B (sodium hydroxide) and A-B indicates, the response increases with increase in the value of factors.

Table No. 4: Analysis of variance (ANOVA) for dependent variable.

Dependant Variable	Source of variation	Degree of Freedom	Mean Square	F-Ratio calculated tabular	p - value
	Regression	3	69.060		
Viscosity (cp)	Residuals	4	9.216	7.49	0.0406
	Total	7			
	R^2	0.849			

Table No. 5: Sodium content of Carboxyethyl *Delonix regia* Gum.

Batch code	Sodium content (% w/w)
CEDRG 1	9.90
CEDRG 2	11.27
CEDRG 3	11.30
CEDRG 4	10.14
CEDRG 5	11.67
CEDRG 6	10.21
CEDRG 7	11.86
CEDRG 8	9.98

Characterization of carboxyethyl *Delonix regia* gum:

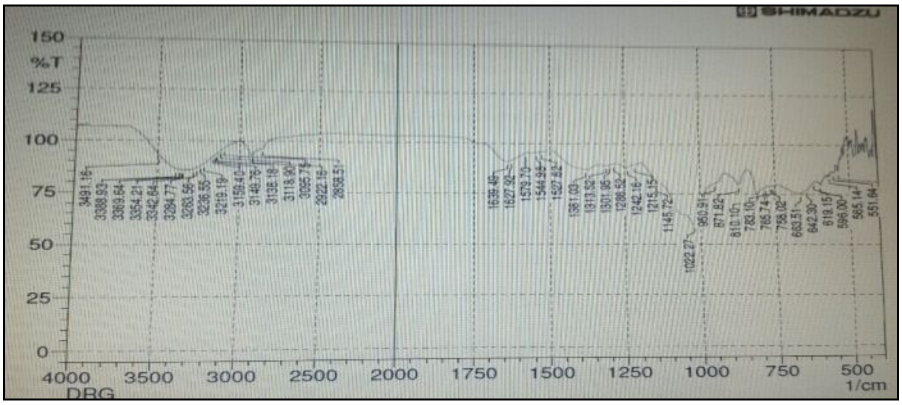
Viscosity:

The viscosities of 1% w/v dispersion of DRG and CEDRG were measured at 25^0C using Brookfield viscometer at 900 rpm. The viscosity of 1 % dispersion of DRG is 130.11 cp while viscosity of all the batches of CEDRG ranges between 16.31 to 31.19 cp (Table No. 2). Thus marked decrease in viscosity was observed after formation of carboxyethyl derivative of DRG.

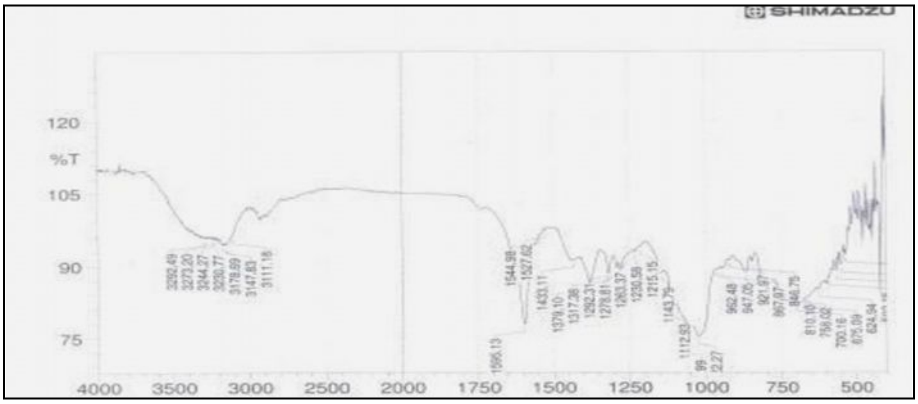
FTIR Spectroscopy:

FTIR spectra of DRG showed very strong bands between 3200-3491 cm^{-1} is assigned to OH bond stretching, while the sharp absorption band at 2922.16 cm^{-1} represents C-H stretching of $–CH_2$. The bands at 1740.57 cm^{-1} and 1698.40 cm^{-1}may be due to ring stretching of mannose and galactose. Bands at 1313.52 cm^{-1} and 1381.03 cm^{-1} may be due to symmetrical deformation of $-CH_2$ and C-OH groups. The band at 1022.27 cm^{-1} represents bending of CH_2-O-CH_2, shown in Figure 1 (a). Sarangapani *et al.,* 2012 have found presence of OH stretching band at 3430.09 cm^{-1}, C-H stretching band at 2924.52 cm^{-1} and bands at 1637.32 and 1657.13 cm^{-1} due to ring stretching of galactose and mannose in FTIR spectrum of *Delonix regia* gum.

FTIR spectra of optimized CEDRG (Figure 1, b) showed presence of strong peaks at 1595.13 $cm^{-1}$and 1433.11 cm^{-1}. This indicates presence of carboxylate ion (-COO) peak, which was not present in spectra of DRG. Thus derivatization of DRG to CEDRG was confirmed.



(a)



(b)

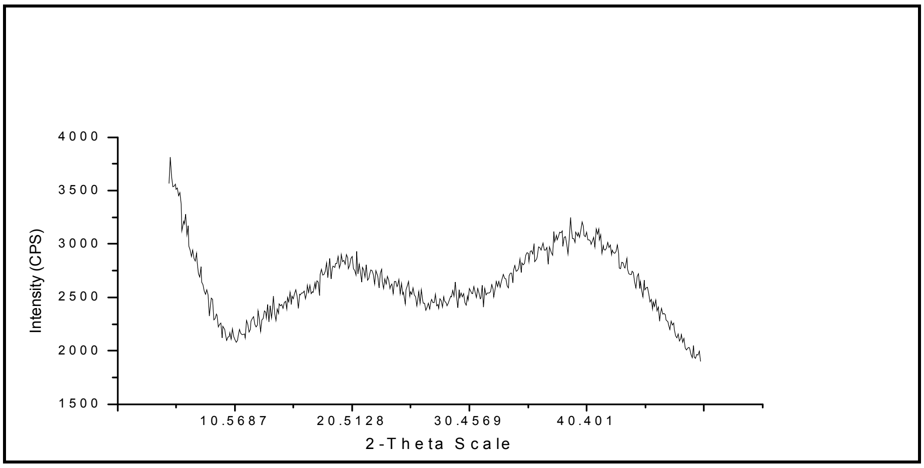
Figure 1: FTIR spectra of (a) DRG and (b) CEDRG

Sodium Content:

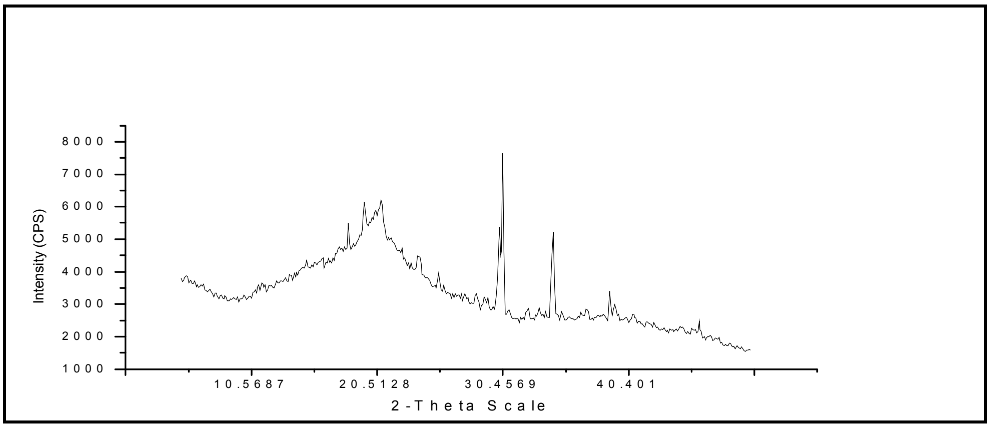
Sodium content was determined using non-aqueous titration method. For all batches of CEDRG, sodium content was found in a range of 9.90 to 11.86 % w/w (Table No. 5).

X-ray Diffraction Studies:

X-ray diffractogram for DRG showed low intensity peaks, indicated the amorphous nature of the gum (Figure 3, a). However X-ray diffractogram for optimized CEDRG showed intense peaks at 2θequal to 19.41, 20.81, 30.45, 30.55, 34.43, and 38.90 which indicates crystalline nature of the derivative (Figure 3, b).



(a)

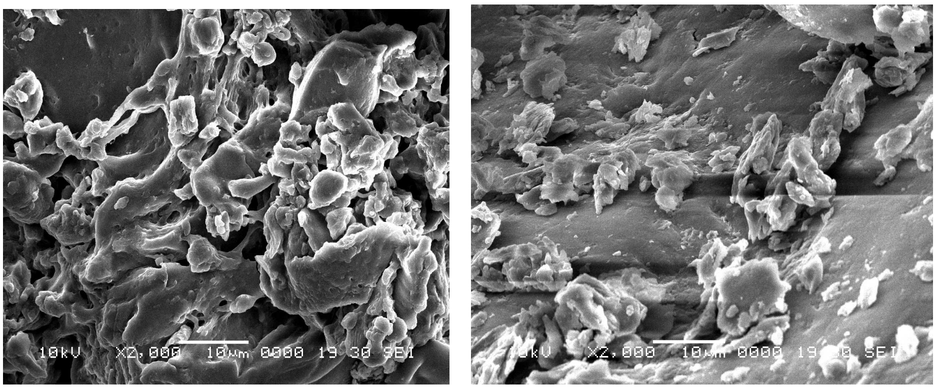


(b)

Figure 3: X-Ray Diffractogram for (a) DRG and CEDRG

Scanning Electron Microscopy:

The morphological features of DRG and CEDRG were studied with a scanning electron microscopy. The biological and botanical source of a pharmaceutical material serves as a determining factor in the granule shape, size and morphology. SEM photographs of DRG (Figure 4, a) powder showed gum like mass which is devoid of crystalline structure; however SEM photographs of CEDRG powder (Figure 4, b) showed change in shape of particles and crystalline nature of the derivative.



(a) **(b)**
Figure 4: SEM photographs of (a) DRG and (b) CEDRG

Conclusion

From the above results it is concluded that carboxyethyl *Delonix regia* gum samples were successfully synthesized from *Delonix regia* gum in presence of aqueous solution of NaOH under etherification with 2-chloropropionic acid. CEDRG can be used as drug delivery carrier to form multi unit dosage form such as beads, micro particles, interpenetrating polymer network microspheres etc.

Aknowledgement

The authors are thankful to the Management and Principal, TVES'S College of Pharmacy, Faizpur for their support during the research.

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