

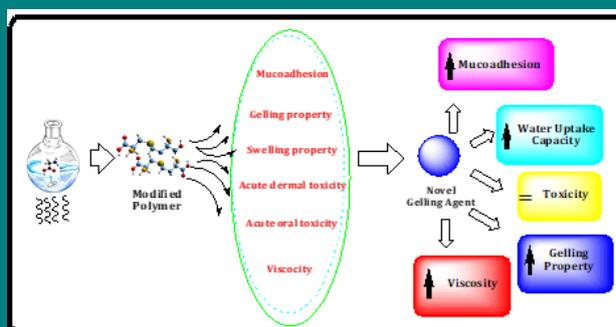
## Poly Acetyl Amine Grafted Xanthan Gum as A Novel Polymeric Carrier for Drug Delivery System

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**Abstract:** The goal of this research is to develop a modified polymer with superior physicochemical characteristics that can be used to a variety of drug delivery systems in contrast to parent polymer systems. The manufacture of modified xanthan gum and improving its desired physicochemical characteristics are the specific goals of this work. Chloroacetyl chloride and ammonia were used in this study to successfully conjugate the acetyl amine group to xanthan gum (XG). The FTIR, <sup>1</sup>H-NMR, DSC, XRD, and SEM techniques were used to characterize the alteration. Together with acute oral and cutaneous toxicity, the physicochemical characteristics—such as mucoadhesive nature, viscosity, gelling, and swelling behavior—were ascertained. The development of poly-acetyl amine grafting on xanthan gum (MXG) was validated by proton NMR and FTIR spectra. DSC and XRD were used to validate MXG's crystallinity. Along with size and form, SEM pictures show that the smooth polyhedral structure of xanthan gum altered to a rough, spongy surface in MXG. When compared to native XG, acetylamine-grafted XG (MXG) demonstrated superior physicochemical characteristics, such as increased gelling capacity, mucoadhesion, and viscosity. Because of its greater hydrophilicity, MXG exhibited longer gelling stability and quicker water absorption, even though it formed gels at a lower concentration (0.6% vs. 1.2% for XG). Superior mucoadhesive strength and detachment force were demonstrated by ex vivo investigations. With an LD<sub>50</sub> > 2 g/kg, toxicity tests showed that MXG was safe for topical and oral use. These results point to the potential of MXG as an effective carrier in oral and topical medication delivery systems.



**Keywords:** Poly-Acetylamine Grafted Xanthan Gum, Polymer Modification, Modified Xanthan Gum, Mucoadhesive Polymer, Gelling Agent, Viscosity Modifier.

### Introduction

Repeating monosaccharide units connected by glycosidic linkages form polysaccharides, which are polymeric carbohydrate structures. They are highly preferred for their nontoxicity, cost-effectiveness, biocompatibility, and biodegradability, and they come from renewable sources such as microbes, plants, and animals [1]. They are therefore employed in a wide range of sectors, such as agriculture, food, medicine, biomedicine, and cosmetics [2]. Nevertheless, polysaccharides also have some disadvantages that restrict their potential uses [3].

Sugars like fructose, mannose, and glucose are typically found in exo-polysaccharides, including microbial gums [4]. With a molecular weight of  $2 \times 10^6$  to  $20 \times 10^6$  Da, xanthan gum (XG), a fermentation product of the Gram-negative bacterium *Xanthomonas campestris*, is the second most widely used microbial polysaccharide after dextran. It has a trisaccharide side chain and a glucopyranose glucan backbone, which give it special rheological and physicochemical characteristics. The greatest commercial uses are provided by this. Among these natural polymers, xanthan gum has garnered a lot of attention in the last 20 years. The gram-negative bacteria *Xanthomonas campestris* ferments to produce XG [5]. With a pendant trisaccharide side chain connected to an alternative glucose

residue by a 1,3 linkage, XG is made up of a 1,4-D-glucopyranose glucan backbone [6]. Many inner mannose units contain an acetyl group in locations 0–6 in the main chain, while many XG units have a pyruvate linkage in places 4 and 6 (terminal mannose) [7]. Its glucuronic acid side chain gives it polymeric characteristics. When dissolved in water, its polar groups, such as hydroxy and carboxyl, create hydrogen bonds both inside and between molecules [8]. Because of these hydrogen bonding interactions and its large molecular weight, it has a high inherent viscosity at low concentrations [9]. It may be employed as a thickening agent [10], a drug reducer in oil drilling [11], a food stabilizer [12], and a drug reducer in pharmaceuticals [13] due to its high viscosity and lack of reaction with pH salts.

Factors including uncontrolled hydration rates, weak gelling characteristics, low heat stability, and vulnerability to microbial contamination restrict the unaltered form of xanthan gum (XG). Chemically altering XG via amidation, etherification, esterification, and cross-linking has been the subject of much research in an effort to get around these issues [14]. In XG, the carboxyl and hydroxyl groups are essential locations for chemical reactions such as amidation, acetylation, etherification, and esterification (Fig. 1) [15]. The enhanced hydrophilicity and

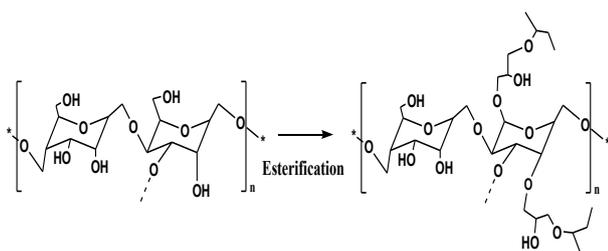
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increased surface area of polyvinyl alcohol (PVA) combined with modified XG, which permits more interaction with aqueous environments, resulted in higher adsorption performance. They are able to retain and absorb chemicals better because of synergistic interactions and also form a complex network structure. [16], while oleamidopropyl dimethyl amine XG [17], triisopropanolamine XG [18], hexadecyl [19, 20] and tetradecyl XG [21] boosted viscosity. By changing its structure, making it more hydrophilic, and boosting its swelling qualities, esterification of xanthan gum improves drug release. When ester groups are added, hydrogen bonds are broken, resulting in a more amorphous and hydrophilic matrix that promotes increased swelling and water penetration and speeds up drug diffusion. Further optimizing drug release profiles under various situations [22, 23] and sustained release [24, 25] are improved drug-polymer interactions and pH-responsive behavior.

The development of amide bonds, which are used in a variety of applications, including strengthening and stabilizing pharmaceutical formulations' thickness [26] and promoting mucoadhesion [27, 28], may be centered on the carboxyl functional group. The hydroxy group of XG may react with aldehyde to create an acetyl bond in an acidic environment. The material became thicker and more soluble as a result [29]. Sulfoxyamine XG is also used in ophthalmic drug delivery systems to improve mucoadhesion, gelling, and viscosity [30, 31]. Dry heating, annealing, moisture, and heat treatment are examples of physical and mechanical changes that change the physicochemical characteristics of sulfoxyamine XG and increase its range of uses [32–35].



**Figure (1):** Esterification of xanthan gum.

Although XG modification has advanced, little is known about the production of acetyl amine-modified XG and its particular uses in mucoadhesive drug delivery systems. By synthesizing and analyzing acetyl amine-grafted XG and assessing its potential as a mucoadhesive polymer for oral and cutaneous drug delivery applications, the current work aims to close this gap. This work sheds light on the structural, physicochemical, and functional improvements made possible by acetyl amine grafting by using methods including FTIR, NMR, DSC, XRD, and SEM.

In order to overcome the inherent drawbacks of native XG, including its low solubility, uncontrolled hydration, and limited mechanical stability, this work presents a unique method of altering XG by grafting an acetyl amine group onto its backbone. The novel aspect is the special synthesis process that uses ammonia and chloroacetyl chloride to functionalize XG at certain locations, improving its physicochemical characteristics.

Because to the enhanced mucoadhesion, viscosity, and gelling qualities provided by the grafted acetyl amine group, MXG may form gels at much lower concentrations than native XG (0.6% vs. 1.2%). Its hydrophilicity and swelling capacity are also improved by this alteration, which improves performance in drug delivery applications.

Although XG has been modified in a number of studies to improve its qualities, the majority of reported changes include hydrophobic or thiolated derivatives, which are intended to

improve features such as stability, mucoadhesion, and viscosity. By grafting acetyl amine groups onto the XG backbone, on the other hand, this work presents a novel alteration that hasn't been widely used in the literature.

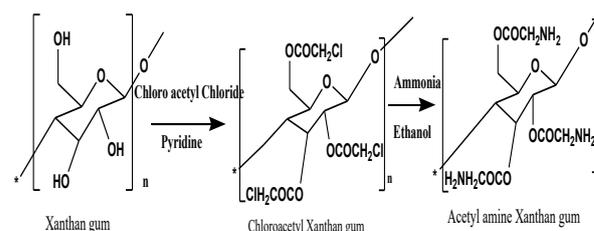
## Materials and Methods

**Materials:** The study's ingredients, which included ammonia solution, xanthan gum, and chloroacetyl chloride, were acquired from Merck in Darmstadt, Germany; Sigma-Aldrich in St. Louis, Missouri, USA; and Loba Chemie Pvt. Ltd. in Mumbai, India. Additionally, rectified spirit was purchased from Merck in Darmstadt, Germany.

### Synthesis

**Preparation of Chloroacetyl Xanthan gum:** 10 g of xanthan gum were dissolved in one hundred milliliters of pyridine to create chloroacetyl xanthan gum, as seen in Fig. 2. 5 mL of chloroacetyl chloride was added to the mixture gradually while being constantly stirred. After two more hours of stirring, the reaction was left to digest overnight. After filtering the resultant combination, the residue was cleaned with rectified spirit and allowed to dry.

**Preparation of acetyl amine xanthan gum:** A 50 mL rectified spirit flask was filled with 10 g of chloro-acetyl xanthan gum. Shaking, the 10 ml ammonia solution was added. The stirring mixture was filtered and cleaned with rectified spirit after two hours. After drying, the solid residue was put to use for other tasks. Fig. 2 provides the schematic presentation [36].



**Figure (2):** Synthesis of both Chloroacetyl and Acetyl amine xanthan gum.

### Characterization

**Fourier Transform Infrared Spectroscopy (FT-IR):** A BRUKER FT-IR alpha ATR spectrometer was used to determine the FTIR spectra for MXG and XG. A little quantity of the powdered material was put directly onto the ATR crystal, and spectra were captured with a resolution of  $4\text{ cm}^{-1}$  in the  $4000\text{--}400\text{ cm}^{-1}$  range. For each sample, thirty-two scans were gathered in order to guarantee precise spectral data. To verify that XG had been successfully modified, characteristic absorption bands that corresponded to the functional groups (hydroxyl, acetyl, and amine) were examined.

**Proton Nuclear Magnetic Resonance ( $^1\text{H-NMR}$ ):** At 300 MHz, a VARIAN MERCURY YH-300 NMR Spectrometer was used to acquire the  $^1\text{H-NMR}$  spectra of MXG and XG.  $\text{D}_2\text{O}$  served as the solvent for both XG and acetyl amine-modified xanthan gum, while TMS served as the internal standard. To examine proton environments and validate the acetyl amine alteration of the XG structure, 0.5 mL of  $\text{D}_2\text{O}$  was used to dissolve around 20 mg of each sample, and the spectra were then recorded.

**Differential Scanning Calorimetry (DSC):** With a TA Instruments Q2000 DSC, the thermal characteristics of XG and MXG were examined. A nitrogen flow of 50 mL/min was used to heat samples weighing around 5–10 mg from  $40^\circ\text{C}$  to  $300^\circ\text{C}$  at a heating rate of  $10^\circ\text{C}/\text{min}$  in aluminum pans. To evaluate the effect of acetyl amine modification on the thermal behavior of

XG, the thermograms were examined to identify thermal transitions, such as melting temperatures and glass transitions.

**X-ray Diffraction (XRD):** A Bruker AXS D8 Advance X-ray diffractometer was used to perform X-ray diffraction studies on XG and MXG utilizing copper K $\alpha$ -radiation ( $\lambda = 1.5406 \text{ \AA}$ ) generated at 35 mA and 40 kV. With a step size of  $0.02^\circ$  and a scan rate of  $2^\circ/\text{min}$ , the diffraction patterns were captured throughout a  $2\theta$  range of  $3^\circ$  to  $60^\circ$ . To determine the crystallinity of MXG in comparison to XG, the XRD data was examined.

**Scanning Electron Microscopy (SEM):** A JEOL JSM-6360LV scanning electron microscope was used to examine the surface morphology and form of XG and MXG. For five minutes, both samples were gold-coated to guarantee conductivity and prevent charge accumulation. To investigate the variations in surface structure and morphology between XG and MXG, electron micrographs were collected at different magnifications using a SEM operating at 10 kV.

### Physiochemical Evaluation

**Evaluation of mucoadhesive strength of gel:** Utilizing a modified physical balance method, the comparative mucoadhesive strength of MXG gel was assessed. After being removed from the slaughterhouse, the goat's buccal mucosa was cleansed to get rid of any loose tissue and underlying fat. Distilled water and simulated saliva with a pH of 6.8 at  $37^\circ\text{C}$  were used to wash the membrane. A standard and test mucoadhesive agent solution (1% w/v) was used to coat the vial with a rubber closure. Nylon thread was used to secure one end of the vial, and a mechanism was included to increase the weight at the other end. To remove the mucous from the glass plate, weight was recorded at certain intervals. The ex vivo bioadhesion time and detachment force were determined by measuring the weight and time needed to separate from the buccal mucosa, respectively. An average of six observations was used to report the outcome [37]. The adhesive force was computed as:

**Viscosity:** Using spindle L4 at 50 and 100 rpm speeds, the viscosity of xanthan gum was measured and adjusted using Brookfield viscometers (Brookfield DV-E Viscometer) [38].

**Gelling Property:** The previously mentioned procedure was used to determine the gelling characteristic. A test tube was filled with a solution of acetylamine XG and XG (0.2–1%, w/v) in water, which was then left overnight. By tilting the test tubes at a  $90^\circ$  angle, the gel's consistency was assessed, and they were classified as solutions, viscous solutions, or gels [36] based on visual examination.

**Gelling Capacity:** Through the use of a solution drop in a vial containing freshly made 2 ml of imitated tear fluid, the gelling capability of XG and MXG was investigated. A visual inspection of the gelling period was conducted [36].

**Swelling Index:** Water absorption of modified xanthan gum was measured by gravimetry. A 30 mg disc with a diameter of 5.0 mm, made using a hydraulic single punch press, was placed on a tiny mesh and submerged in a saliva solution (pH 6.75 at  $37\pm 0.5^\circ\text{C}$ ) in a beaker. The disc was weighed at predetermined intervals. Before taking measurements, extra water was removed from the bulging discs [39]. The following formula was used to determine water uptake:

$$\text{Water uptake (mg)} = \{W_t - W_o\}$$

Where,  $W_t$ =Disc Wt. at given time;  $W_o$ =Initial Wt.

### Toxicological Study

**Acute Oral toxicity:** It was carried out in accordance with OECD norms [40]. To put it briefly, as advised by regulatory

requirements, healthy young adult female albino rats were employed in toxicological experiments due to their uniformity, sensitivity to toxins, and standardization. They were all between the ages of 8 and 12 weeks and weighed  $140 \pm 10 \text{ g}$ . A temperature of  $22 \pm 3^\circ\text{C}$  and a relative humidity of 50% were maintained in the room. Laboratory foods, lots of drinking water, and artificial lighting with 12 hours of light and 12 hours of darkness were all given. Before the trial started, the animals were housed in a lab setting for ten days. Rats were marked for individual identification after being chosen at random for the research. Prior to dosing, the animals were fasted. Charts were used to determine the dosage. For six hours, the test drug was given in smaller doses. Three animals per treatment level were employed in each experimental group. According to the flow charts in Annexure 2, 2000 mg/kg body weight is the chosen dosage level to be employed as the first dose. Following dosing, animals were monitored for the first half hour and then at various points during the first 24 hours. For 14 days, special care was provided during the first four hours and then every day after that. Every observation was methodically documented, and each animal's unique record was kept. Other findings are being documented, including changes in the eyes, mucous membranes, skin and hair, tremors, convulsions, salivation, diarrhea, lethargy, coma, and patterns of behavior, respiration, and circulation. On the day of the test substance's administration, or just before, and at least once per week thereafter, the individual weights of the animals were recorded [41].

**Acute dermal toxicity:** It was carried out in accordance with OECD-434. The young adult female albino rats utilized were healthy, weighing  $140 \pm 10 \text{ g}$  and aged between 8 and 12 weeks. Twenty-four hours into the investigation, test animals were taken out of the dorsal part of the trunk by shaving or trimming their fur. The area used to remove animal hair was determined by the weight of each individual animal. For a whole day, the test sample, non-irritating tape, and a porous gauze dressing were placed to a region that made up around 10% of the body's surface area. To preserve the test sample and the gauze covering, the test site was covered; water was used to remove the remaining sample after exposure. A beginning dosage of 2000 mg/fixd was determined according on the chart. For 14 days, all animals were routinely watched. The animals were watched for the first half hour, especially for the first four hours, and then every day for the next fourteen days. Every observation was methodically documented, with separate files kept for every species. Changes in the respiratory, circulatory, autonomic, and central nervous systems, as well as somatomotor activity and behavioral patterns, skin and hair, eyes, and mucous membranes, are among the other observations being documented. On the day of or right before the test material was administered, as well as at least once per week following that, the individual weights of the animals were measured [42].

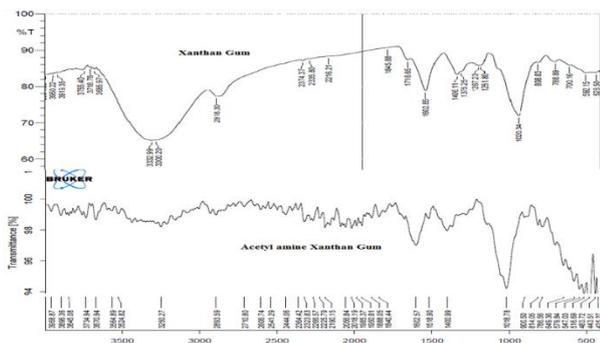
## Results and Discussion

**Synthesis:** It was discovered that acetyl amine xanthan gum had a melting point of  $210^\circ\text{C}$ , chloroacetyl xanthan gum had a melting point of  $165\text{--}170^\circ\text{C}$ , and xanthan gum had a decomposition point of  $220^\circ\text{C}$  shows how the product is formed.

### Characterization

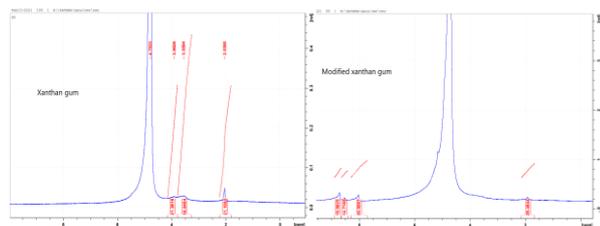
**Fourier transforms infrared spectroscopy:** A BRUKAR FT-IR alpha ATR spectrometer was used to record the FT-IR spectrum analysis of XG and MXG, which is shown in Fig. 3. Due to the varying degrees of bonding between the OH groups, xanthan gum displays a broad band of absorption at  $3332 \text{ cm}^{-1}$ . For CH aliphatic stretching, the peak was found at  $2918 \text{ cm}^{-1}$ . The asymmetric stretching of carboxylate ions and the carboxyl

group stretching of alkyl esters are responsible for the absorption band at 1716 and 1602  $\text{cm}^{-1}$ . The C-O-C of cyclic ether and the CH of methyl show absorption bands at 1020  $\text{cm}^{-1}$  and 1406, respectively, as a result of bending vibrations. Because of the  $\text{NH}_2$  group, MXG shows an absorption band at 3250  $\text{cm}^{-1}$ . When substitution occurs on the hydroxyl group of xanthan gum, the wide shape that results from the OH group transforms into acute spikes. The bending of alkanes and the stretching of CH caused the peak to be seen at 2893  $\text{cm}^{-1}$  and 1400  $\text{cm}^{-1}$ . The stretching of cyclic ether caused the peak at 1018  $\text{cm}^{-1}$  to be seen. The concentration of hydroxyl functions in the XG decreased as a result of a decrease in the strength of the hydroxyl group signal. These modifications aligned with earlier studies by Baig *et al.* on the use of acylation methods to functionalize xanthan gum [43].



**Figure (3):** Fourier transform infrared spectra of Xanthan gum and Modified xanthan gum.

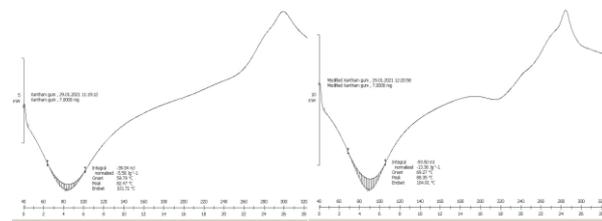
#### Proton Nuclear Magnetic Resonance



**Figure (4):**  $^1\text{H}$ NMR spectra of Xanthan gum and Modified Xanthan gum.

The effective alteration of native xanthan gum was confirmed by the  $^1\text{H}$ NMR study. Fig. 4 displays the XG and MXG spectra. The methyl ( $-\text{CH}_3$ ) protons of the acetate group in XG are represented by major peaks around  $\sim 2$  ppm in the  $^1\text{H}$  NMR spectra of Xanthan gum (XG) and modified xanthan gum (MXG). In MXG, there is a possible signal near  $\sim 4$  ppm that could represent  $-\text{OCH}_2\text{-Cl}$  protons from modification. Protons next to electronegative elements like chlorine seem closer to  $\sim 4$  ppm, whereas methyl groups near electron-withdrawing groups resonate at  $\sim 2$  ppm. These changes are consistent with predicted chemical environments. This implies that XG and MXG differ structurally, especially in terms of functional group changes.

#### Differential Scanning Calorimetry (DSC)

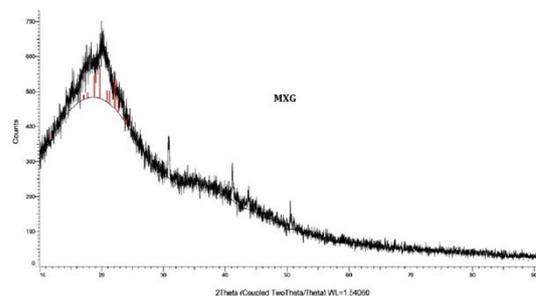


**Figure (5):** DSC of Xanthan gum and Modified Xanthan gum.

The thermogram of MXG has an endothermic peak at 88.35 $^{\circ}\text{C}$ , while that of XG displays a wide endotherm at 82.47 $^{\circ}\text{C}$ , according to the DSC spectrum. Fig. 5 displays the DSC curve

for amorphous material, which is comparable to that of XG. An additional endothermic peak at 220 $^{\circ}\text{C}$  was noted in the case of MXG, which could be the result of the change.

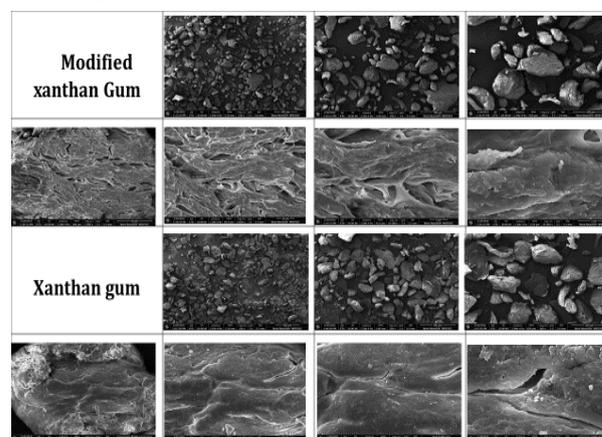
#### X-ray diffraction



**Figure (6):** X-ray diffraction spectra of Modified xanthan gum.

The results of the X-ray examination and the DSC investigation were consistent. An uncommon crystalline type diffractogram is shown in the XRD spectra of MXG in Fig. 6, with the characteristic peak showing up at  $2\theta$  equal to 19 $^{\circ}$ , 30 $^{\circ}$ , 41 $^{\circ}$ , and 51 $^{\circ}$ .

#### Scanning Electron Microscopy



**Figure (7):** SEM photographs of Modified Xanthan gum and Xanthan gum.

While MXG has a spongy, rough surface, the SEM images of XG in Fig. 7 reveal a smooth surface with a polyhedral structure. The original xanthan gum's size and shape changed after polydactyl amine was substituted on xanthan, according to MXG's SEM.

#### Physicochemical Evaluation

**Mucoadhesion property:** While xanthan gum (a plain polymer) demonstrated 0.0133N adhesion force to separate the plate in reaction to test material towards the mucus in 16.83 seconds, the modified polymer demonstrated 0.0703 N adhesion force to separate the 1% w/v coated plate from the mucus in 33.91 seconds. The detachment of the modified polymer had a water weight of 14.33, whereas that of xanthan was 2.74. According to this finding, xanthan gum treated with acetylamine shows the longest detachment time and the highest water weight for detachment. Compared to regular xanthan gum, it takes the most force. These results align with previous studies by Patel *et al.* that shown enhanced mucoadhesive qualities of modified xanthan gums (Fig. 8) [44].

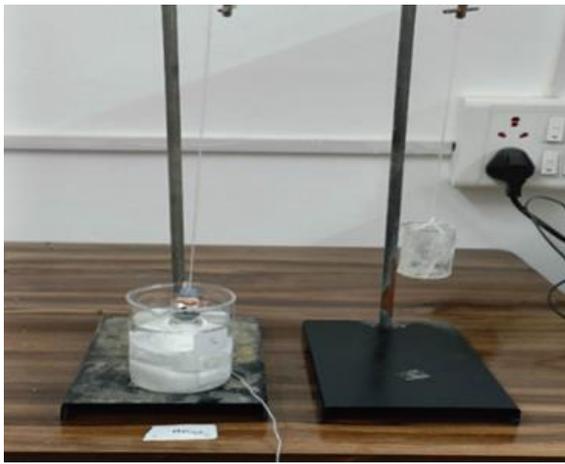


Figure (8): Shear stress (in Newton) measurement of Modified polymer.

The main component of mucus is mucin, also known as mucous glycoprotein, which includes surface residues that are negatively charged. As a result, the cationic primary amine group of xanthan gum and the negatively charged mucus interact ionotically, giving the gum better mucoadhesive qualities than ordinary xanthan gum. Primary amines in xanthan gum are positively charged because the pH is lower than their pKa (9–10), but mucins in mucus are negatively charged because of deprotonated acidic groups (pKa 2-3) at physiological pH (~6.8). Strong ionic interactions are made possible by these charges, which improve modified xanthan gum's mucoadhesion [45].

**Viscosity:** As seen in Fig. 9, the acetyl amine grafting on xanthan gum changed its viscosity. Aqueous solutions of XG and MXG show pseudo-plastic flow characteristics, which means that the fluid flows more readily when it is agitated or forced harder. Nevertheless, MXG had higher viscosity. It was shown that decreased speed and temperature, together with increasing polymeric concentration, enhanced the viscosity of XG and MXG. However, the cationic amine character (acetyl amine group) that MXG contributes to its backbone chain may be the cause of its increased viscosity. This is because it reduces coulombic repulsion and increases the participation of the backbone chain.

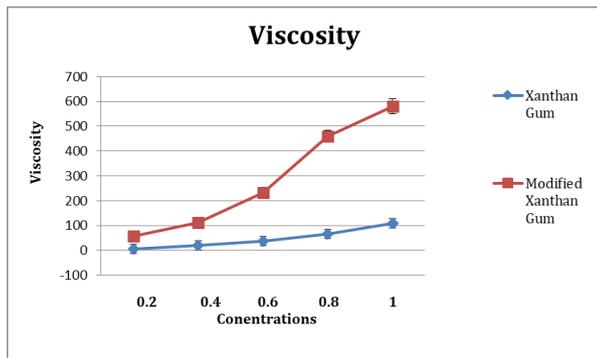


Figure (9): Comparative viscosity study of Xanthan gum and Modified Xanthan gum. Illustration values are the mean of three experiments  $\pm$ SD.

**Gelling Property and Gelling Capacity Study:** The gelling property and gelling capacity findings are compiled in Table 1. The gelling property of MXG is doubled compared to that of the parent XG. While MXG stayed the same for over 12 hours, the 1% gel made from xanthan gum was dispersed throughout the fluid in about one hour.

Table (1): Comparative Gelling Property and Gelling Capacity study of Xanthan gum and Modified xanthan gum.

Parameters		XG	MXG
Gelling property (% w/v)	Solution	0.2	0.2
	Viscous	0.8	0.4
	Gel	1.2	0.6

Parameters		XG	MXG
The gelling capacity of the solution	Less than 1 hrs	1	0.6
	Less than 8 hrs	-	0.8
	More than 8 hrs	-	0.9
	More than 12 hrs	-	1

**Swelling Index:** By tracking variations in the disc's weight over time, swelling tests were carried out. Within 10 minutes, MXG displayed a disc expanding weight of 0.6 gm, whereas XG displayed a weight of 0.3 gm. Because of the strong hydrophilicity and capillary action of the MXG surface, water was absorbed by MXG during the swelling process at a very rapid and consistent rate, as seen in Fig. 10. The SEM picture shows that the surface of MXG is spongy and rough. These networked pores played a key role in encouraging rapid swelling and efficient water retention. A polymer's swelling degree indicates how effectively it can absorb biological fluids or water, which has an immediate effect on its porosity and structure. The amount of drug that may be implanted in the polymer matrix is determined by this characteristic, which affects drug loading. By controlling the drug's diffusion rate as the polymer swells and forms pathways for the drug to depart, it also regulates drug release.

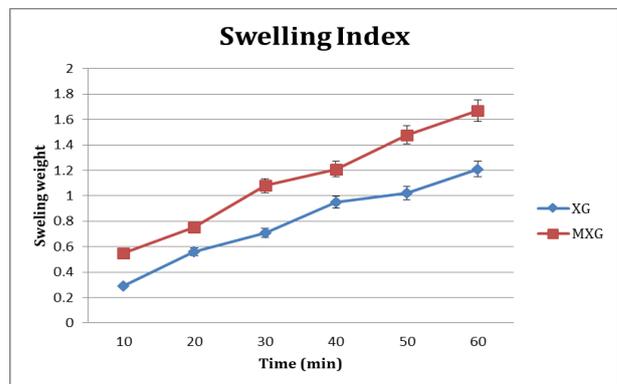


Figure (10): Swelling behavior of Xanthan gum and Modified xanthan gum in simulated saliva solution, Illustration values are the mean of three experiments  $\pm$ SD.

### Toxicological study

**Acute Oral Toxicity:** Due to its low or negligible harmful effects, xanthan gum is typically regarded as safe for use in food applications. Modified xanthan gum's acute oral toxicity was investigated using OECD guideline 423. When choosing the dosage, 2000 mg/kg weight from the Annex. 2c flow chart was taken into account. Three group animals did not exhibit any mortality at this dosing level. MXG has less acute toxicity than xanthan gum, as evidenced by the fact that the modified xanthan gum's Lethal amount, 50% (LD<sub>50</sub>), which is the amount of a chemical that causes death in 50% of a test population, was greater than 2000 mg/kg. These results are in line with research on xanthan gum compounds by Kumar et al., which shown little toxicity at large dosages [46].

Table (2): Toxicological Results and Indicators of Toxicity.

Indicator	Observation	Negative Response (Yes/No)
Eyes and Mucous Membranes	No irritation or discoloration.	No
Tremors	No tremors observed.	No
Convulsions	No convulsions detected.	No
Salivation	No excessive salivation.	No
Diarrhea	No signs of diarrhea.	No
Lethargy	No signs of lethargy.	No
Coma	No signs of coma.	No
Respiratory Distress	No breathing difficulties.	No
Circulatory Disturbances	No circulatory issues.	No
Behavioral Changes	Normal activity observed.	No
Eyes and Mucous Membranes	No irritation or discoloration.	No

**Acute dermal Toxicity:** MXG's acute cutaneous toxicity was also assessed using OECD guideline 434. At the 2000 mg/kg dosage level, no mortality was seen when the gel was administered to the rat's skin. The eyes, fur, and skin were all unchanged. The animal did not exhibit other symptoms including tremors, convulsions, salivation, diarrhea, drowsiness, or coma.

**Acute dermal Toxicity:** MXG's acute cutaneous toxicity was also assessed using OECD guideline 434. At the 2000 mg/kg dosage level, no mortality was seen when the gel was administered to the rat's skin. The eyes, fur, and skin were all unchanged. The animal did not exhibit other symptoms including tremors, convulsions, salivation, diarrhea, drowsiness, or coma.

## Conclusion

Chloroacetyl chloride was applied to xanthan gum in a basic environment, followed by a reaction with ammonia to effectively conjugate the acetyl amine group on xanthan gum. FTIR, <sup>1</sup>H-NMR, DSC, XRD, and SEM spectra validated the characterization of the alteration. The spectra that were acquired show that XG was successfully modified. XG grafted with acetylamine exhibited better physicochemical characteristics than normal XG. Because of the cationic acetylamino group on the backbone of XG, MXG was shown to have improved viscosity and mucoadhesion. When compared to plain polymer, the findings of ex vivo bioadhesion experiments show a considerable increase in mucosal detachment time, suggesting that acetylamine-grafted polymer exhibits more mucoadhesion. XG needs a 1.2% concentration, while MXG from gel requires a 0.6% concentration. The gelling capability was also enhanced, as seen by the 1% gel of MXG staying in the solution for almost 12 hours. Because MXG has a greater hydrophilicity and capillary action, it absorbs water very quickly. More mucoadhesion qualities were indicated by the force of MXG detachment being larger than XG. MXG's LD<sub>50</sub> value was greater than 2 g/kg, suggesting reduced oral toxicity; no alterations were seen following skin application, suggesting dermal application safety. As a result, this grafted polymer may be well suited as a possible delivery mechanism for a variety of oral and topical medications. To further increase its therapeutic potential, future studies might investigate the creation of MXG-based formulations for mucosal therapy, wound healing, and targeted drug administration. Its future conversion into medicinal uses would also depend on studies into its long-term safety and effectiveness in clinical settings.

## Disclosure Statement

- **Ethics approval and consent to participate:** The Research Ethics Committee of GIPER, Limb, Satara, Maharashtra, India, authorized all experimental methods in this work in accordance with the Institutional Animal Care standards of NC3Rs (ethical code: GIPER/IEC/2020-21/03 dated 27/01/2021). The "Principles of laboratory animal care" (NIH publication No. 85-23, amended 1985) and, where relevant, particular national legislation were complied with, according to all authors. The relevant ethics committee has reviewed and approved each experiment.
- **Consent for publication:** Not applicable
- **Availability of data and materials:** The raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
- **Author's contribution:** The authors confirm contribution to the paper as follows: study conception and design: Singla NS, theoretical calculations and modeling: Singla NS; data analysis and validation, Patil MV. Draft manuscript preparation: Patil MV. All authors reviewed the results and approved the final version of the manuscript.

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