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SYNTHESIS OF CARBOXYMETHYL *DELONIX REGIA* GUM USING NON AQUEOUS METHOD THROUGH OPTIMIZATION AND ITS CHARACTERIZATION.

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ABSTRACT

Delonix regia gum (DRG) is a natural polysaccharide obtained from the endosperm of Delonix regia seeds, chemically classified as galactomannan. The objective of the present study was to derivatise DRG to carboxymethylated Delonix regia gum (CMDRG) using non aqueous method and to overcome limitations of natural polymer viz, fall in viscosity on storage, susceptible to microbial contamination, and to improve solution clarity. DRG was subjected to carboxymethylation using optimization technique (2^3 factorial design) by altering concentration of sodium chloroacetate (SCA), sodium bicarbonate and temperature of reaction mixture in presence of ethanol, while the degree of substitution (DS) and viscosity were selected as response variables. Optimized batch was characterized by the FTIR and differential scanning calorimetry. The X-ray diffraction study showed an amorphous nature in DRG and crystalline nature in CMCRG. The carboxymethylated Delonix regia gum (CMDRG) may provide an efficient alternative approach for the oral delivery of hydrophilic macromolecules.

KEY WORDS: ***Delonix regia*** **gum (DRG), carboxymethylated** ***Delonix regia*** **gum (CMDRG), galactomannan and degree of substitution (DS).**

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INTRODUCTION

Delonix regia is a species of flowering plant from the family *Leguminosae* and sub family *Fabaceae*[1]. *Delonix regia seed* gum is a natural polysaccharide obtained from the endosperm of *Delonix regia* seeds, chemically classified as galactomannan. It 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)[2]. Natural gum polysaccharides are promising biodegradable materials for use in drug delivery systems. But these materials have certain drawbacks, like uncontrolled rate of hydration, thickening, drop in viscosity on storage, microbial contamination and require some modifications to overcome these problems. Carboxymethylation of gums increase their hydrophilicity and solution clarity and makes them more soluble in aqueous systems[3]. Chemical modification provides an efficient route not only for removing such drawbacks but also for improving swelling and solubilization[4]. Hence to overcome these drawbacks, the present work was aimed to develop carboxymethylated *Delonix regia* gum (CMDRG) using a mild alkali Sodium bi carbonate ($NaHCO_3$,) sodium chloroacetate (SCA) in presence of ethanol, hence the possibility of alkaline degradation caused by strong sodium hydroxide (NaOH) could be minimized, and removal of $NaHCO_3$ was easier. Modification of hydrophilic backbone of these biopolymers diversifies and enhances its applications and functionality. Some researchers had synthesized carboxymethyl derivatives of guar gum[4, 5], *Delonix regia* gum[6] using SCA and (NaOH) in aqueous environment. Also investigations have been performed on carboxymethylation of galactomannans such as guar, tara and locust bean gum using monochloroacetic acid and $NaHCO_3$ in dry state[7]. Carboxymethylation is most widely studied conversion of naturally occurring biopolymers to produce commercially important biopolymers with promising properties[8].

MATERIALS AND METHODS

Dried seed pods of *Delonix regia* were collected from the local area of Baramati, Maharashtra and authenticated from Biological Survey of India, Pune. Sodium chloroacetate (Purity 99%, National Chemicals), sodium bicarbonate was purchased from Loba Chemie Pvt. Ltd. Mumbai and all other chemicals and solvents used were of analytical grade.

(i) Isolation and Purification of Seed Gum

Dried pods were imbibed in the water for an overnight and then dried in sunlight to separate the seeds from the pods. The seeds (500g) were boiled in the distilled water using pressure cooker for 1 h. Seed coat was then removed by hand. The gum part (endosperm) was separated from the dicotyledons. The endosperm was dried in an oven at 45°C for 12 h and then was grounded in the multimill. The resulting powder was passed through 60 # sieve. The isolated seed gum was purified by suspending it (100 gm) in water (1 L) at 70 ^{o} C for 2 h. The suspension was squeezed using several folds of muslin cloth to separate the marc from the filtrate. The filtered sample was precipitated in absolute ethanol, dried at 50 ^{o} C for 12 h in an oven, and milled. The powder was passed through 80 # sieve[9].

(ii) Study Design

Carboxymethylation of DRG was carried out by applying 2^3 factorial design using Design Expert Version 8.0.7.1. Derivatives were prepared by varying the reaction parameters such as concentration of $NaHCO_3$, SCA and temperature as shown in Table 1 and then subjected to determination of degree of substitution and viscosity. Carboxymethylation was confirmed by FTIR analysis.

(iii) Synthesis of Carboxymethylated Delonix regia Gum

Synthesis of carboxymethylated *Delonix regia* gum was carried out by a slight modification of the methods suggested by Paramita D *et al.* For this 10 g of finely powdered purified DRG and $NaHCO_3$ were mixed well in a pestle mortar. To this 25 ml ethanol was added, transferred to a flask fitted with a thermometer and mechanical stirrer. The reaction mixture was stirred for 30 min. Then solid sodium chloroacetate was added to it and heated to a

specified temperature (Table I) with continuous stirring for 3 h. The reaction mixture was cooled and neutralized to pH 7 using glacial acetic acid. The resulting product was repeatedly washed with methanol: water (80:20) followed by methanol and dried at 50 ^{O}C for 5-6 h. Dried CMDRG powder was subjected to further analysis[10-11].

Table I
2^3 Factorial Design for Carboxymethylation of Delonix regia Gum.

Batch Code	Factor 1	Factor 2	Factor 3	Response 1	Response 2
	Temperature(deg C)	Sodium bicarbonate (g)	Sodium chloroacetate (g)	Degree of substitution	Viscosity (cp)
CMDRG 1	75	6	3.2	0.341	20.88
CMDRG 2	75	10	3.2	0.419	30.23
CMDRG 3	75	6	3.6	0.367	25.3
CMDRG 4	65	10	3.2	0.378	22.1
CMDRG 5	75	10	3.6	0.451	35.52
CMDRG 6	65	6	3.6	0.356	21.54
CMDRG 7	65	10	3.6	0.44	35.88
CMDRG 8	65	6	3.2	0.325	18.31

Optimization, Data Analysis and Desirability Function

The data obtained after carrying different runs, all the batches were analyzed using design expert software 8.07.1 version. Optimization of CMDRG was carried out using response surface method (RSM). Polynomial models employing quadratic terms were generated for both response variables. Also contour plots were constructed using the output files generated by the software. The significance of the parameters on the variables was assessed by analysis of variance used for the optimization (ANOVA). The desirability function was used for the optimization. The responses were combined to find a product having the desired characteristics. The desirability function combines all the responses into one variable to predict the optimum level for the independent variables[12-13]. Further, the optimized batch of CMDRG was selected (Table II) and subjected to further characterization.

Table II
Formula for Optimized CMDRG batch.

Factor 1	Factor 2	Factor 3	Response 1	Response 2	Desirability
Temperature (deg C)	Sodium bicarbonate (g)	Sodium chloroacetate (g)	Degree of substitution	Viscosity (cp)	
75	10	3.6	0.4508	36.035	0.999

Characterization of Carboxymethylated Delonix regia Gum

The resulting product was subjected to characterization techniques based on, degree of substitution, FTIR spectroscopy, Differential Scanning calorimetry (DSC) and X-ray diffraction studies.

Determination of Degree of Substitution

Conversion of CMDRG to the acid form:

CMDRG (5g) was transferred to acid form by slurring the CMDRG in 75 ml of ethyl alcohol (95%). A 3-6 ml 70% Nitric acid (HNO3) was added and stirred for 20 minutes. While stirring the slurry was heated to boil for 5 minutes, then the heat was removed, and the stirring continued for 15-20 minutes. The cooled mixture was filtered and the residue was washed with three 75 ml aliquots of 80% aqueous methanol to remove salts and excess acid. Finally it was washed with methanol and dried in oven at 60^{0}C overnight.

Determination of the Degree of Substitution via Titration

The dried acid form samples made in the previous step (0.5g) were transferred to 200 ml flasks and suspended in distilled water (50 ml) until they dissolved completely. An excess of 0.5 N NaOH (12.5 ml) solution was added with stirring and stirring was continued for 15 more minutes before solution was heated to boil for 15- 30 min. While the solution was hot excess NaOH was back titrated with 0.5 N HCl

to a phenolphthalein end point. The amount of acid consumed was recorded and the D.S. was calculated using following equation[14] and listed in Table 1.

D.S. = 0.162 A/ (1-0.058A)

Where A = (BC-DE)/F

A= acid consumed per gram of sample

B= NaOH solution Added, ml

C= Normality of NaOH

D= HCl required for titration of the excess NaOH, ml,

E= Normality of HCl,

F= CMG used, g

162= grams molecular mass of anhydroglucose unit of Gum, and

58= net increase in molecular mass of anhydroglucose unit for each carboxymethyl group substituted.

Determination of Viscosity

The viscosity of 1 % dispersion of all batches of CMDRG was measured at 25^0C using Brookfield viscometer (Cap 1000+visco) at 900 revolutions per minute[15].

FTIR Spectroscopy

Fourier transform infrared (FTIR) spectra of DRG and optimized CMDRG were recorded[4] on BRUKER FTIR spectrophotometer over the wave number range from 4000-400 cm^{-1} as shown in Figure1.

Differential Scanning Calorimetry (DSC)

Thermograms of DRG and CMDRG were obtained (DSC-METTLER Toledo, Switzerland) for the determination of glass transition temperature (Tg), shown in Figure 2. About 5 mg of sample was placed in aluminium and scanned over a temperature range (20-300 ^{0}C).[15]

X-ray Diffraction Studies

XRD patterns were obtained (BRUKER D8 Advanced) for DRG and optimized CMDRG, shown in Figure 3. Powder sample were scanned in the range 5-55^0 of 2 theta.[15]

RESULTS AND DISCUSSION

Characterization of carboxymethylated Delonix regia gum:

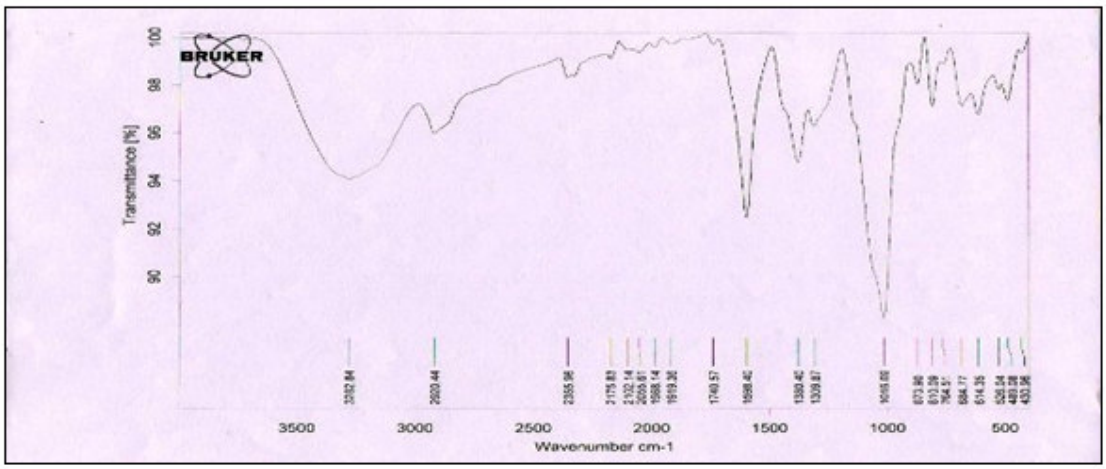
Degree of substitution:

Degree of substitution represents the number of hydroxyl groups on each anhydroglucose unit (AGU) that has been substituted by carboxymethyl groups. During determination of DS via titration, the CMDRG was converted to the acid form by a treatment of CMDRG dispersed in ethanol with concentrated HNO_3. It is then treated with NaOH of known molarity. The excess of NaOH is back titrated permitting to calculate the DS. For all the batches of CMDRG, value of DS was found between 0.325-0.425, shown in Table I.

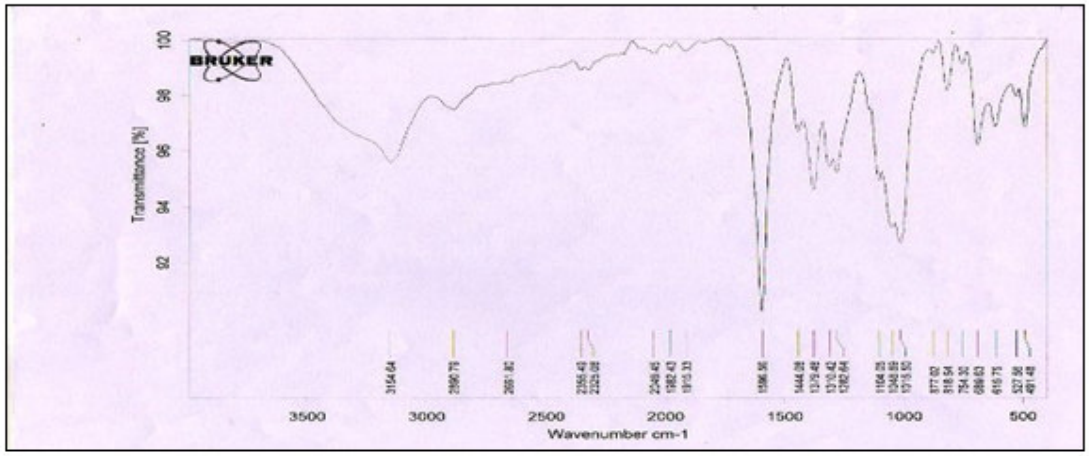
Viscosity

The value of viscosity for all the batches ranges between 18.31- 35.88 cp. Increase in viscosity with increase in DS was observed.

FTIR spectroscopy



(a)

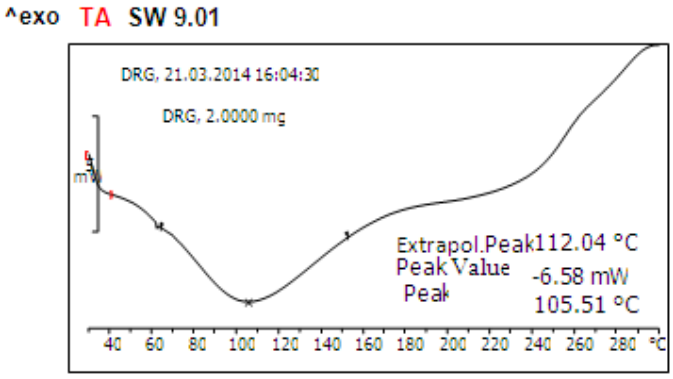


(b)

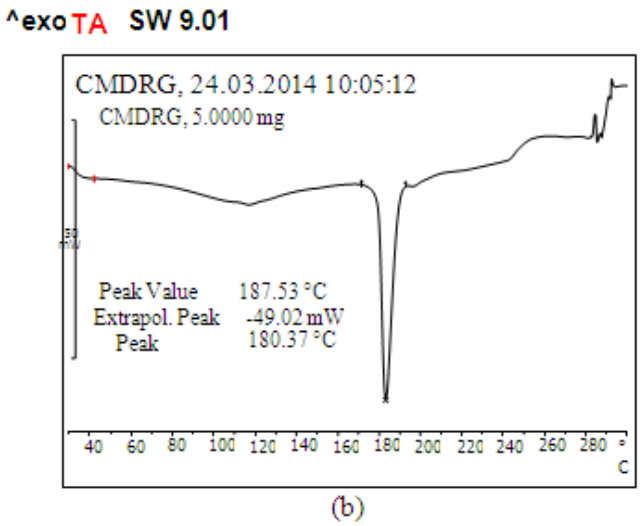
Figure1
FTIR spectra of (a) DRG and (b) CMDRG

DRG and CMDRG samples were analyzed by FTIR. The spectrums were shown in Figure 1. In FTIR spectra for DRG, band at 3311.78 cm^{-1} is due to O-H stretching vibration, band at 2923.44 cm^{-1} is due to C-H stretching of $-CH_2$ group. The bands at 1740.57 cm^{-1} and 1698.40 cm^{-1}may be due to ring structure of mannose and galactose. Bands at 1330.40 cm^{-1} and 1309.87 cm^{-1} may be due to symmetrical deformation of $-CH_2$ and C-OH groups. The band at 1016.08 cm^{-1} is due represents $-CH_2$ twisting vibration. FTIR spectra of CMDRG showed a strong intensity peak at 1596.56 cm^{-1} and 1444.09 cm^{-1}, may be due to incorporation of carboxymethyl groups into the DRG molecule, which were not present in spectra of DRG. Thus carboxymethylation of DRG was confirmed by FTIR analysis.

Differential Scanning Calorimetry (DSC)



(a)

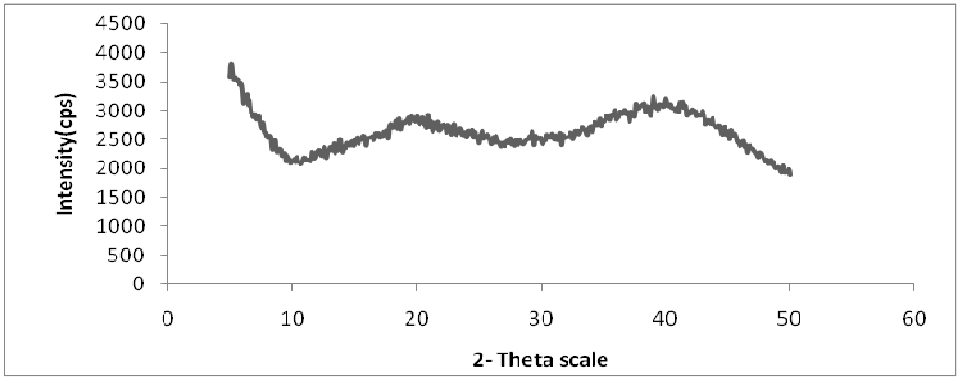


(b)

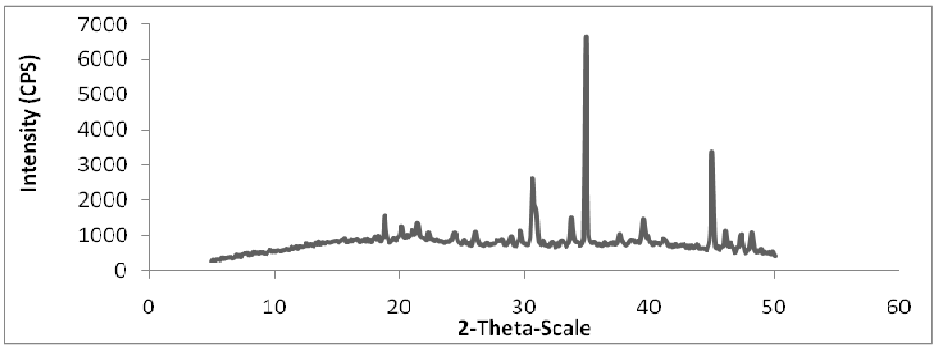
Figure 2
DSC Thermogram of (a) DRG and (b) CMDRG.

DSC was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC of DRG (Figure 2, a) showed broad endothermic peak at 105.51 ^{0}C which indicates melting point and amorphous nature of DRG. Figure 2, (b) showed a sharp endothermic peak at 180.27 ^{0} C, indicating melting point and crystalline nature of CMDRG.

X-ray diffraction studies



(a)



(b)

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

In X-ray diffractogram for DRG as shown in Figure 3 (a), low intensity peaks were observed which confirms the amorphous nature of DRG. But strong intensity peaks were observed between 18.82 to 48.05^{o} (2 theta) in X-ray diffractogram for CMDRG as shown in Figure 3(b). It indicates the crystalline nature of carboxymethylated gum.

Optimization, Data Analysis and Desirability Function

Factorial design for three factors at 2- levels was chosen as the experimental design. The effect on degree of substitution (Y_1) was observed to be significant by ANOVA and the polynomial equation in terms of coded factors was found as follows:

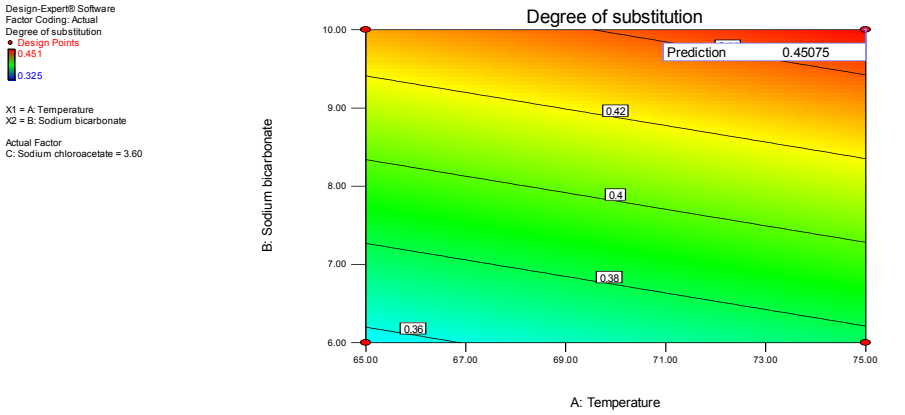
Table III
Analysis of variance (ANOVA) for dependent variables

Dependent Variable	Source of variation	Degree of freedom	Mean square	F- Ratio calculated tabular	P- value
Degree of substitution	Regression	3	0.004935		
	Residuals	4	0.00012	41.083	0.0018
	Total	7			
	R2	0.969			
Viscosity (cp)	Regression	3	97.26		
	Residuals	4	8.734	11.35	0.0207
	Total	7			
	R2	0.893			

Y_{DS} = 0.34+0.0099A + 0.373B + 0.0189C + 0.00313AB - 0.0044AC + 0.00463BC – 0.0031ABC

$Y_{Viscosity}$ = 26.22 + 1.762A + 4.712B + 3.34C + 0.18AB – 0.912AC + 1.427BC – 1.21ABC

From the regression equation of degree of substitution positive sign for A, B, C and the interaction terms A-B and B-C means their combined increase in value increase the degree of substitution and viscosity while increase in A-C, decrease the degree of substitution and viscosity. The interaction terms showed how the response changes when two factors were simultaneously changed. The relationship between the dependent and independent variables was further elucided using contour plots as shown in figure 4.


Design-Expert® Software
Factor Coding: Actual
Degree of substitution
Design Points
0.451
0.325
X1 = A: Temperature
X2 = B: Sodium bicarbonate
Actual Factor
C: Sodium chloroacetate = 3.60
Degree of substitution
Prediction 0.45075
0.42
0.4
0.38
0.36
B: Sodium bicarbonate
A: Temperature
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8.00
7.00
6.00
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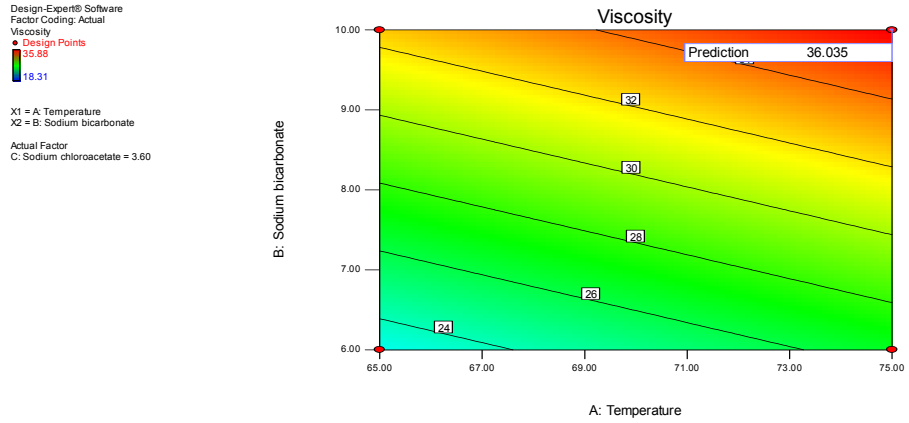
67.00
69.00
71.00
73.00
75.00


Design-Expert® Software
Factor Coding: Actual
Viscosity
Design Points
35.88
18.31
X1 = A: Temperature
X2 = B: Sodium bicarbonate
Actual Factor
C: Sodium chloroacetate = 3.60
Viscosity
Prediction 36.035
32
30
28
26
24
B: Sodium bicarbonate
A: Temperature
10.00
9.00
8.00
7.00
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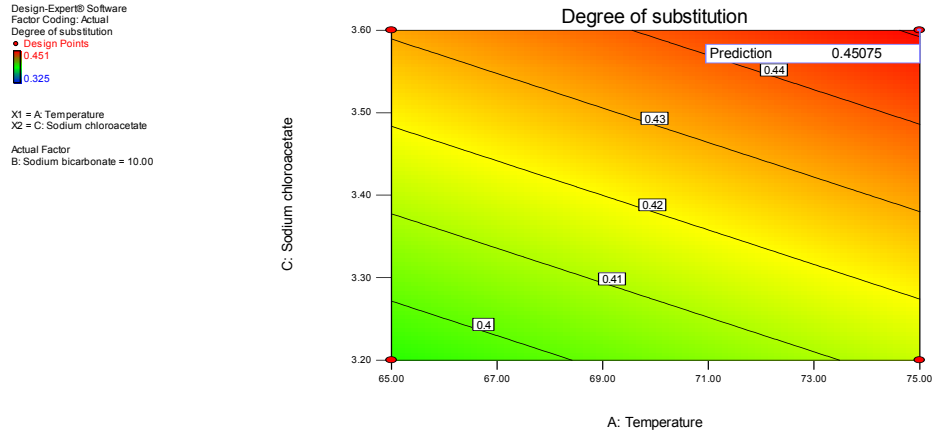
65.00
67.00
69.00
71.00
73.00
75.00


Design-Expert® Software
Factor Coding: Actual
Degree of substitution
Design Points
0.451
0.325
X1 = A: Temperature
X2 = C: Sodium chloroacetate
Actual Factor
B: Sodium bicarbonate = 10.00
Degree of substitution
Prediction 0.45075
0.44
0.43
0.42
0.41
0.4
C: Sodium chloroacetate
A: Temperature
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3.40
3.30
3.20
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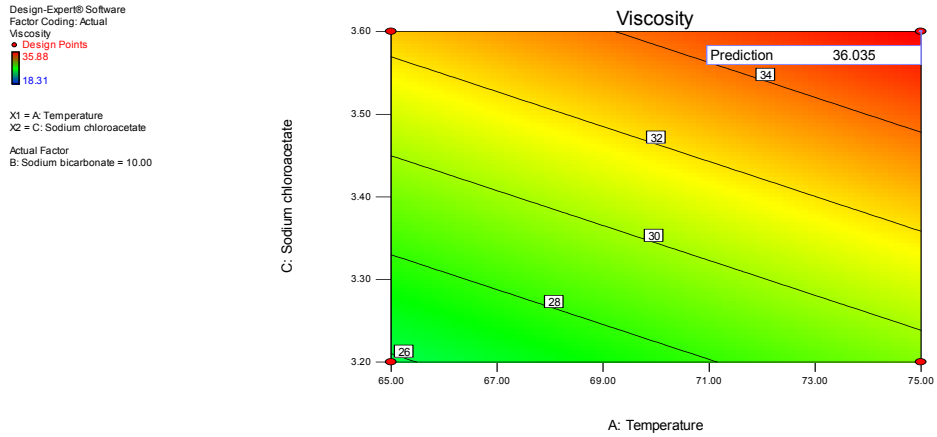
67.00
69.00
71.00
73.00
75.00


Design-Expert® Software
Factor Coding: Actual
Viscosity
Design Points
35.88
18.31
X1 = A: Temperature
X2 = C: Sodium chloroacetate
Actual Factor
B: Sodium bicarbonate = 10.00
Viscosity
Prediction 36.035
34
32
30
28
26
C: Sodium chloroacetate
A: Temperature
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3.40
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71.00
73.00
75.00

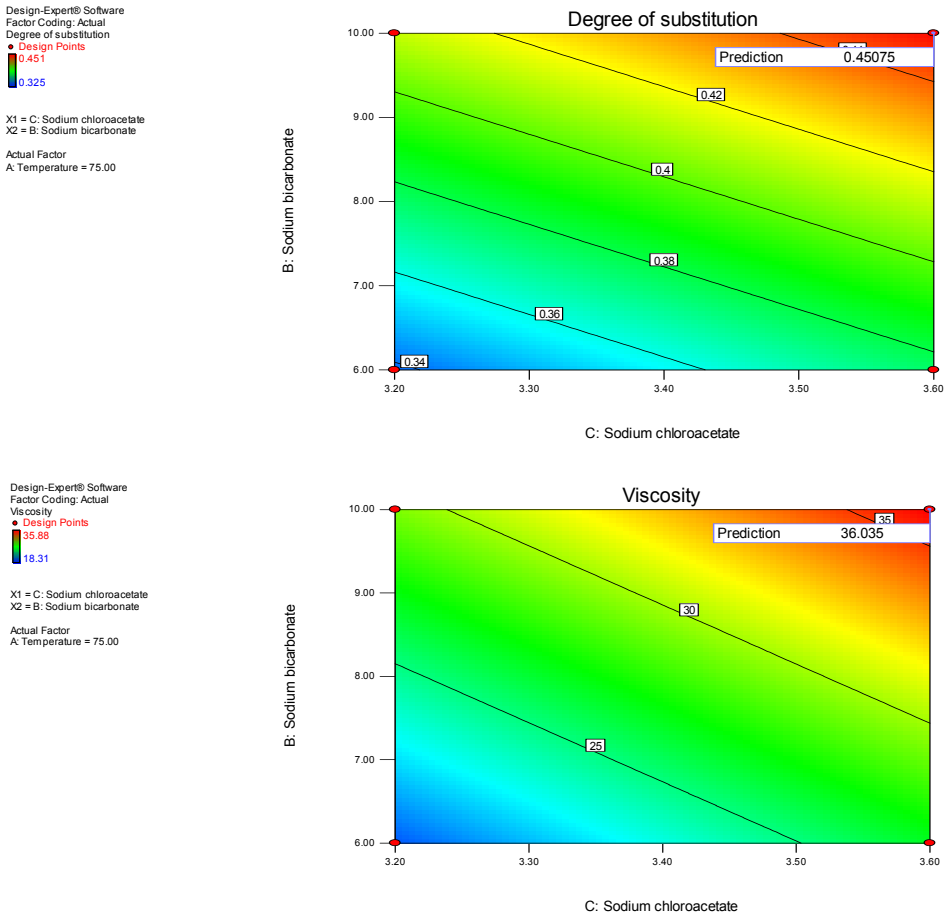

Figure 4

Contour plots showing relationship between the dependent and independent variables.

The final selection of the optimized batch was done by after considering the DS and viscosity through desirability analysis. The desirability function combines all the responses into one variable to predict the optimum levels for independent variables. A desirability value of 0 represents an unacceptable for the responses and the value of 1 represents the most desired value for the responses. Further the optimized formulation as selected by the design was compared with prepared formulation for DS and viscosity. There were no significant variations in results.

CONCLUSION

From the above results it was concluded that CMDRG samples were successfully synthesized using non aqueous method, in presence of $NaHCO_3$ and etherifying agent SCA. In this process, less quantity of organic solvent was used as compare to other proposed methods. Thus the use of large quantity of solvent which is expensive was avoided. Moreover traditional methods including water medium and dry processes suffer from very low DS and paste forming. These drawbacks are overcome in this process. CMDRG can be used as a promising derivative for further studies as gelling, viscosity building, suspending, film forming and matrix forming agent. Furthermore CMDRG can have wider pharmaceutical applications as drug delivery carriers by grafting or crosslinking compounds of interest to form multi unit dosage form such as beads, micro particles microspheres etc.

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