SEPARATION AND PURIFICATION OF CARBOXYMETHYL CELLULOSE FROM *SPINACIA OLERACEA* FOR USE IN PHARMACEUTICAL DOSAGE FORM

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Abstract

Synthesis of carboxymethyl cellulose (CMC) is carried out by using 3^2 factorial designs by using Design Expert software. The optimized conditions for CMC production with Design Expert software predicted the maximum viscosity of 3422.84 cP, degree of substitution of 0.386 and yield of 76.03% at NaOH concentration of 30% and Chloroacetic acid amount of 20 g. After optimization reaction condition was carried out experimentally obtained viscosity was 3421cP, degree of substitution was 0.38 and yield was 76%. Optimized Carboxymethyl cellulose was studied for qualitative test, physicochemical characterization. Instrumental analysis was performed for both cellulose and carboxymethyl cellulose from *Spinacia oleracea*. Instrumental analysis to confirmed effect after conversion of cellulose into carboxymethyl cellulose by carboxymethylation. In which cellulose and carboxymethyl cellulose were analyzed by FTIR, SEM, XRD, TGA and DSC. Application of carboxymethyl cellulose in pharmaceutical dosage form was carried out in which prepared carboxymethyl cellulose was compared to marketed CMC.

Keywords: Carboxymethyl cellulose, Spinach, Sodium Carboxymethyl cellulose, Cellulose, CMC, *Spinacia oleracea*, Na CMC, Film, Suspending agent, Emulsifying agent, Binding agent, Binder.

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1. INTRODUCTION

Globally, huge amounts of plant waste are created today. Despite being the most abundant and renewable source of organic materials available today, significant amounts of these materials are still not utilized properly. Thus, the production of valuable items from plant wastes can assist in reducing environmental issues. More than 90% (w/w) of plant wastes are composed of carbohydrate polymers, which can be altered through biochemical and chemical processes to make products like starch, cellulose, cotton linter, bagasse fiber, etc. [1].

Natural plant fibers are composed primarily of the polymer's cellulose, hemicelluloses, and lignin as well as a few extractives. Plant cell walls are primarily composed of hydrophilic cellulose, which supports the physical stability of the cells. As a result, cellulose is the organic compound that is most widely available on earth. Cellulose has a high degree of crystallinity and a high molecular weight, and it does not dissolve in water [2].

The abundant cellulose is made up of repeating β–D glucopyranose linkages, which can be converted into high-value cellulose esters and ethers [3]. In water, cellulose does not dissolve. However, some cellulose derivatives, like carboxymethyl cellulose, can be dissolved in water [4-6]. Cellulose and its derivatives, ether and ester, are among the excipients often utilized in industrially product and pharmaceutical preparations for a variety of uses [7]. CMC has been produced among all of these modified cellulosic materials primarily because of its broad commercial applications. Carboxymethylation of cellulose a versatile modification which being able to access components that are water soluble [8].

Man-made modified cellulose (CMC) is an anionic polysaccharide that is water-soluble, linear, and long-chained and is produced when Monochloroacetic acid (MCA) reacts with alkali cellulose [9]. The carboxymethyl cellulose (CMC) is a hydrophilic polymer, also known as sodium carboxylic methylcellulose (sodium CMC). These polymers are carbohydrates, which are synthetic cellulose derivatives [10].

CMC is an anionic linear polysaccharide that is a cellulose derivative that is very viscous, nontoxic, non-allergenic, and biodegradable. CMC's capacity to bind and absorb water is made possible by the presence of hydroxyl and carboxylic groups in the compound [10-13].

Cellulose is activated using aqueous NaOH in a slurry of an organic solvent, where it combines with the cellulose and monochloroacetic acid (MCA) to form CMC. The hydroxyl groups of the cellulose chains are stimulated and transformed into a more reactive alkaline state as the first stage in the carboxymethylation process, known as an alkalization as in equation (i). [14]

$$\text{CLL-OH} + \text{NaOH} + \text{H}_2\text{O} \longrightarrow \text{CLL-ONa} + \text{H}_2\text{O} \quad \text{(i)}$$

After that, CMC is obtained through an etherification as in equation (ii), and sodium glycolate is produced as a byproduct of a side reaction as in equation (iii).

$$\text{CLL-ONa} + \text{ClCH}_2\text{COOH} \longrightarrow \text{CLL-OCH}_2\text{COO}^-\text{Na}^+ + \text{NaCl} \quad \text{(ii)}$$

$$\text{NaOH} + \text{ClCH}_2\text{COOH} \longrightarrow \text{HOCH}_2\text{COO}^-\text{Na}^+ + \text{NaCl} \quad \text{(iii)}$$

Due to its simple production, carboxymethyl cellulose (CMC) is the most significant and widely known water-soluble cellulose derivative. As a result, it has a wide range of uses that include those in food, medicine, adhesives, lubricants, insecticides, textiles, detergents, ceramics, cements, paper, and coating [15]. CMC utilizes as binders, stabilizers, emulsifiers, film-forming components, reliable carriers, etc. in a variety of pharmaceutical applications [16]. This research work will be conducted to achieve following objectives, to prepare carboxymethyl cellulose from Spinach (*Spinacia oleracea*). Characterization of CMC for determining its further applications in pharmaceutical dosage form.

1. MATERIAL AND METHODS

1.1 Materials

Spinacia oleracea (Spinach) plant procured from local market. Chemicals used during the present study were Sodium Hydroxide, Ethanol, Sodium Hypochlorite, Methanol, Chloroacetic acid, Isopropanol, Acetic acid, Hydrochloric acid, Copper sulphate, Castor oil, Acetone, Nitric acid, silver nitrate, Chloroform, Sucrose, Sodium Carboxymethyl cellulose, Corn starch, Magnesium stearate, Paracetamol, Benzoic acid from LOBA chemie, Mumbai, India.

1.2 Extraction of Cellulose

The Spinach plant was sliced into smaller portions. After being sun-dried, Spinach was milled into a fine powder and put through it with a 20-mesh sieve. The cellulose powder was then boiled in 8% NaOH at a cellulose to solvent ratio of 1:20 (w/v) for 3.5 hours at 100°C. The resulting black slurry was filtered, and the solid portion was then washed with distilled water and bleached with 5% NaOCl for 3 hours at 30°C. The bleached cellulose was filtered and cleaned again with distilled water until the smell of sodium hypochlorite was eliminated. After that it was dried in an oven at 60°C [17-21].

1.3 Identification Test for Cellulose:

- For both cold and boiling water, cellulose was insoluble.
- Cellulose was not given colored with iodine. Cellulose was turned blue or bluish green by the further addition of strong sulfuric acid.
- In 80% (v/v) sulfuric acid, cellulose can be dissolved.
- It was insoluble in 66% (v/v) sulfuric acid in the cold, but when heated, it becomes soluble. When sulfuric acid was diluted, cellulose was insoluble [22].

2.4 Synthesis of Carboxymethyl Cellulose

2.4.1 Synthesis of Carboxymethyl cellulose

The following ingredients were combined in a beaker for 1.5 hours: 10 gram of cellulose powder, 100 ml of various sodium hydroxide (NaOH) concentrations, and 350 ml of isopropanol. Various concentration of Chloroacetic acid was added to begin the carboxymethylation reaction, which was then constantly stirred for 1.5 hours. The mixture-containing beaker was covered with aluminium foil and heated to 55°C for 3.5 hours. The solution divided into two phases after heating. After that the liquid phase was eliminated, the solid phase was suspended in 66.67 ml of absolute methanol for an overnight period, neutralized it with using acetic acid (90% v/v), and then filtered using a Buchner funnel. The finished product was rinsed five times by soaking in 200 ml of ethanol (70% v/v) for 10 minutes to get rid of unwanted byproducts, and then it was cleaned once again with 200 ml of pure methanol. The resultant CMC was dried for 12 hours in an oven at 55°C [23-24].

2.4.2 Design of the Experiment

For optimization of CMC production was constructed using 3^2 Factorial design. DESIGN EXPERT (Stat-Ease Inc., USA) was used to examine the data. NaOH (X1) and Chloroacetic acid (X2) were employed as independent variables (factors) at three levels (1, 0, +1). The Degree of Substitution (Y1), Viscocity (Y2), and % Yield (Y3) were the dependent variables (response). (Table no. 1).

Table no. 1: Factors and levels of process parameter for synthesis CMC

Factors	Level		
	Low (-1)	Medium (0)	High (+1)
NaOH (% w/v)	20	30	40
Chloroacetic acid (gm)	10	15	20

2.4.3 Evaluation of Carboxymethyl Cellulose

Batches obtained for synthesis of carboxymethyl cellulose using 3^2 Factorial Design were further evaluated by degree of substitution, viscosity, carboxymethyl cellulose yield as dependent variable (response)

Degree of Substitution

0.5gm of dried produced CMC was gently ashed between 450 and 550°C for 24 hours to determine the degree of substitution (DS). After that, 100 ml of distilled water was used to dissolve the ash. Using methyl red as an indicator, 20 ml of this solution was titrated with 0.1 N sulfuric acid. The solution was boiled after reaching the first end point and then titrated to a sharp end point. The following equation was used to determine the degree of substitution **[**25]

$$\text{Degree of Substitution (DS)} = \frac{0.162 \times B}{1 - 0.08 \times B}$$

where, B= (0.1× b)/a, b was the volume (in ml) of 0.1 N sulfuric acid and a was the mass of pure CMC in grams.

Carboxymethyl cellulose yield

CMC yield was measured on dry weight basis. The yield value was calculated by dividing the net weight of dried CMC by the net weight of dried cellulose [26].

$$\text{CMC yield} = \frac{W}{W_0} \times 100$$

where W was weight of oven dried CMC (g) and W_0 was the weight of oven dried cellulose (g)

Viscosity

Preparation was done for the 1% CMC solution in distilled water. A Brookfield DV-E viscometer was used to measure the viscosity of a solution made of up to 1 g of CMC that had been weighed, dissolved in 100 ml of distilled water, and mixed until homogeneous [27].

2.5 Characterization of Carboxymethyl Cellulose

Carboxymethyl cellulose from *Spinacia oleracea* which is prepared by using optimized condition given by Design of Expert was further studied for qualitative test, physicochemical characterization, instrumental analysis.

2.5.1 Qualitative Test for CMC

A beaker glass was filled with up to 0.5 g of CMC, and 50 ml of distilled water was then added. To homogenize the mixture, heat it for 20 minutes at 60 to 70°C while stirring occasionally. Use it as a test solution after cooling it down. It was separated into three reaction tubes.

Tube 1: Add 10 ml of acetone and 5 ml of test solution. Gently shake the container and check to see (+) if any white flocculants forms.

Tube II: Add 5 ml $CuSO_4$ and 5 ml of the test solution. Gently shake the container and check to see (+) if any light blue flocculant forms.

Tube III: Add 1 ml of the test solution, 1 ml of distilled water to dilute it, and 5 drops of 1-naphthol. Place tube on slope, add 2 ml of sulfuric acid, and then check to see (+) if a reddish-purple ring form [28].

2.5.2 Physicochemical Characterization of CMC

Organoleptic Properties

CMC was examined for its organoleptic properties like colour, texture and odor [29].

Moisture content

An open sample container containing 3 g of the sample was heated in an oven at 105 °C for 2 hours. After cooling, the sample was weighed. The sample was then placed back in the oven for an additional 30 minutes, allowed to cool, and then weighed again. This process was carried out repeatedly until the mass loss was not more than 5 mg during a 30-minute drying period. The following formula was used to determine the percent moisture, M [30]:

$$\text{Moisture content \%} = \frac{\text{Mass loss on heating (g)} \times 100}{\text{Sample used (g)}}$$

Solubility

In 3% (w/v) CMC in water, quantitative solubility tests were performed. To achieve a homogeneous suspension, the liquid was agitated for 16 hours at room temperature. After that, the sample was centrifuged at 1500 rpm for 10 minutes. After centrifugation, a pipette was used to transfer 25 ml of the clear supernatant into a dry, pre-weighed sample bottle. The weighing bottle was dried in an oven at 105°C until it had a constant weight (approx. 24 hrs.). It was then weighed after cooling in a desiccator. Using the following equation, the solubility was determined [30]:

$$\text{Solubility of sample in 3\% (w/v) aqueous solution} = \frac{Wd \times 100}{25 \times 0.03}$$

Where: Wd = weight of dried soluble sample in 25 ml

CMC Ash Content

By igniting a 1gm sample at 550° C in a muffle furnace, the sample was ashed. The following equation was used to determine the ash content:

$$\text{Ash content} = \frac{A}{B} \times 100$$

Where, A is mass of ash and B is mass of moisture free specimen

pH

A pH meter was used to determine the pH of the CMC sample. 50 ml of distilled water and 1 g of CMC powder were stirred for 10 minutes. The pH of the final solution was measured and noted [31].

CMC content

1.5 g of CMC was added to 100 ml of 80% aqueous methanol, agitated for 10 minutes, and then filtered to determine the CMC content. To acquire pure CMC, the cake was washed with 100 ml of new 80% aqueous methanol and dried. The following formula was used to determine the CMC content [32].

$$\text{CMC content (\%)} = \frac{W}{W_0} \times 100$$

Where, W_0 (g) is the weight of sample before washing and W (g) is the weight of washed sample.

NaCl content

2 g of CMC was added to 250 ml of 65% methanol and left for 5 hours to determine the amount of NaCl. Diluted HNO_3 was used to neutralize 100 ml of liquid phase and titrated with 0.1N $AgNO_3$ solution [33].

NaCl (%) = 1.461V/m

Where, V (ml) is the amount of AgNO3 and m (g) is the weight of dried sample.

Flow property of Carboxymethyl Cellulose

Several tests were conducted to determine the flow characteristics of CMC, including bulk and tap densities, angles of repose, Carr's index, and Hausner ratio. Thorough explanations of these tests were given below.

Bulk density and Tap density

A calibrated 100 ml graduation cylinder was filled with the appropriate amount of CMC powder (g), which was then placed in a bulk density device. The cylinder was softly tapped to get the estimated occupied volume V_0. The cylinder was then tapped 500 times to get the tap density, which was then determined using the relationship below.

$$\text{Bulk density (BD)} = \frac{w}{V_0}$$

$$\text{Tap density (TD)} = \frac{w}{V_{500}}$$

Where, w is the CMC powder's weight, V_0 was its volume before tapping, and V_{500} was CMC powder volume after 500 taps.

Angle of repose

Using a funnel, the angle of repose of the CMC powder was calculated. The funnel was first fixed with a funnel holder. The CMC powder was allowed to flow freely through the funnel to form a cone. A measuring scale was used to record the cone's height and diameter, which were then calculated using the formula,

$$\text{Tan } \theta = \frac{2h}{D}$$

Where, D was the cone's diameter and h were the cone's height

Carr's index and Hausner ratio

The value of tap and bulk density were used to determine the Carr's index (CI) and the Hausner ratio (HR) [34]

$$\text{CI} = \frac{\text{tap density} - \text{bulk density} \times 100}{\text{tap density}}$$

$$\text{HR} = \frac{\text{tap density}}{\text{bulk density}}$$

2.5.3Instrumental Analysis

Instrumental analysis was performed for both synthesized cellulose and optimized carboxymethyl cellulose from *Spinacia oleracea*. Instrumental analysis to confirmed effect after conversion of cellulose into carboxymethyl cellulose by carboxymethylation. In which cellulose and carboxymethyl cellulose were analyzed by FTIR, SEM, XRD, TGA and DSC.

Fourier transformed infrared spectroscopy (FTIR)

Shimadzu IR DRS 8000A was used to record the infrared spectra of cellulose and carboxymethyl cellulose from *Spinacia oleracea*. 1 mg of sample and 99 mg of potassium bromide were used to prepared pellets. Transmission was measured between 4000 and 400 cm^{-1} in wavelength range.

Scanning electron microscopy (SEM)

Using a Scanning Electron Microscope (SEM), model number S3700N, Hitachi, Japan, the morphological structure of cellulose and carboxymethyl cellulose from *Spinacia oleracea* were analyzed.

X-ray diffraction (XRD)

The crystalline and amorphous forms of cellulose and carboxymethyl cellulose from *Spinacia oleracea* were observed using XRD analysis. X-ray Diffractometer was used to analyze it.

Thermogravimetric analysis (TGA)

Thermogravimetric (TGA) studies of the cellulose and carboxymethyl cellulose from *Spinacia oleracea* were recorded using a DTG 60, Shimadzu, with threshold samples of 12 mg on a platinum pan, in a nitrogen atmosphere. A 20°C/min heating rate was used to raise the temperature from the ambient temperature to 900 °C.

Differential Scanning Calorimetry (DSC)

Analysis of the phase transitions of cellulose and CMS samples from *Spinacia oleracea* were done using Differential Scanning Calorimetry. Model DSC 60 was utilized to record DSC. 40° to 400° C were used to heat aluminium pans containing 2–10 mg of material. There was a 10 °C/min heat rate set. The tests were conducted using nitrogen gas flowing at a 50 ml/min rate.

2.6 Application of CMC in Pharmaceutical Dosage Form

2.6.1Binding Agent in Solid Dosage Form

Preparation of tablets

The formula given in Table no. 2 was used to prepare the various batches F1–F6 of paracetamol granules using varying concentrations of synthesised CMC from *Spinacia oleracea* and NaCMC. In this, NaCMC were utilized as standard binders for comparison. Using a mortar and pestle, the desired amounts of paracetamol, lactose, and corn starch were dry mixed for five minutes. The mixture was then moistened with the proper quantity of binder solution, which was made with various concentrations of prepared CMC and the chosen standard binders massed separately with enough water. After another 5 minutes of massing, the wet mass was manually granulated through a mesh 16 sieve and then dried for 3–4 hours at 50°C in a hot air oven. Dried granules were passed through a mesh 16 sieve and collected granules, which were then passed through 22 sieves. Using tablet punching machines, the tablets were compressed [35-38].

Table no. 2: Formulation of different batches of paracetamol tablet using Prepared CMC and NaCMC as binder

Ingredients	Batches					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Drug (Paracetamol)	250	250	250	250	250	250
Prepared CMC	12.5	25	50	-	-	-
NaCMC	-	-	-	12.5	25	50
Corn starch	100	100	100	100	100	100
Lactose	122.5	110	85	122.5	110	85
Magnesium stearate	10	10	10	10	10	10
Talc	5	5	5	5	5	5
Total (mg/tablet)	500	500	500	500	500	500

Prepared CMC with 2.5, 5 and 10% binder were present in formulation batches F1, F2 and F3, respectively.

NaCMC with a 2.5, 5 and 10% binder were present in formulation batches F4, F5, and F6, respectively.

Physical Evaluation for granules

The granules of paracetamol as prepared above were evaluated for bulk density, tapped density, angle of repose and Carr's index as mentioned in previous section.

Evaluation for tablet

Hardness

Monsanto's hardness tester was used to measure the hardness of tablets from each batch.

Friability

A Roche Friabilator was used to determine the friability of tablets. Six tablets from each batch were weighed, put into the Friabilator drum, and subjected to 100 revolutions in 4 minutes. After dusting and reweighing the tablets, the percentage of friability was determined using the formula shown below [39].

Disintegration test

The disintegration medium was a 600 ml volume of distilled water. The disintegration apparatus's medium was heated up to a temperature of 37 °C. The apparatus was set up to run at 30 cycles per minute. The tablet cylinders were each filled with three tablets from a batch, and the apparatus was then turned on. The amount of time it required for the last tablet or a piece of it to pass through the mesh and into the medium for disintegration was noted [40].

2.6.2As Suspending Agent

Preparation of Paracetamol Suspensions

20 ml of simple syrup were used to triturate 0.25g of sodium carboxymethyl cellulose (NaCMC) powder with 10g of paracetamol to create a smooth paste. After adding 2ml of benzoic acid solution, 1ml of amaranth solution gradually with stirring constantly and 50ml of chloroform water (double strength) was added and mixed.

The mixture was poured into a stoppered measuring cylinder with a 100 ml capacity, made up to volume with distilled water, and vigorously shaken for two minutes (producing 0.25%w/v of

the composition). The process was performed with sodium CMC powder at 0.5% w/v and 1% w/v. The above-mentioned process was repeated using prepared CMC made from *Spinacia oleracea* at concentrations of 0.25%w/v, 0.5%w/v, and 1%w/v.

Evaluation of Suspensions
Physical Test:
The produced suspensions were monitored for physical changes such aggregation, caking, and crystal growth development over the period of 4 weeks, every day.

Redispersibility:
Each suspension was kept in separate tubes with a fixed volume (25 ml) and kept at room temperature. A tube was shaken regularly every 24 hours to check for deposits and to see how easily the sediment could be redispersed. Records of the observations were made.

Determination of Sedimentation Volume
For 4 days at 35°C, each suspension (25 ml) was kept in a 25 ml stoppered measuring cylinder, and observations were conducted every 24 hours. The following equation was then used to determine the sedimentation volume, F (%) [41].

$$F = \frac{Vu}{Vo} \times 100$$

Where, Vo was the total volume of the suspension and Vu was the volume of the sediment.

Measurement of Viscosity:
The viscosity of the samples was determined using the Brookfield DV-E viscometer, at 30 rpm.

Flow rate:
Each suspension sample's time to flow through a 10 ml pipette was measured and calculated using the following equation [29]:

$$\text{Flow rate} = \frac{\text{Volume of pipette (ml)}}{\text{Flow time (sec.)}}$$

2.6.3 As Emulsifying Agent
Preparation of emulsion:
The required quantity of prepared CMC from *Spinacia oleracea* was dissolved in distilled water to produce aqueous CMC solutions with concentrations ranging from 0.5, 0.75, and 1% w/v. First, 90 ml of solutions containing 0.5%w/v, 0.75%w/v, and 1%w/v CMC were taken (named F1, F2 and F3, respectively). For 30 minutes, each solution was homogenized at 5000 rpm with an addition of 10 ml castor oil. Emulsions that had been prepared were kept in sealed containers until they could be tested further. The above-mentioned method was used for comparison batches of emulsions using standard NaCMC at concentrations of 0.5%w/v, 0.75%w/v, and 1%w/v (named F4, F5 and F6, respectively).

Evaluation of emulsion:
Determination of emulsion viscosity:
The viscosity of the emulsions was determined using Brookfield viscometer.

pH measurement:
pH was determined using digital laboratory pH meter [42].

Emulsion stability:
Emulsion stability measurement is necessary to show how stable an emulsion is while being stored. 10 ml of the prepared emulsions were centrifuged at 5000 rpm for 5 minutes to measure the stability of the emulsions. The separated water and oil phases of the emulsions were visible after centrifuging. Following equation used to determine the stability of an emulsion,
Emulsion stability (%) = [(Vo - V) / Vo] × 100

Where, Vo is the volume of emulsion that will be centrifuged in ml, and V is the volume of phase given off in ml. [43,44].

2.6.4Film Forming Ability
Preparation of film
3 gm of NaCMC were dissolved in 100 ml of distilled water and mixed continuously at 80 °C with magnetic stirring for 10 minutes to prepare the film-forming solutions. The solution was transferred on petri dish after cooling to 25°C.
The film was allowed to dry for 36 hours at room temperature. The dry film was then peeled and stored until use in a sealed plastic bag. The above procedure was repeated with CMC synthesized from *Spinacia oleraceae* for preparation of film [45].

Evaluation of Film
Weight of film
Using a digital weighing balance, the weight of each film was measured [46].

Thickness of film
By using a vernier caliper, the thickness of the films was measured at three distinct locations on the film, and the average was determined [47].

Folding Endurance:
The folding endurance measured manually for the prepared film. A strip of film is cut evenly and folded at the same place till it breaks [48].

3 RESULTS AND DISCUSSION

3.1 Cellulose

The yield of cellulose extracted from the Spinach powder was measured based on the dry weight basis. After alkaline treatment and bleaching the yield of cellulose extracted from Spinach was found to be 9.39%. The identification test gives positive results for extracted cellulose

3.2 Synthesis of Carboxymethylcellulose Using 3^2 Factorial Design

Results of Synthesis of Carboxymethyl cellulose with Design of Experiment software using 3^2 Factorial Design were shown in table no. 3.

Table no. 3: Results of 3^2 factorial design for synthesis pf carboxymethyl cellulose

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	**Run**	**A: NaOH %w/v**	**B: ChloroaceticAcid Gm**	**Viscosityc P**	**Degree of Substitution**	**Yield %**
1	1	20	10	2800	0.24	56
9	2	40	20	2750	0.33	72
5	3	30	15	3250	0.37	74
2	4	30	10	3100	0.34	71
10	5	30	15	3250	0.37	74
6	6	40	15	2500	0.31	70
3	7	40	10	2000	0.28	69
7	8	20	20	2900	0.29	61
8	9	30	20	3400	0.38	76
4	10	20	15	2850	0.26	58

3.3 Optimization of Synthesis of Carboxymethyl Cellulose

Analysis of Variance (ANOVA) for Quadratic model of Viscosity

The analysis of variance for the response viscosity was summarized in Table no. 4 and 3D surface of viscosity shown in Fig. no. 1.

Table no. 4: ANOVA for Response 1: Viscosity

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	1.523E+06	5	3.047E+05	96.35	0.0003	significant
A-NaOH	2.817E+05	1	2.817E+05	89.07	0.0007	
B-ChloroaceticAcid	2.204E+05	1	2.204E+05	69.70	0.0011	
AB	1.056E+05	1	1.056E+05	33.40	0.0045	
A^2	8.703E+05	1	8.703E+05	275.21	<0.0001	
B^2	2976.19	1	2976.19	0.9412	0.3869	
Residual	12648.81	4	3162.20			
Lack of Fit	12648.81	3	4216.27			
Pure Error	0.0000	1	0.0000			
Cor Total	1.536E+06	9				
R^2	0.9918					
Adjusted R^2	0.9815					
Predicted R^2	0.9151					
Adeq Precision	31.5259					

The Model F-value of 96.35 implies the model was significant. There was only a 0.03% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2 were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. The Predicted R^2 of 0.9151 was in reasonable agreement with the Adjusted R^2 of 0.9815; i.e., the difference was less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 31.526 indicates an adequate signal. This model could be used to navigate the design space.

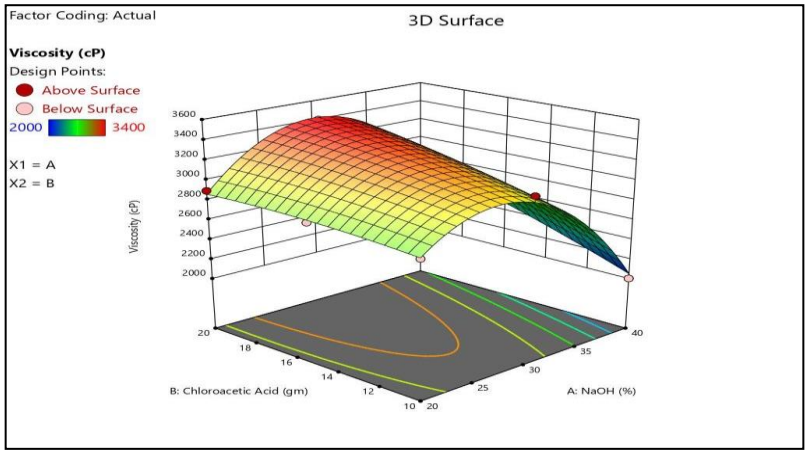

Fig. no. 1: 3D surface for Viscosity

Analysis of Variance (ANOVA) for Quadratic model of degree of substitution

The analysis of variance for the response degree of substitution was summarized in Table no. 5 and 3D surface for degree of substitution shown in Fig. no. 2

Table no. 5: ANOVA for Response 2: Degree of Substitution

Source	Sum of Squares	df	Mean Square	F-value	p-value	Significance
Model	0.0215	5	0.0043	138.83	0.0001	significant
A-NaOH	0.0028	1	0.0028	91.00	0.0007	
B-ChloroaceticAcid	0.0033	1	0.0033	105.54	0.0005	
AB	0.0000	1	0.0000	0.0000	1.0000	
A^2	0.0147	1	0.0147	473.88	<0.0001	
B^2	0.0000	1	0.0000	1.38	0.3046	
Residual	0.0001	4	0.0000			
Lack of Fit	0.0001	3	0.0000			
Pure Error	0.0000	1	0.0000			
Cor Total	0.0216	9				
R^2	0.9943					
Adjusted R^2	0.9871					
Predicted R^2	0.9481					
Adeq Precision	34.2547					

The Model F-value of 138.83 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms were significant. In this case A, B, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms were not significant. The Predicted R^2 of 0.9481 was in reasonable agreement with the Adjusted R^2 of 0.9871; i.e. the difference was less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 34.255 indicates an adequate signal. This model could be used to navigate the design space.

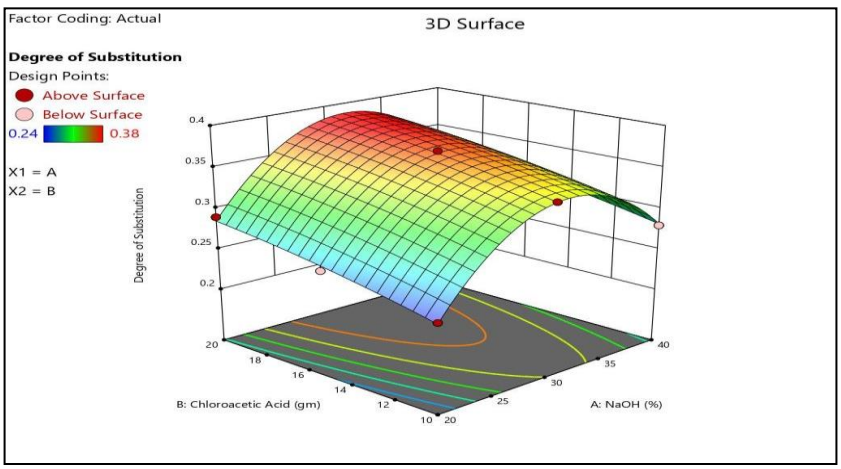

Fig. no. 2: 3D surface for degree of substitution

Analysis of Variance (ANOVA) for Quadratic model of yield

The analysis of variance for the response yield was summarized in was summarized in Table no 6 and 3D surface of yield shown in Fig. no. 3.

Table no. 6: ANOVA for Response 3: Yield

Source	Sum ofSquares	df	Mean Square	F-value	p-value	Significance
Model	458.00	5	91.60	404.96	< 0.0001	significant
A-NaOH	216.00	1	216.00	954.95	< 0.0001	
B-ChloroaceticAcid	28.17	1	28.17	124.53	0.0004	
AB	1.0000	1	1.0000	4.42	0.1033	
A^2	207.43	1	207.43	917.05	< 0.0001	
B^2	0.0119	1	0.0119	0.0526	0.8298	
Residual	0.9048	4	0.2262			
Lack of Fit	0.9048	3	0.3016			
Pure Error	0.0000	1	0.0000			
Cor Total	458.90	9				
R^2	0.9980					
Adjusted R^2	0.9956					
Predicted R^2	0.9836					
Adeq Precision	55.0006					

The Model F-value of 404.96 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2 were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. The Predicted R^2 of 0.9836 was in reasonable agreement with the Adjusted R^2 of 0.9956; i.e., the difference was less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 was desirable. Your ratio of 55.001 indicates an adequate signal. This model could be used to navigate the design space.

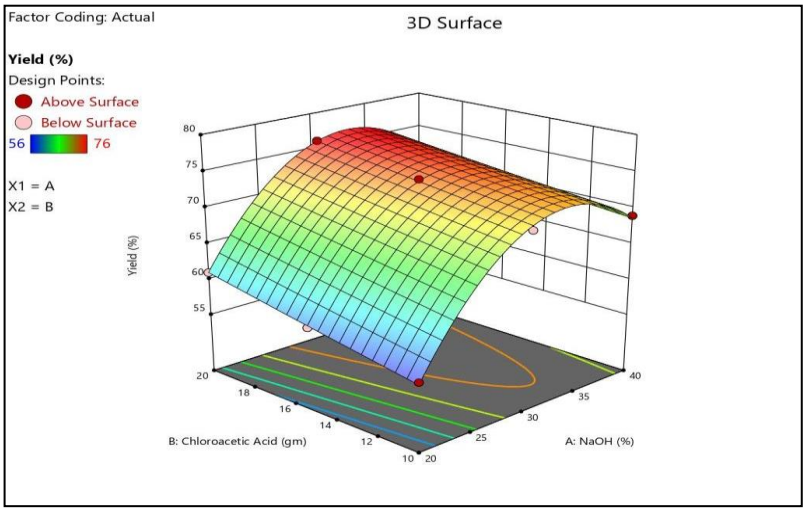

Fig. no. 3: 3D surface for yield

Polynomial Equation

In the polynomial, a positive sign indicated a synergistic effect and a negative sign indicated an antagonistic effect.

Table no. 7: Polynomial equation for viscosity, degree of substitution and yield

Response	NaOH-Chloroacetic acid
Viscosity	$Y1= +3267.86 - 216.67*X_1 + 191.67*X_2 +162.50* X_1*X_2 – 610.71 X_1^2 – 35.71X_2^2$
Degree of Substitution	$Y2= +0.3671 +0.0217*X_1 + 0.0233*X_2 +0.0000* X_1*X_2 – 0.0793 X_1^2 – 0.0043X_2^2$
Yield	$Y3= +73.71 + 6.00*X_1 + 2.17*X_2 – 0.5000* X_1*X_2 – 9.43 X_1^2 + 0.0714X_2^2$

Where, Y1 was viscosity response, Y2 was degree of substitution response, Y3 was yield response, X_1 was value of level of concentration of NaOH and X_2 was the value of level of concentration of Chloroacetic acid

Optimization of model for synthesis of CMC

The optimized conditions for CMC production with Design Expert software determine the optimum values of independent variables to the aim of maximization of Viscosity, degree of substitution and Yield. The software predicted the maximum amount of 3422.84 for Viscocity, 0.386 for degree of substitution and 76.03% for yield at NaOH concentration of 30%, Chloroacetic acid amount of 20 g.

To verify the software results, the carboxy methylation process was experimentally done at the optimum condition at NaOH concentration of 30%, Chloroacetic acid amount of 20 g. Comparison of the experimental viscosity (3421± 1), degree of substitution (0.38 ±0.005) and yield (76%) with the predicted value indicated only a small difference.

3.4 Characterization of Carboxymethyl Cellulose

3.4.1 Qualitative Test for CMC

Through qualitative investigation, it was proven that CMC synthesized gives positive results.

3.4.2 Physicochemical Characterization of CMC

Results of physicochemical characterization of CMC shown in Table no. 8.

Table no. 8: Physicochemical Characterization of prepared CMC

Characteristics	Prepared CMC
Organoleptic test	Odorless, off whiteand powder texture
Moisture content (%)	9.65 ± 0.05
Ash content (%)	12.4 ± 0.36
Solubility	Partially soluble in water.
% Yield	76± 0.05
Degree of substitution	0.38± 0.005
Viscosity (cP)	3421 ± 1
pH	7.17 ± 0.015
CMC content (%)	97.63 ± 0.55
NaCl content (%)	0.94 ± 0.015

Flow property of Carboxymethyl cellulose

Flow property of prepared CMC from *Spinacia oleracea* showed almost similar behavior with the marketed CMC. These flow property of CMC which is compare with marketed CMC shown in Table no. 9.

Table no. 9.: Flow property of prepared CMC and marketed CMC

Characteristics	Prepared CMC	Marketed CMC
Bulk density (g/ml)	0.26 ± 0.01	0.263 ± 0.02
Tap density (g/ml)	0.316 ± 0.002	0.317 ± 0.0015
Angle of repose(Degree)	33.90 ± 0.36	31.46 ± 0.34
Carr's index (%)	14.51 ± 0.50	11.05 ± 0.58
Hausner ratio	1.14 ± 0.127	1.18 ± 0.051

Values expressed as Mean ± SD (n=3)

Fourier transformed infrared spectroscopy (FTIR) analysis

Fig. no. 4. shows overlay FTIR spectra of the cellulose from *Spinacia oleracea*, CMC from *Spinacia oleracea* and standard CMC. Similar functional groups found in cellulose and each CMC include hydroxyl group (-OH stretching) at 3650-3200 cm-1, carbonyl group (-COO group) at 1680-1630 cm-1, hydrocarbon group (-CH_2 scissoring) at 1450-1400 cm-1, and ether group (-O- stretching) at 1200-1000 cm-1 shown in fig. no. 4. When compared to the cellulose from *Spinacia oleracea* in Fig. no. 4, the carbonyl group (-COO), methyl group (-CH_2) and ether group (-O-) all significantly increased in the CMC from *Spinacia oleracea*. These outcomes show that the cellulose molecules undergone carboxymethylation. CMC from *Spinacia oleracea* when compared to those Standard in Fig. no. 4 it shows the similar functional group.

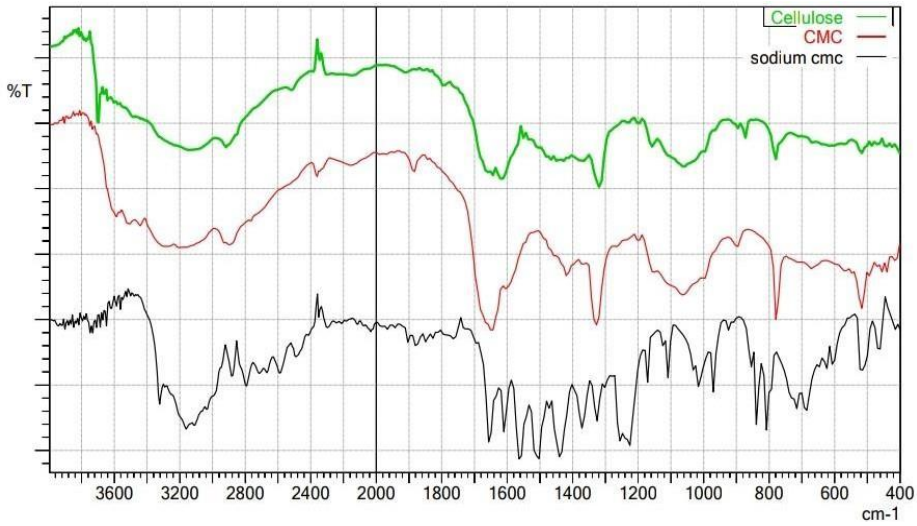

Fig. no. 4: Overlay FTIR spectrum of cellulose from *Spinacia oleracea* (green), carboxymethyl cellulose from *Spinacia oleracea* (red) and standard CMC (black)

Scanning electron microscopy (SEM) analysis

The SEM of cellulose from *Spinacia oleracea* in Fig. no. 5 was totally elongated with fibrils of varied thicknesses and lengths. SEM of the cellulose revealed that the fibrils packed structures were distinct from one another. The SEM of CMC from *Spinacia oleracea* in Fig. 6, which reveals that it had a rough surface, a partly round fibril, was not elongated like cellulose structures, and resembled a cluster of tightly packed atoms. SEM micrograph of prepared CMC in Fig. no. 6 shows clear differences in comparison to SEM micrograph of cellulose in Fig. no. 5 which shows isolated cellulose was further treated with sodium hydroxide during carboxymethylation, thus the ruptured surface was obtained from the synthesized CMC.

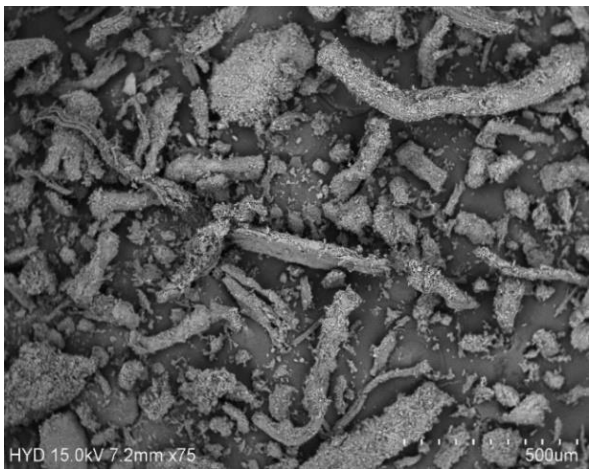

Fig. no. 5: Scanning electron micrographs of cellulose from *Spinacia oleracea*

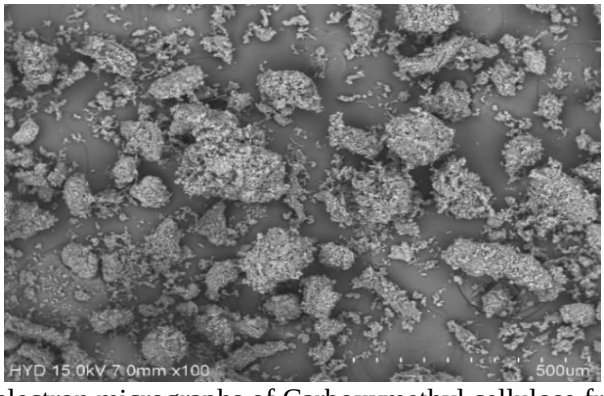

Fig. no. 6: Scanning electron micrographs of Carboxymethyl cellulose from *Spinacia oleracea*

X-ray diffraction (XRD) analysis

The X-ray diffractogram of the cellulose from *Spinacia oleracea* in Fig. no. 7 showed sharp peaks at 2θ = 14.21°, 14.83°, 19.98°, 21.92°, 29.29°, and 32°, indicating the presence of crystalline phases on the cellulose structure. The X-ray diffractogram of CMC from *Spinacia oleracea* in Fig. no. 8 shows clear differences in comparison to Fig. no. 7, in which Fig. no. 8 shows less number of peaks were found after carboxymethylation in comparison with the diffractogram of cellulose. It was seen from the significant reduction in peak intensity at 2 θ= 14.21° and 19.98°. Additionally, it was noted that the extracted cellulose peak at 2θ = 14.83°, 21.92°, and 29.29° disappeared in CMC. This resulted from the transformation of cellulose during carboxymethylation, which caused the crystallinity of CMC to decrease.

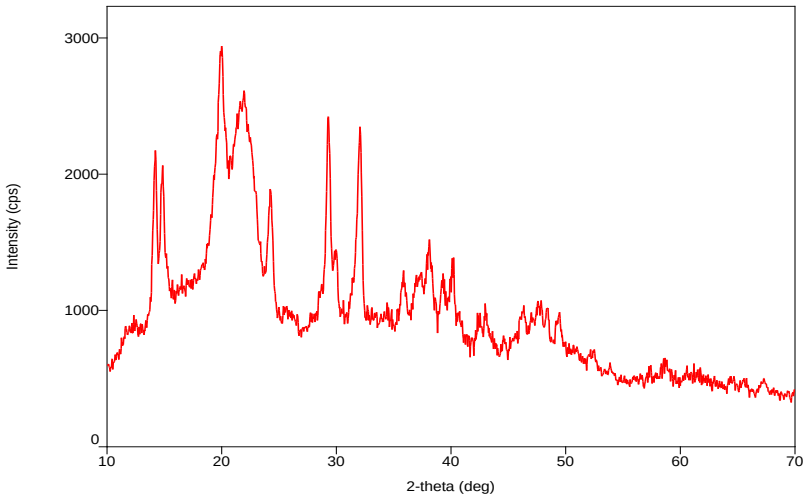

Fig. no. 7: X-ray diffractograms of Cellulose from *Spinacia oleracea*

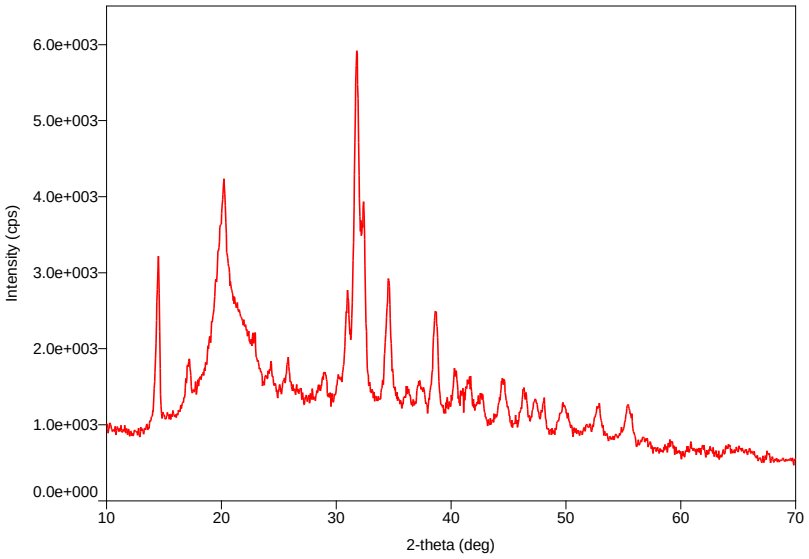

Fig. no. 8: X-ray diffractograms of Carboxymethyl cellulose from *Spinacia oleracea*

Thermogravimetric analysis (TGA) analysis

As shown in Fig. no. 9, the cellulose from *Spinacia oleracea* was shown to be degrading between 300 and 400°C. However, cellulose began to degrade at about 300°C, and weight loss of the cellulose was seen between 350 and 400°C. The final change, which was caused by the breakdown of cellulose, was also seen after 380°C. According to Fig. no. 10, the prepared CMC from *Spinacia oleracea* began to decompose at temperatures above 200°C. The initial stage was attributed to the moisture's

release of the CMC as hydrogen-bound water. The second and third stages of decomposition took place at 350°C and 550°C, respectively.

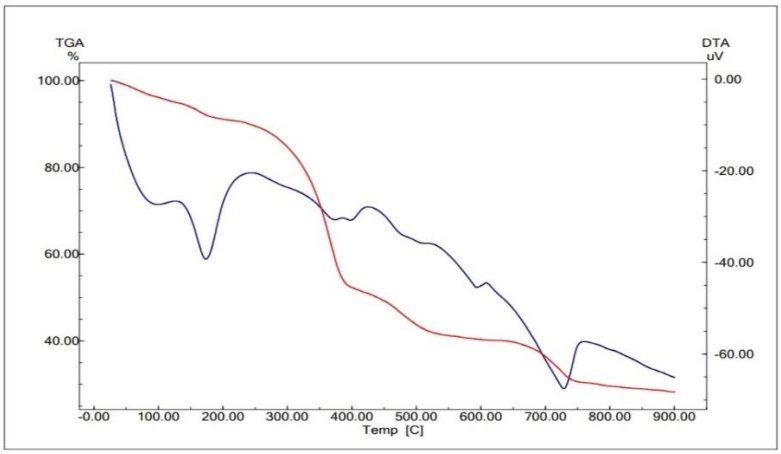

Fig. no. 9: TGA curve of cellulose from *Spinacia oleracea*

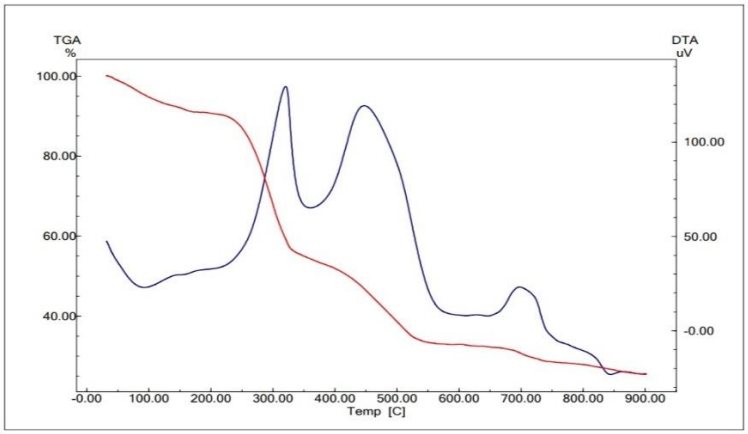

Fig. no. 10: TGA curve of carboxymethyl cellulose from *Spinacia oleracea*

Differential Scanning Calorimetry (DSC) Analysis

Fig. no. 11 and 12 shows DSC curve that indicates the energy consumption property of Cellulose and CMC from *Spinacia oleracea* respectively. As seen in Fig.no. 11 DSC profile of Cellulose shows an endothermic peak at 133.5 °C. The DSC curve of CMC shows peak at 112.70°C in Fig. no. 12. The DSC curve of CMC in Fig. no. 12 shows clear differences in comparison to Fig. no. 11 which shows the new peak of CMC at 112.70°C as compare to cellulose because of substitution of carboxymethyl group and they were comparable.

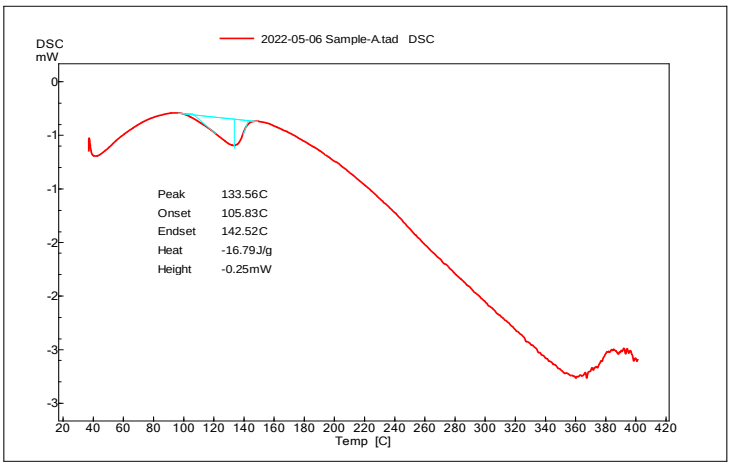

Fig. no. 11: DSC thermogram of cellulose from *Spinacia oleracea*

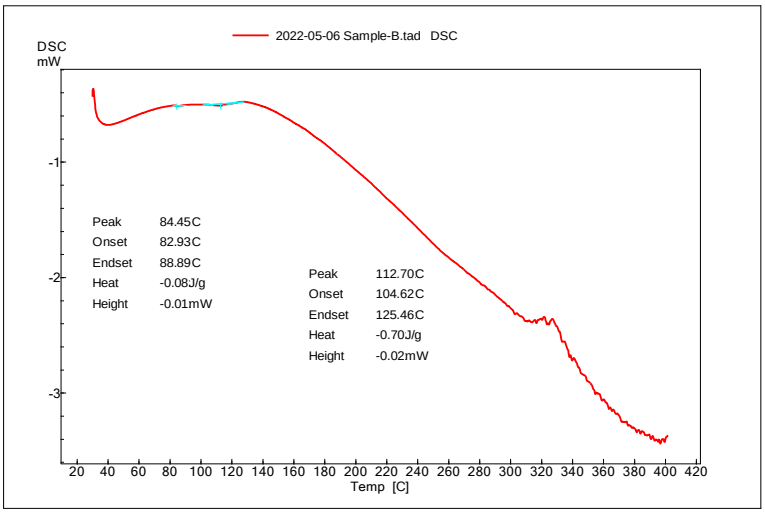

Fig. no. 12: DSC thermogram of Carboxymethyl cellulose from *Spinacia oleracea*

3.5 Applications of CMC In Pharmaceutical Dosage Form

3.5.1 Binding Agent in Solid Dosage Form

Bulk density, tap density, angle of repose, and Carr's index were used to analyses the granules' flow properties, and it was observed that all batches had good flow properties. The results were presented in Table no. 10. Tablet hardness and disintegration time increased with increasing binder concentration in all batches of tablets. As the binder concentration increased, the friability values decreased in all batches. Therefore, based on the results, it is possible to apply CMC from *Spinacia oleracea* as a binding agent in solid dosage form. The results were shown in Table no. 11.

Table. no. 10: Evaluation of granules

Binding Agent	Concentration % w/w	Bulk Density (g/ml)	Tap Density (g/ml)	Angle of repose (degree)	Carr's index (%)
Prepared CMC	2.5	0.291 ±0.0026	0.332 ±0.001	32.27 ±0.304	11.40 ±0.364
	5	0.293 ±0.0025	0.340 ±0.0015	33.00 ± 0.500	14.14 ±0.315
	10	0.294 ±0.0037	0.349 ±0.002	33.47 ±0.424	15 ±0.500
NaCMC	2.5	0.332 ±0.003	0.377 ±0.0025	31.29 ±0.261	11.5 ±0.404
	5	0.335 ±0.0047	0.381 ±0.0023	32.77 ±0.376	12.16 ±0.351
	10	0.334 ±0.0032	0.392 ±0.0041	33.50 ±0.555	14.7 ±0.360

Values expressed as Mean ± SD (n=3)

Table no. 11: Evaluation of Tablets

Binding Agent	Concentration % w/w	Hardness (kg/cm^2)	Friability (%)	Disintegration (min)
Prepared CMC	2.5	5.40 ± 0.015	0.87 ± 0.025	11.58 ± 0.026
	5	5.66 ± 0.026	0.73 ± 0.03	13.30 ± 0.09
	10	6.13 ± 0.01	0.61 ± 0.032	16.72 ± 0.052
NaCMC	2.5	5.92 ± 0.02	0.85 ± 0.04	12.09 ± 0.081
	5	6.35 ± 0.05	0.73 ± 0.045	14.85 ± 0.098
	10	6.84 ± 0.04	0.60 ± 0.05	17.89 ± 0.02

Values expressed as Mean ± SD (n=3)

3.5.2 As Suspending Agent

Physical test:

During the first 48 hours, the suspensions were monitored; no particle aggregation, caking, or crystal growth development were observed.

Redispersibility:

To determine the redispersibility, the suspensions were shaken every 24 hours for several days. Particles quickly settled in suspensions containing prepared CMC and sodium CMC at concentrations of 0.25 and 0.5%, respectively. However, 1% concentrations of prepared CMC or sodium CMC in suspensions observed slow and gradual particle settling.

Determination of sedimentation volume, viscosity and flow rate:

Results of determination of sedimentation volume, viscosity and flow rate shown in Table

no. 12. Results of sedimentation volume shows that increasing the concentration of prepared CMC or sodium CMC increases in the percentage of sedimentation volume. The result shows that viscosity gradually increases along with the concentration of the suspending agent increases. The results showed that flow rate steadily reduces when prepared CMC or sodium CMC concentration increases. The results obtained from physical test, redispersibility, sedimentation volume, viscosity and flow rate therefore have indicated that, the CMC prepared from *Spinacia oleracea* has the potential to be used as suspending agent.

Table no. 12: Determination of sedimentation volume, viscosity and flow rate

Parameters	Prepared CMC			NaCMC		
	(0.25%w/v)	(0.5%w/v)	(1%w/v)	(0.25%w/v)	(0.5%w/v)	(1%w/v)
Sedimentation volume (%)	45.3 ± 0.264	68.43 ± 0.404	90.26 ± 0.305	39.4 ± 0.2	76.6 ±0.529	91.83 ±0.152
Viscosity(cP)	260.3 ± 1.52	291.6 ± 2.08	320 ± 2.64	399 ± 1	420.3 ± 2.30	476.3 ± 2.51
Flow Rate (ml/sec)	1.40 ± 0.015	0.44 ± 0.02	0.16 ± 0.025	1.13 ± 0.152	0.28 ± 0.026	0.11 ± 0.03

Values expressed as Mean ± SD (n=3)

3.5.3 As Emulsifying Agent

Evaluation parameter results of viscosity, pH and emulsion stability for various concentrations of prepared CMC and NaCMC emulsions were depicted in Table no. 13. According to the results for viscosity, viscosity gradually increases as prepared CMC or sodium CMC concentration increases. The pH range for prepared CMC emulsions for varying concentrations were found to be 6.99 to 7.08, and the pH range for NaCMC emulsions for varying concentrations were found to be 6.92 to 7.01. The results for emulsion stability indicated that as prepared CMC or NaCMC concentrations increased, emulsion stability also increased. It was also observed that a higher concentration of emulsifying agent results in better and more stable emulsions; this data is shown in Table no. 13.

The results obtained from viscosity, pH and emulsion stability have indicated that, the CMC prepared from *Spinacia oleracea* has the potential to be used as an emulsifying agent.

Table no. 13: Evaluation parameters of prepared CMC and NaCMC Emulsions

Parameters	Prepared CMC			NaCMC		
	F1 (0.5%w/v)	F2 (0.75%w/v)	F3 (1%w/v)	F5 (0.5%w/v)	F6 (0.75%w/v)	F7 (1%w/v)
Viscosity (cP)	567 ± 3.05	611 ± 2.08	627± 3	582 ± 1.52	639 ± 2.51	704 ± 2.64
pH	6.99 ± 0.02	7.02 ± 0.015	7.08 ± 0.01	6.92 ± 0.025	6.97± 0.015	7.01 ± 0.032
Emulsion Stability (%)	97.5 ± 0.5	98.5 ± 0.5	99.83 ± 0.28	97.83 ± 0.288	99.5 ± 0.5	99.8 ± 0.23

Values expressed as Mean ± SD (n=3)

3.5.4 Film Forming Ability

Evaluation parameters results of films of prepared CMC and NaCMC were depicted in Table no. 14. Evaluation results of prepared CMC film that are weight of film, thickness of film and folding endurance were shows near about comparable results as compared standard NaCMC film. Prepared CMC from *Spinacia oleracea* formed film and film was evaluated. Therefore, CMC prepared from *Spinacia oleracea* have film forming ability.

Table No.14: Evaluation of films of prepared CMC and NaCMC

Parameters	Prepared CMC	NaCMC
Weight of film (mg)	40 ± 0.208	39 ± 0.305
Thickness of film (mm)	0.14 ± 0.01	0.10 ± 0.015
Folding Endurance	87 ± 1.52	98±2

Values expressed as Mean ± SD (n=3)

4 CONCLUSION

The cellulose was extracted from *Spinacia oleracea* and its was converted into carboxymethyl cellulose. Yield of cellulose and carboxymethyl cellulose from *Spinacia oleracea* were obtained 9.39% and 76% respectively. The Prepared CMC was characterized and applications was studied in pharmaceutical

dosage form. In application in pharmaceutical dosage form, prepared CMC was compared to marketed CMC it shows that prepared CMC used as binding agent in solid dosage form, suspending agent, emulsifying agent and film forming ability in pharmaceutical dosage form. From the above observation we can say that we achieved our aim and objectives.

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