RESEARCH ARTICLE

Synthesis and Characterization of Hydroxypropyl *Sesbania* Galactamannan Seed Gum for Pharmaceutical Application

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ABSTRACT

The core focus of current research is chemical polysaccharide modification in pharmaceutical applications. The gum is made from the endosperm of *Sesbania grandiflora* Plant seeds that belongs to family Leguminosae. Both water-soluble and water-insoluble gum were present in the *Sesbania* seed powder; the water-soluble gum was removed during purification, yielding a 30% purification yield. In order to increase the applications of partially hydroxypropyl *Sesbania* gum, the modifications indicated here entail adding hydroxypropyl groups to the molecule under a variety of different conditions. Among the factors that were looked at were the etherifying agent concentration, alkaline volume, and preparation medium parameters, including the reaction time and temperature. The degree of substitution (DS) was raised, which boosted the unaltered gum's solubility, stability, and viscosity. Increases in an etherifying agent and alkali concentration, volume, reaction duration, and temperature increase DS from 0.4 to 0.7. The finished product was characterized using IR spectroscopy, differential scanning calorimetry, X-ray diffraction, scanning electron microscopy, rheologic property, solubility, swelling index, and gel fraction analysis of batch F1 as an improved batch. The alternate method for developing drug-loaded nanoparticles for controlled release dosages form by suing hydroxypropyl *Sesbania* gum.

Keywords: *Sesbania* gum, Hydroxypropylation, Chemical modification, Degree of substitution, Viscosity, Solubility.

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INTRODUCTION

Polysaccharide gums are among the most popular industry components and have become the subject of much research regarding their long-term sustainability, biodegradability and biological safety.[1] A few drawbacks, however accompany the use of gums. They include the potential of microbial contamination, changing rates of hydration, influenced by pH soluble content, thickening up, and viscosity loss on storage are a few of these. Gums can be chemically altered to reduce these limitations while simultaneously increasing their solubility and viscosity.[2]

According to Duke *et al.,* the endosperm, or outermost layer, of a seed of the species *Sesbania grandiflora* (Leguminosae) is used to make *Sesbania* gum. According to Faroogi *et al.*, *Sesbania* seeds are composed of a coat 6.9 to 18.9%, endosperm 40 to 42% and germ about 51.1%.

The outermost layer of seed is made up of galactose side chain residues linked by -(1-6) and a mannan backbone connected by -(1-4) glycosidic connections, which is known as galactomannan. According to one study, the ratio of galactose to mannose produced by the acid hydrolysis of *Sesbania* galactamannan gum was 1.2:2.2 as opposed to 1:3.9 for locust bean (carob), and for tara gum 1:2, and 1:3. It is believed that the varying degrees of branching are what produce the variances in the characteristics of galactamannan gums. More side groups reduce the amount of molecular bonding and improve the cold-water dispersion of gum, as reported as.[3,4]

Galactamannan, sometimes referred to as galactose side chain residues and a mannan backbone coupled by -(1-4) glycosidic linkages, make up the endosperm. In contrast to the ratios of 1:3.9 for locust bean (carob), 1:2, and 1:3 for Tara gum, one study found that the ratio of galactose to mannose generated by the acid hydrolysis of *Sesbania* galactamannan gum was 1.2:2.2. The differences in properties of galactamannan gums are assumed to be caused by the varied degree of branching.[5]

The reagents utilized and the reaction conditions have a significant impact on how effective the hydroxy propylation reaction is. Due to its accessible structure, the amorphous area

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of the cellulose is where the etherification reaction mostly takes place. The proportion of the etherifying reagent that reacts with or is substituted on cellulose is used to measure the reaction's efficiency. The remaining etherifying agent is consumed to create byproducts when it does not react with cellulose. The presence of an alkaline catalysts, the etherifying agent, and the probability of propylene oxide-cellulose alcoholate nucleophile contacts strongly influence the efficiency of etherification. Elevated reaction temperature promotes alkaline catalyst diffusion and etherifying reagent penetration into the cellulose reaction site, reducing reagent usage. Several methods have been developed to produce lower substitution hydroxyalkyl ethers of cellulose in an aqueous form. Using non-aqueous medium or under dry circumstances might produce high levels of substitution in carbohydrates.[6-8] *Sesbania* gum's low solubility is a significant barrier to its use. To create derivatives soluble in water across a larger pH range, special emphasis has been made to its chemical modification and depolymerization.[9] Alkaline reagents are therefore excellent catalysts for the synthesis of alkali cellulose. After this alkalization phase, cellulose must interact with propylene oxide via a two-dimensional substitution mechanism to yield hydroxypropyl cellulose.[10]

The study's primary goal was to completely solubilize galactamannan gum by extracting gum from locally available seeds and applying it to chemical alteration via hydroxypropylation, This may be accomplished by enhancing the various reaction parameters, such as temperature, propylene oxide concentration, reaction time, and alkali concentration.

MATERIAL AND METHODS

Material

The powder of *Sesbania* seed was procured from Vinayak Industries, Ahmadabad, Gujarat, India. propylene oxide procured from Hymedia; and analytical-grade chemicals such as glacial acetic acid, sodium hydroxide, isopropyl alcohol, and acetone were used.

Purification

The approach outlined by.[11] is modified by this process. For 20 mL of boiling 80% (v/v) ethanol was used to treat 1 g of raw *S. grandiflora* gum about 10 minutes. The resulting slurry was washed on glass filter no. 3 with ethanol, acetone, and ether in that sequence. The obtained substance was mixed with distilled water (100 mL) and given a one-hour hydration period. After 15 minutes of mixing with a magnetic stirrer, it underwent 15 minutes of centrifugation at 1500 rpm. A cold acetone solution in two volumes was used to precipitate the supernatant. At room temperature, the polymer mixture was centrifuged for 1.5 hours at 6000 rpm after being redissolved in hot water (80°C). Two litres of ethanol were used to precipitate the supernatant. On a glass filter no. 4, after being collected, the precipitate washed using both ethanol and acetone before it was dried with hot air.

Synthesis of Hydroxypropylation of Sesbnia Gum

Sesbania was hydroxypropylated using a two-step process (A and B).

Step A: A material : liquor ratio of 1:20 is used, 1-gm *Sesbania* samples were individually dispersed in 20 mL sodium hydroxide solutions containing 15% NaOH. They were then stirred for 1-hour at room temperature.

Step B: Reaction of etherification, formed alkali *Sesbania* was then given 4 mL of cold isopropyl alcohol, followed by 1.15 mLof cold propylene oxide (0°C), and the reaction beaker was carefully covered with aluminium foil. At a temperature of 60°C for two hours, a magnetic stirrer was used to homogenize the reaction mixture. The final product of the reaction was neutralized with glacial acetic acid, precipitated, and multiple times washed with acetone before being dried and crushed.[12]

Characterization of Modified *Sesbania* Gum

Degree of substitution of modified Sesbania gum

The degree of substitution (DS), or the proportion of hydroxypropyl groups within the *Sesbania* chain, was calculated using the conductimetric technique (Campana-Filho, 2005) (Figure 1).

In 100 mL of 0.05 M HCl, around 0.1 g of CMS was dissolved, the increase in pH to 2.0–2.2 by adding 0.1 M NaOH. The DS was determined using the following equation and titration up to pH 11.5 with 0.1 M NaOH.

$$\mathrm{DS} = \frac{\mathrm{V2} - \mathrm{V1} \times \mathrm{DD}}{V3 - V1}$$

where DS is the amount of HPS substitution (mol per mole of all monomers), DD is the amount of *Sesbania* gum de-acetylation, and V1, V2, V3 is the volume of NaOH. D.S., as indicated in Table 1, increased with a rise in sodium hydroxide and propylene oxide concentrations.

Swelling and gel fraction studies

Onuki Y *et al.*'s described technique as the foundation for the swelling and gel fraction investigations conducted.

Samples containing 0.01 g of modified *Sesbania* gum were put in little plates and carefully placed within glass flasks. Each glass flask was gently filled with a total of 60 mL of distilled water. At room temperature, the samples were soaked for 2 hours. After carefully removing the surplus solution, the gelled samples still in the glass container were weighed. After three days of lyophilization, the gelled samples were once more weighed. Using Eqs. (1) Swelling Ration and Eqs. (2), Percentage of gel fraction computed.

$$\boldsymbol{Swelling\ ratio} = \frac{Wwater}{Wgel} \quad \text{.....(Eqs..1)}$$

$$\boldsymbol{Percentage\ gel\ fraction} = \left(\frac{Wgel}{Wsolid}\right) \boldsymbol{x\ 100} \quad \text{.....(Eqs..2)}$$

(water- Sample weight after 2 h soaking , Wgel- Sample weight after dry in Oven, and Wsolid – sample initial weight)

Table 1: Synthesis parameter for hydroxypropylation

Batch no	*Naoh conc (%)*	*NaOH volume (Ml/gm)*	*Propylene oxid volume In (ml/gm)*	*Temp (°C)*	*Time of reaction (minutes)*	*Degree of substitution*
HPS 1	15	20	1.15	60	60	0.4
HPS 2	20	20	1.50	70	120	0.5
HPS 3	30	20	1.70	80	180	0.6
HPS 4	40	20	2.00	90	240	0.7

FTIR analysis

Japan's Shimadzu, IR Affinity-1 IR spectra between 4500 and 400 cm^{-1} were recorded using FTIR spectroscopy on SG and CMS materials.

DSC analysis

DSC thermograms of GG and HPS samples were captured by Using a differential scanning calorimeter (Q10 V9.0 Build 275, TA Systems, USA). A common aluminum pan was used to crimp around 7 to 8 mg of sample, which was then heated to temperatures between 40 and 3000°C.

X-RD Analysis

The powder XRD patterns were identified using the Bruker diffractometer (D-8) Advance and Cu-k radiation. The step size used for diffractograms was 0.01, the scanning speed was one time every step, and the chart speed was 2°/2 cm per 2θ.

Scanning Electron Microscope

The materials' SEM pictures were recorded using a field emission scanning electron microscope (Hitachi, S-4800, Japan) with a 20 kV accelerating voltage.

Solubility

According to the Indian Pharmacopoeia volume 1(2007), the solubility of SG and HPS was examined in various organic solvents and water.

- *Determination of solubility in water*

A thick jelly is produced when 1-gm SG is swirled with 100 mL of water; an added extra water 150 mL results in a colloidal dispersion. *Sesbiana* Gum powder generates a cloudy solution rather than dissolving in water to produce a clear solution. While 1-g of HPS in 100 mL of water results in a viscous, clear, and yellowish solution, HPS is soluble in water.

- *Determination of solubility in organic solvents*

Inorganic solvents can virtually not dissolve the gum HPS, which is water soluble. But in other solvents, it either swells up or becomes partially soluble. Usually, substances that contain polar groups or are miscible with water have some sort of action. The solubility behavior with common organic solvents is shown in Table 2. Data were obtained by mixing 100 mL of each solvent with 1-g each of SG and HPS at 25°C.

- *Determination of rheological properties*

Using spindle number 1 on a Brookfield digital viscometer with model number LVDV 2P230, the rheological characteristics of the samples were ascertained. Prior to each measurement, the sample temperature was kept at 25°C.

The following procedure was used to produce samples at various concentrations (0.1 to 1.0%) in distilled water; 1-gm of the sample was moistened with 2 mL of rectified spirit (95%) before being diluted with 100 mL of distilled water that was kept at 25°C. To allow the gum to expand, the sample was agitated for 10 minutes at 1000 rpm using a laboratory stirrer, and then let to stand for 1-hour. Table 3 displays the results of the rheological investigation.

RESULTS AND DISCUSSION

Hydroxypropylation of *Sesbania* Gum

Different alkali concentrations, propylene oxide concentrations, temperatures, and reaction times were used during the hydroxypropylation reaction. *Sesbania* gum is hydroxypropylated in a two-step process. The gum's hydroxyl group and sodium hydroxide combine in the first step to form an alkoxide group. In the second stage, isopropyl alcohol reacts with alkali *Sesbania*, and a reaction between gum alkoxides and A hydroxypropyl group forms as a consequence of propylene oxide and these responses are as follow.

Effect of NaOH concentration and volume on DS is given in Table 1, together with the degree of substitution of modified *Sesbania* gum mentioned in the above approach. It demonstrates that the degree of substitution increases as NaOH concentration and propylene oxide volume rise. Thus, the amount of propylene oxide and NaOH concentrations determined the degree of substitution (Figure 2). The temperature rose from 60 to 90°C, followed by a rise in HPS substitution from 0.4 to 0.7 (Figure 3). Reaction time also impacts DS; DS rose as reaction time increased (Figure 4).

Investigations on Swelling and Gel Fraction

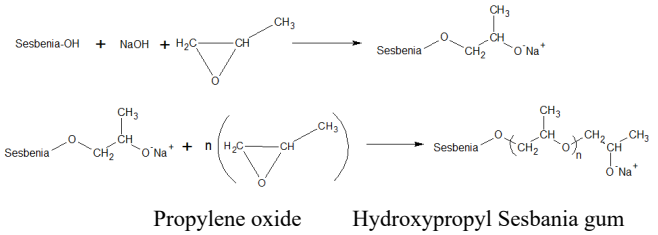
Investigations were conducted using a procedure that had already been reported. Swelling ratios across various batches are shown in Table 4. Both batch HPS1 and batch HPS4 had HPS values of 3.26 and 2.13, accordingly. This indicated the swelling ratio increased as the DS rose; this impact was visible.

Table 2: Solubility behaviour of SG and HPS in organic solvents

Solvent	*Ethanol*	*Methanol*	*Hot water*	*Dichloromethane*	*Water*	*Acetic acid*	*Acetone*
SG	Insoluble	Insoluble	Slightly Soluble	Insoluble	Partially Soluble	Insoluble	Partially Soluble
HPS	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	soluble

Table 3: The characterization of Rheology

Concentration	*Viscosity in cps*				
	SG	*HPS1*	*HPS 2*	*HPS3*	*HPS 4*
0.1%	30	5	7	11	16
0.2%	100	15	18	21	26
0.3%	230	27	32	42	49
0.4%	500	43	46	51	56
0.5%	700	54	58	68	77
0.6%	850	59	66	79	82
0.7%	1010	70	73	85	91
0.8%	1200	75	88	99	108
0.9%	1600	85	98	109	119
1.0%	1900	93	108	122	132



Propylene oxide Hydroxypropyl Sesbania gum

Figure 1: Degree of substitution of modified *Sesbania* gum

Gel fraction from various batches as shown in Table 4. It was 68.6 in batch HPS 4 and 84.4 in batch HPS 1, respectively. This demonstrated that the gel fraction decreased as the DS level increased.

The modified *Sesbania* gum hydroxypropylation depends on increasing the level of substitution. Raj S. B. *et al.* and Kooijman *et al.* The fundamental motivation for researching the gel fraction % is that hydroxypropylated gum's water solubility rises as it undergoes substitution, which diminishes the gum's gelling capabilities.[13,14]

Determination of Solubility

The following Table 2 displays the solubility of HPS in an organic solvent. However, HPS was somewhat soluble in isopropanol, whereas SG and HPS were hydrophobic in organic solvent. The modification of SG into HPS enhanced its solubility. When HPS powder is dissolved in water, it results in a transparent and viscous solution. Since each batch's degree of substitution differs so does its solubility in water, indicating that solubility varies depending on substitution level.

Determination of Rheological Properties

On the basis of a methodology that had previously been published,[15] rheological characteristics were investigated.

According to rheological characteristics, the water viscosity increases with an increase in concentration of Modified *Sesbania* seed gum powder (HPS). Increased gum powder content led to an increase in viscosity in all batches. Another result was that batch F4's viscosity was higher than that of the other batches as a result of the DS effect, as well as variations in the concentrations of propylene oxide and

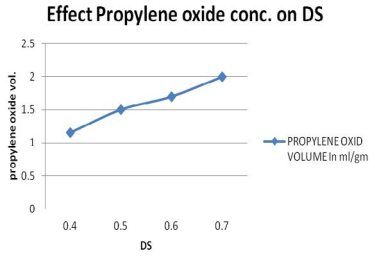


Figure 2: Propylene oxide conc. Effect on DS

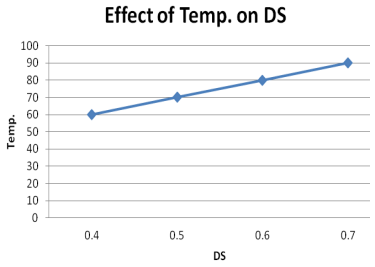


Figure 3: Temp Effect on DS

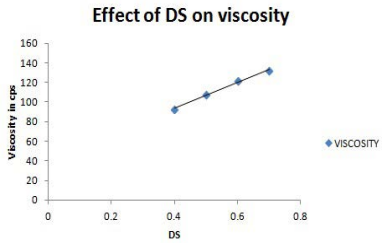


Figure 4: Reaction time Effect on DS

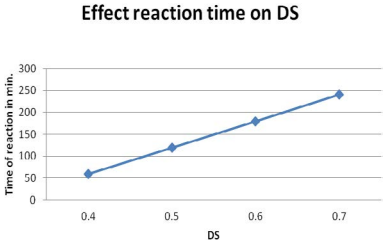


Figure 5: Effect of DS on Viscosity

Table 4: Response of purified and hydroxypropylated *Sesbania gum* (swelling ratio and gel fraction)

Batch	*Swelling ration*	*Gel fraction (%)*
SG	1.90	90.6
HPS 1	2.13	84.4
HPS 2	2.51	78.2
HPS 3	2.83	74.8
HPS 4	3.26	68.6

NaOH used in each batch. The more hydroxypropyl groups that the SG's hydroxyl group replaced for, the more the rise in DS's effect raised the viscosity of the HPS solution. The hydroxypropyl group functions as a hydrophilic group, increasing DS and CMS's capacity to immobilise water in a system. According to Figures 5 and 6, Table 3 demonstrated that the DS and Concentration HPS powder impacted the HPS viscosity.

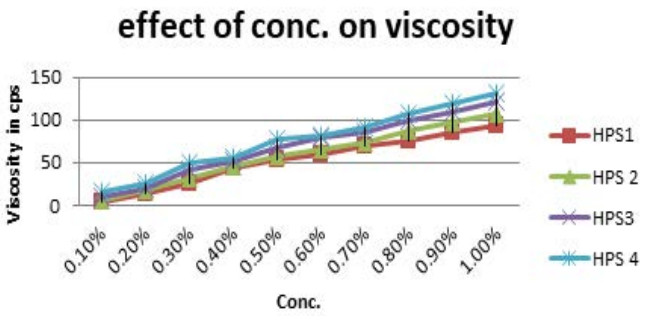


Figure 6: Effect of conc. on viscosity

FTIR Analysis

Figures 7 and 8 demonstrate the combined IR spectra of carboxymethyl *Sesbania* (CMS) and *Sesbania* gum (SG).[16] Stretching of the OH bond is responsible for the strong and wise absorption peak band at 3491 cm^{-1}, whereas CH group stretching is responsible for a sharp absorption at 2920 cm^{-1}. The OH bnd initiate in water fragments is what causes the absorption band to occur at 1649 cm^{-1}. The CH2-O-CH2 group bending is placed in the 1009 cm^{-1} frequency range, while the CH2 bend group is allocated to absorption at 1383 cm^{-1}.

According to the FTIR Spectra of hydroxypropyl *Sesbania*, certain groups of OH were hydroxypropylated as evidenced by the lower strength at 3418 cm^{-1} Due to stretching -OH. Both C-O stretching and C-H stretching are attributed to the region about 2922 cm^{-1}. The vibration of the CH2 scissor is located in the band at 1446 cm^{-1}. Furthermore, it should be observed that at 1374 cm^{-1}, CH bending of CH3 (symmetric) and OH in-plane bending vibration simultaneously emerged.

DSC

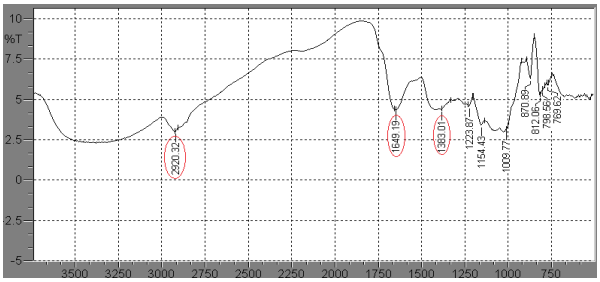
Figures 9 and 10 show the DSC thermograms of SG and CMS, respectively. One distinct endothermic peak was visible on the thermograms of the SG and HPS at 136.10 and 202.21°C, respectively.[17] That suggested that because of the high degradation threshold, HPS was more stable than SG. Additionally, due to the breakdown of the hydroxypropyl groups included in the polymer moiety, the thermal degradation of hydroxypropyl *Sesbania* gum manifests within a temperature range of 202.21°C. This demonstrated the weight loss of SG at 136.10°C and HPS at 202.21°C, while the weight loss of CMS at a significant degree further supported the introduction of hydroxypropyl groups.

X-RD Analysis

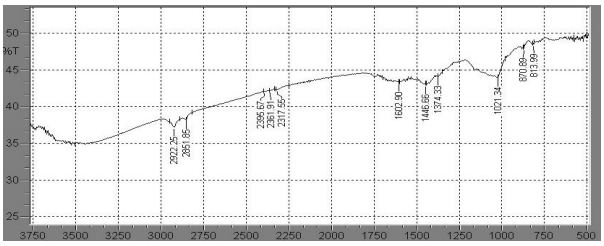
The XRD patterns for SG and HPS are shown in Figures 11 and 12. At a diffraction angle of 19.210°, specific peaks for SG showed in the XRD pattern with intensities exceeding 300, indicating SG's crystalline nature. Compared to SG the intensity of the typical peaks for HPS was lower, suggesting that HPS was becoming more amorphous.

Scanning Electron Microscopy

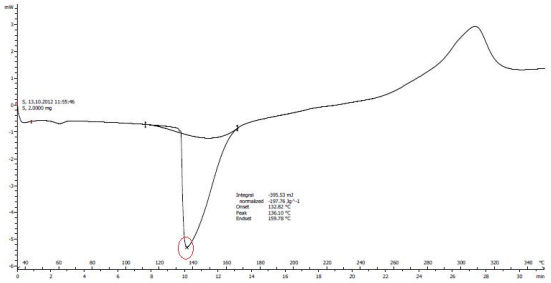
Figures 13 and 14 display the SEM images of SG and HPS, respectively. The considerably smaller and more extended



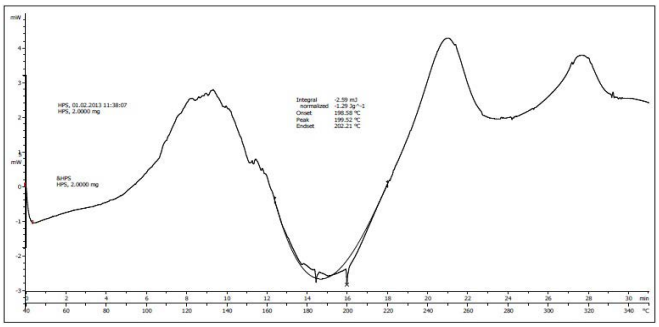
Figures 7: FTIR spectra of SG



Figures 8: FTIR Spectra of HPS



Figures 9: DSC thermograms of SG



Figures 10: DSC thermograms of HPS

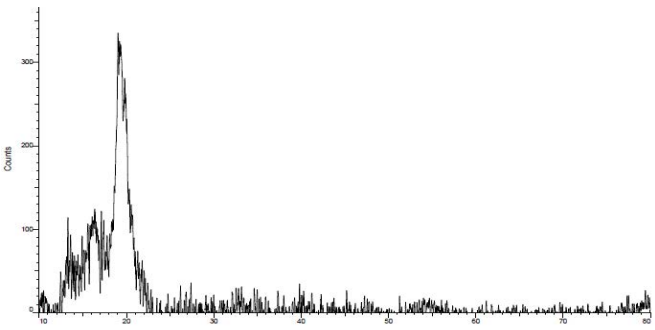


Figure 11: XRPD of SG

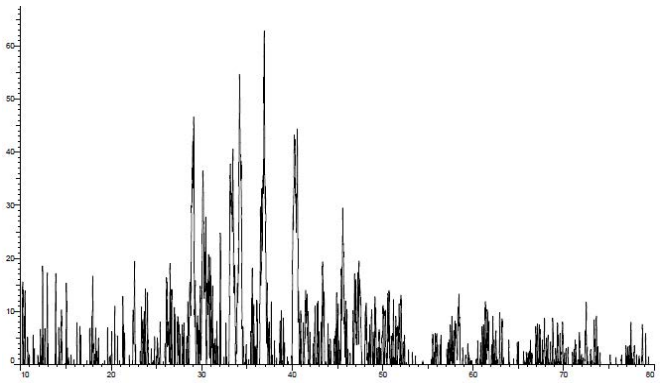


Figure 12: XRPD of HPS

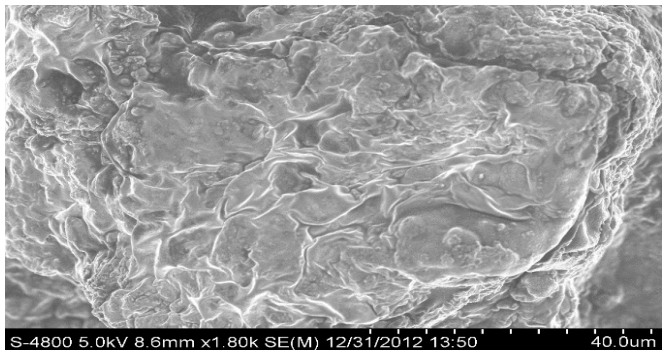


Figure 13: Sg sem analysis

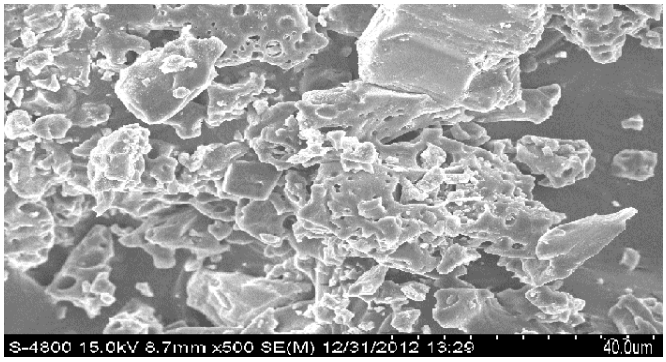


Figure 14: Hps sem analysis

crystals of SG and the multifarious, slippery textured granules of HPS were discovered by SEM analysis.[12,18] Particle size decrease was seen in the scenario of SG. It was evident that SG particles changed from smaller crystalline forms to small crystalline structures. Granule aggregation was seen in the case of HPS. It was obviously of an amorphous character.

CONCLUSION

Different etherifying agent and alkali concentrations were used throughout the hydroxypropylation process to produce hydroxypropyl *Sesbania* galactamannan gum with varying degrees of substitution. It was discovered that the concentration of the etherifying agent rose with the degree of substitution, and the extent of substitution resulted in an increase in viscosity. According to studies, unpolished *Sesbania* seed gum's stability, solubility, and viscosity are improved by hydroxypropylation and purification. By utilising of IR Spectrocsopy, X-ray diffraction FE-SEM and DSC analysis to show the processes' viability. The resulting compounds can also be used as drug-delivery vehicles in a variety of biological contexts.

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