

# Extraction and Characterization of Aloe Glucomannan: Assessing Its Flocculation Capability

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## Abstract

Acemannan, a highly acetylated glucomannan, was extracted from fresh *Aloe vera* leaves by ethanol fractionation, resulting in a concentration increase of more than threefold. The presence of acemannan was confirmed using FTIR and <sup>1</sup>H NMR analysis, revealing an average molecular weight of 780 kDa. The flocculating activity of the fractionated aloe gel polysaccharide was assessed through settling tests in a 1% (w/v) bentonite suspension. The results demonstrated that the aloe polysaccharide exhibited remarkable stability within a temperature range of 20–70 °C. The maximal flocculation rate at different pH levels ranged from 93% to 97%, with an optimal dose for maximum flocculation rate between 0.25 mg/mL. Notably, the minimum dose required for flocculation was achieved at a pH of 3, attributed to the compression of electrostatic repulsion on the surface of bentonite particles. However, the flocs obtained under acidic conditions were less dense and compact, exhibiting lower sedimentation velocity compared to those formed under neutral and alkaline pH conditions. Additionally, the addition of salt showed a slight synergistic effect on flocculation, significantly enhancing the sedimentation velocity. This investigation highlights the potential of *Aloe vera* polysaccharide as a natural and edible flocculant, offering promising applications in various industries.

**Keywords:** *Aloe vera* gel, Acemannan, Bioflocculant, Polysaccharide

## 1. Introduction

*Aloe vera*, a highly versatile plant, is renowned for its rich composition of over 200 different substances, with polysaccharides being among its key functional components[1]. Acetylated polysaccharides, such as acemannan, play a crucial role as major biofunctional components among various polysaccharides[2]. While *Aloe vera's* polysaccharides have been linked to various health benefits, their physical properties and functionalities in the realm of chemistry remain relatively underexplored when compared to polysaccharides derived from other sources.

Soluble polysaccharides have found widespread applications in various industrial applications, serving as essential viscosity modifiers, emulsion stabilizers, coagulants, drug delivery agents, adsorbents, dispersants, stabilizers, water-soluble polymer coatings, adhesives[3] and even as a corrosion inhibitor[4]. As such, it is reasonable to expect that aloe polysaccharides may also exhibit a diverse range of physical functionalities, yet limited research has been conducted in this area. Previous studies have explored the coagulation properties of aloe, with a specific focus on the correlation with glucomannan content[5,6]. However, comprehensive investigations into the coagulation properties

and mechanisms of aloe polysaccharides, particularly using sedimentation velocity analysis, remain scarce and warrant further examination.

Recently, there has been growing interest in *Aloe vera* substances as natural coagulants or bioadsorbents for water and wastewater treatment. Studies have evaluated the effectiveness of aloe gel powder and its solution as flocculants for surface water purification and improving drinking water quality by replacing metal-based coagulants[7,8]. *Aloe vera* gel solution has also shown promise in reducing initial turbidity in drinking water[9]. Additionally, *Aloe vera* has been utilized as a natural coagulant for treating palm oil mill effluent[10]. Moreover, research on aloe vera gel extracts for wastewater treatment has demonstrated significant reductions in turbidity, total suspended solids, and COD removal[11]. However, most of these studies have focused on the phenomena rather than identifying coagulation-active components and the flocculation mechanism, as well as limited knowledge about sedimentation rate theory and underlying coagulation mechanisms, suggesting the need for further investigation.

The objective of this study was to isolate and purify polysaccharides from aloe gel and characterize using instrumental analysis. Subsequently, we analyzed bentonite coagulation by the polysaccharides, focusing on flocculation rate and sedimentation velocity. Additionally, we conducted an examination of the influence of pH, cation addition, and thermal treatment on flocculation efficiency, aiming to ascertain the coagulation characteristics of aloe polysaccharides as potential biobased coagulants.

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## 2. Materials and methods

### 2.1. Preparation of aloe polysaccharide

The *Aloe vera* used in this study was sourced from the Jeju farm of KJM Corporation (Seoul, Korea) through domestic cultivation. Fresh aloe vera leaves were provided by the company and stored at 4 °C in a cold room until the commencement of experiments. To prepare the inner gel, the outer layer of aloe leaves was carefully peeled off, and the remaining gel was homogenized using a blender (EBR400, Electrolux, Sweden). The homogenized gel underwent centrifugation at 10,000 rpm for 30 minutes to separate the fibrous components. The resulting supernatant was collected and mixed with four volumes of cold ethanol, allowing it to stand overnight at 4 °C. The precipitates were collected and dissolved in distilled water and subjected to dialysis using a cellulose membrane dialysis bag (MWCO: 12400) against deionized water for 24 hours to remove low molecular weight substances. After the dialysis process, the sample was freeze-dried and finely powdered (200 mesh).

### 2.2. Characterization of aloe polysaccharides: NMR, FTIR, and O-acetyl analysis

To characterize the aloe polysaccharides, a 10 mg sample was dissolved in 0.7 mL of D<sub>2</sub>O. <sup>1</sup>H NMR spectra were recorded at room temperature using a Bruker Model Advance 600 (Karlsruhe, Germany). Additionally, FTIR spectra were acquired using a Bio-Rad Model Excalibur instrument (Cambridge, USA) with a resolution of 4 cm<sup>-1</sup>, covering the range of 500–4000 cm<sup>-1</sup>. The glucomannan content was quantified using the Congo-red method[12], and the O-acetyl content was determined according to the Hestrin method using acetylcholine as the standard solution[13].

### 2.3. Preparation of bentonite clay and flocculation test

The clay used for the flocculation test was bentonite (Daejung Chemicals & Metals Co. Ltd., Siheung, Korea), with montmorillonite as the major mineral component. The bentonite particles had a size of 200 mesh, and the swell ratio was measured to be 10–16 times of its original volume. The moisture content of the bentonite was determined to be within the range of 8–14%. Clay suspensions were prepared by mixing bentonite powder with distilled water at the pre-determined volume, yielding a concentration of 10 g/L. The mixture was thoroughly dispersed, and the large clay particles that immediately precipitated were removed, resulting in homogeneously dispersed suspensions used for the experiments.

The flocculation rate was determined using bentonite suspension [14]. In the experimental setup, 8 mL of 10 g/L bentonite suspension was mixed with 2 mL of the sample solution in a test tube. The mixture was then vigorously stirred using a Vortex mixer for 5 seconds and left to settle for 30 minutes. At specific time intervals (3, 5, 10, and 30 minutes), the absorbance of the supernatant was measured at 550 nm. The analysis was performed in triplicate, and the flocculation rate (%) was calculated using the following formula:

$$\text{Flocculating rate (\%)} = [(B - A) / B] \times 100$$

Here, A and B represent the absorbance of the sample and blank at 550 nm, respectively. In a sedimentation test, the height of the clear interface resulting from the sedimentation was measured as a function of time for all experiments where visible interfaces occurred due to flocculation. The final measurement was taken at 30 minutes. The initial sedimentation velocity (cm/min) was determined by dividing the rate settling with time as the interface height changed linearly over time.

### 2.4. Flocculate morphology

The morphology of the flocculum was observed using an image analysis system and scanning electron microscopy (SEM). The images were captured using the image analysis system connected to a PC with an image-capturing board (Optimas Co., USA). Specifically, 1 mL of the flocculate sample was randomly taken and placed in a glass tube (4 × 3 × 1.5 cm) without fixation. The images were then captured by the CCD camera with a resolution of 640 × 640 pixels and 256 grey levels. To minimize errors, the resolution was adjusted to 400 × 400 pixels.

## 3. Results and discussion

### 3.1. Solubility fractionation of highly acetylated aloe glucomannan

Fresh aloe gel was homogenized and centrifuged to remove insoluble fibers. It was then fractionated using four volumes of cold ethanol at 4 °C. The resulting precipitates were collected, dissolved in distilled water, dialyzed, and freeze-dried. The fraction exhibited a glucomannan content of 508.0 ± 36.0 mg/g dry solid, and an O-acetyl content of 307.8 ± 32.9 mg/g dry solid. These values were 3.5 and 3.3 times higher, respectively, compared the glucomannan content (145.9 ± 5.2 mg/g) and O-acetyl content (93.1 ± 3.3 mg/g) found in aloe gel.

The FTIR spectrum of the fractionate (Figure 1(a)) displayed broad peaks at 3380 cm<sup>-1</sup> (O-H stretching), at 1620 cm<sup>-1</sup> (O-H bending), at 1050 cm<sup>-1</sup> (C-O stretching), and at 2920 cm<sup>-1</sup> (C-H stretching), typical polysaccharides. Additional peaks at 1748–1735 cm<sup>-1</sup> and 1230 cm<sup>-1</sup> indicate C=O stretching and C-O stretching in the O-acetyl group. The <sup>1</sup>H NMR spectroscopy (Figure 1(b)) confirms the presence of acetylated polysaccharides with signals of hydrogens of polysaccharide at 3–4 ppm and acetyl hydrogen at 2.0–2.2 ppm. The gel permeation chromatogram (Figure 1(c)) depicted a single broad peak at an elution time 25.8 minutes and sharp peaks of small molecules beyond the low molecular weight cutoff at elution times of 40–43 minutes. Consequently, the average molecular weight of the aloe gel polysaccharide was approximately 780 kDa. The TGA result (Figure 1(d)) indicated that weight loss occurring in two stages at around 200–630 °C, with the major DTG peaks at 55 °C and 338 °C, corresponding to moisture evaporation and the decomposition temperature range of acemannan.

Taken together, the results reveal that the fractionate contains a highly O-acetylated glucomannan. It is known that aloe gel poly-

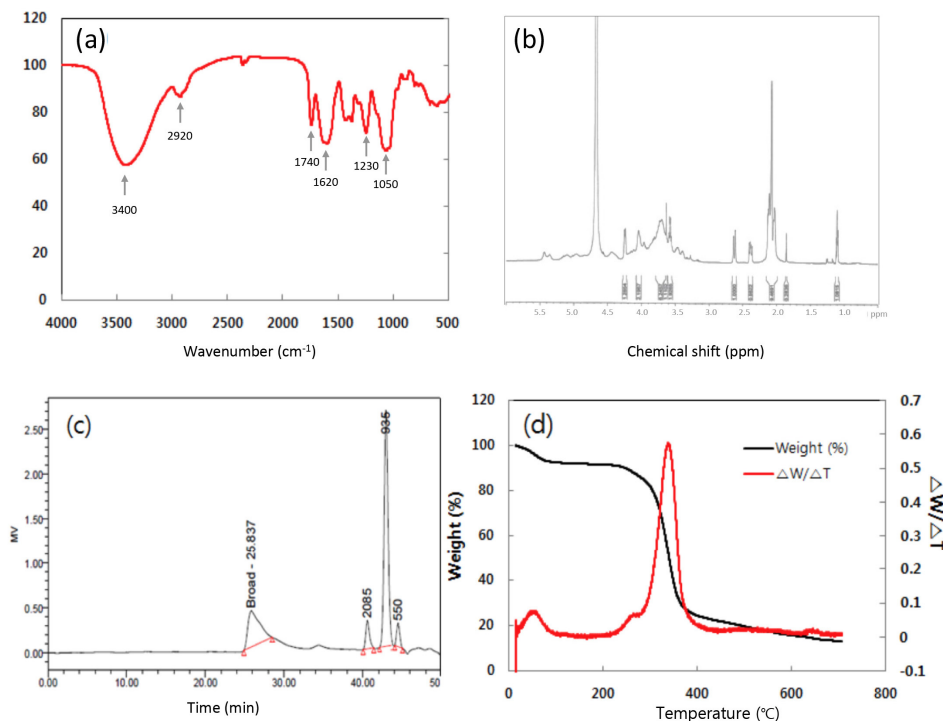


Figure 1. FTIR spectrum (a), <sup>1</sup>H NMR spectrum (b), gel permeation chromatogram (c), and TGA-DTG curve (d) of the fractionated acemannan polysaccharide.

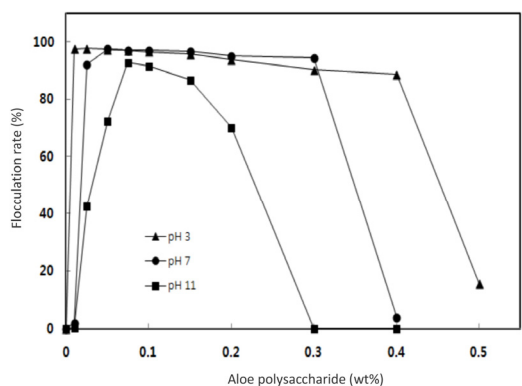


Figure 2. Flocculation rate of 1% bentonite suspension at different pH and varying acemannan polysaccharide levels.

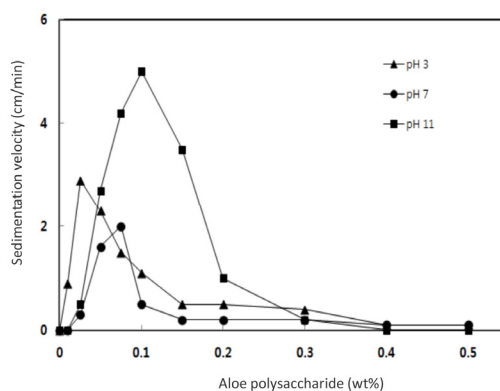


Figure 3. Impact of pH and the acemannan concentrations on initial sedimentation velocity of 1% bentonite suspension.

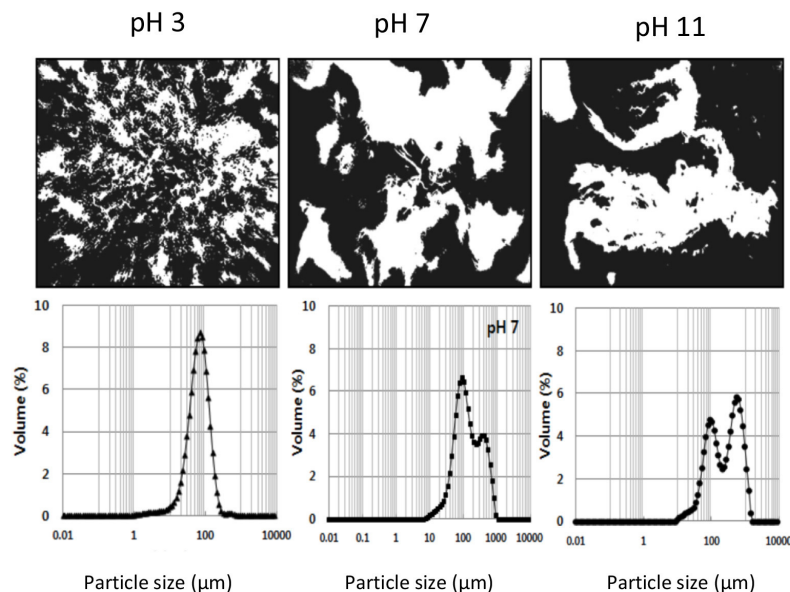
saccharides consist of mucopolysaccharide acemannan, which is an acetylated glucomannan with a backbone of  $\beta$ -1,4-linked mannose units, interspersed by glucose and partially acetylated. However, the degree of O-acetylation varies based on harvesting time and cultivation method. In this study, we present a simple fractionation method that successfully enriches acemannan from aloe gel, demonstrating its potential for enhancing the acemannan content.

### 3.2. Flocculating properties of the fractionated polysaccharide

The evaluation of the flocculating properties of the fractionated acemannan at different pH levels and concentrations revealed significant differences in the flocculation rate and sedimentation velocity (Figure 2). At pH 11, the addition of acemannan significantly increased the

flocculation of bentonite solution, reaching a maximum flocculation efficiency of approximately 93% at a concentration of 0.75 mg/mL. However, efficiency decreased rapidly when the concentration exceeded 1.5 mg/mL. At pH 7, the flocculation efficiency showed a plateau, with more than 95% maintained from 0.5 to 3 mg/L of acemannan concentration. However, beyond this concentration range, the efficiency declined rapidly. At pH 3, the flocculation efficiency exhibited a prolonged plateau, maintaining levels of more than 90% efficiency within an extended concentration range from 0.25 mg/mL to 4 mg/mL of acemannan.

Figure 3 illustrates the effect of acemannan polysaccharide concentration and pH on the initial sedimentation rates. The graph showcases how varying concentrations and pH levels impact the rate at which



**Figure 4. pH and polysaccharide effects on floc morphology and size distribution of the coagulation suspensions of 1% bentonite.**

particles settle during the flocculation process. The settling rates showed notable differences depending on the polysaccharide concentration after the first 1~2 minutes. Notably, the initial flocculation rate, determined from the linear slope, was found to be higher at alkaline pH and lower at pH 3 and 7. Furthermore, the initial flocculation rate demonstrated a highly sensitive dependence on polysaccharide concentration at each pH, resulting in sharp flocculation rate-concentration curves. Interestingly, the concentration associated with the maximum flocculation rate generally fell within the range of concentrations linked to the maximum flocculation efficiency. This observation suggests a strong correlation between the maximum initial flocculation rate and the maximum flocculation efficiency, underscoring the importance of investigating flocculation characteristics, just as studying flocculation efficiency.

On the other hand, Figure 4 represents the flocculum's form, size, and distribution as a function of pH. Consequently, flocculum size generally increased with increasing pH. Furthermore, the flocculum size distribution was unimodal at pH 3, whereas at pH 7 and 11, it showed bimodal distributions with peaks at 100 and 1000  $\mu\text{m}$ , with a larger flocculum distribution observed at pH 11. The flocculum exhibited long chain or mesh-like structures, where higher pH led to the confinement and connection of physically smaller particles, forming larger floccula.

The study demonstrates that an optimal concentration exists for achieving the highest flocculation efficiency at each pH level, aligning well with the importance of pH and concentration being the most crucial variables[15,16]. At low polysaccharide concentrations, the decrease in flocculation efficiency can be attributed to the limited number of aggregation sites available for effective particle binding. Conversely, at high concentrations, re-dispersion occurs, leading to reduced flocculation efficiency[17]. Additionally, increased viscosity at high concentrations is proposed to hinder the flocculation process[18].

It was also found that Alkaline pH 11 showed the highest efficiency and require higher polysaccharide concentration for maximum initial sedimentation rate. Since initial aggregation is typically associated with the maximum available polymer with adsorption capacity, pH could affect the negative surface of bentonite particles. It was suggested that alkaline pH leads to negatively charged broken-edge surfaces, causing low flocculation efficiency due to electrostatic repulsion between surfaces[19].

Furthermore, the maximum flocculation efficiency of the present polysaccharide sample (0.25~0.75 mg/mL) is relatively lower compared to other biological flocculants. For instance, it was reported that extracellular polysaccharide CBF-F26 achieved over 97% maximum efficiency at concentrations above 12 mg/mL[18]. Similarly, increasing flocculation efficiency up to 10~30 mg/L DYU500 concentration was observed[14], decreasing at higher concentrations. Other studies also reported the highest efficiencies at specific concentrations[15,20,21], similar to the present polysaccharide results.

Size distribution of the flocculum is similar to pullulan-based graft copolymers, where a unimodal distribution with smaller size and narrower coagulum size distribution, attributed to cationic samples, suggests neutralization as the main mechanism of particle coagulation. Kaolinite coagula formed through edge-face association are larger than those formed through face-face association[22]. Thus, for bentonite, the larger flocculum size observed in alkaline solutions can be attributed to edge-face association. In contrast, the bimodal distribution with two larger size peaks indicates that some particles undergo coagulation through cross-linking, with grafted chains participating in inter-particle association, resulting in larger floccula[23]. Therefore, at low pH, the unimodal distribution mainly suggests neutralization as the primary mechanism, while at neutral and alkaline pH, coagulation occurs through cross-linking.

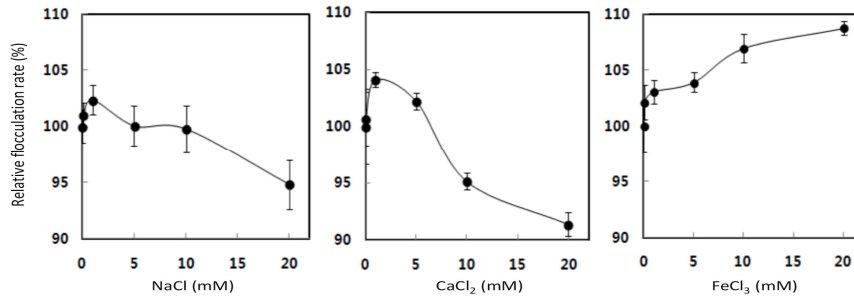


Figure 5. Cation influence on aloe polysaccharide-initiated flocculation of 1% bentonite suspension.

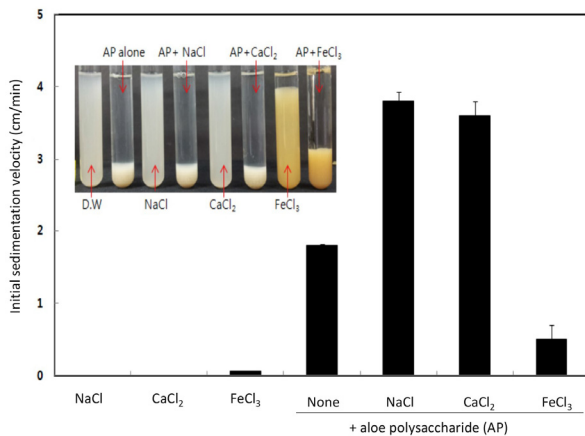


Figure 6. Cation effects on initial sedimentation velocities of 1% bentonite suspension with and without aloe polysaccharide.

### 3.3. Effect of metal ions on flocculating activity

The impact of cations on the flocculating activity of the acemannan fractionate was investigated (Figure 5). The results demonstrated that the flocculating activity appeared to decrease with increasing levels of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  cations. However, enhanced flocculation results were observed at low levels of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and with increasing concentrations of  $\text{Fe}^{3+}$ . The relative flocculation rate exceeding 100% indicates a further clarification of the background turbidities of acemannan and bentonite. Notably, when  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Fe}^{3+}$  were supplemented alone to the bentonite test suspension, no significant changes were observed, except for a slight increase in turbidity caused by  $\text{Fe}^{3+}$  (data not shown).

Additionally, the initial sedimentation velocity during the first 5 minutes was examined (Figure 6). The supplementation of  $\text{Na}^+$  (1 mM) and  $\text{Ca}^{2+}$  (2 mM) doubled the initial sedimentation velocities, while  $\text{Fe}^{3+}$  (20 mM) significantly reduced them, compared to that without cation supplementation. Hence, mono- and divalent cations had a more pronounced effect on the sedimentation rate rather than enhancing the flocculation efficiency. However, despite the initial sedimentation rate being affected, overall flocculation was enhanced by the presence of  $\text{Fe}^{3+}$  supplementation.

Cations are generally known to increase the initial adsorption of coagulants to suspended particles by neutralizing the residual charge of particles and coagulants [24]. The precipitation behavior of  $\text{Fe}^{3+}$  in clay

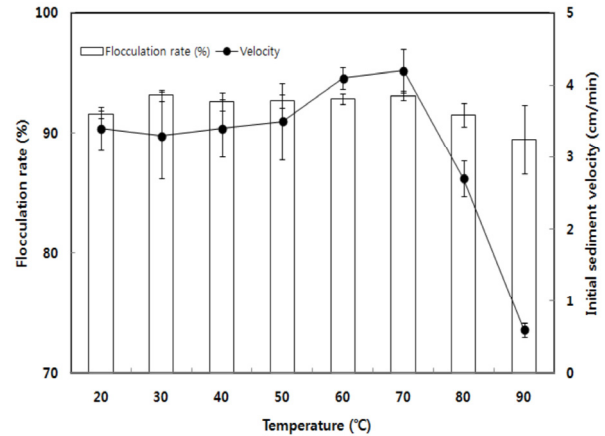


Figure 7. Temperature impact on flocculation rates and initial sedimentation velocities of 1% bentonite suspension with the aloe polysaccharide.

suspensions has been quantitatively explained by the DLVO theory [25]. Therefore, the effect of adding  $\text{Fe}^{3+}$  to the bentonite suspension in this experiment can be attributed to the strong repulsion caused by the electrical double layer around the bentonite particles. As the concentration of  $\text{Fe}^{3+}$  increases, the electrical double layer is compressed, leading to the coagulation and sedimentation of dispersed suspensions, resulting in a higher aggregation efficiency.

Overall, the study suggests that cations have varying effects on the flocculating activity of the acemannan fractionate, with  $\text{Fe}^{3+}$  demonstrating an enhancing role in overall flocculation efficiency despite its impact on initial sedimentation rates. These findings contribute to a deeper understanding of the flocculation process and can aid in optimizing flocculation strategies for various applications.

### 3.4. Effect of heat treatment on aggregation properties

The impact of heat treatment on the flocculating property was investigated by subjecting the polysaccharide to temperatures ranging from 20 °C to 90 °C for 30 minutes. Figure 7 showed no significant changes in the flocculation efficiency and the initial sedimentation velocity within this temperature range. However, the initial sedimentation rate exhibited an increase of approximately 20% from 3.3–3.5 cm/min at 20–50 °C to 4.1–4.2 cm/min at 60–70 °C, before sharply decreasing after reaching 80 °C, with a final rate of 0.6 cm/min at 90 °C. This

finding suggests that the acemannan fractionate, like other carbohydrate coagulants, exhibits relatively stable characteristics under heat.

Generally, carbohydrate-protein coagulants are known to be thermally unstable due to protein structure denaturation[14]. However, polysaccharides tend to exhibit structural stability even at relatively high temperatures. Polysaccharides in aloe gel are degraded by aloe's own enzymes at low-temperature ranges (40–60 °C) during heat treatment, while thermal decomposition occurs at high-temperature ranges (80–90 °C), making 70 °C the temperature that offers maximum stability[26]. Similarly, the structural changes such as deacetylation and decomposition of galactose residues of acemannan occurred during low-temperature sterilization (65–85 °C, 15 or 25 minutes) of acemannan, with significant effects observed at 85 °C[27].

In general, polymer coagulants are classified as anionic, cationic, or nonionic, and can be synthetic or natural polymers. Synthetic polymers are preferred for their lower dosage requirements for effective coagulation, but they may contain toxic monomers from synthesis and additives. Conversely, natural polymers are known for their non-toxicity, biodegradability, and good stability under shear stress[23]. Extracellular polysaccharides, particularly as biological coagulants, are gaining attention due to their non-toxic and biodegradable nature.

This study focused on highly acetylated aloe glucomannan, a polysaccharide component in *Aloe vera*, and its potential as a flocculant. The fractionation process enriched the glucomannan content significantly. The polysaccharide showed efficient flocculating properties at specific pH levels and concentrations. Metal ions and heat treatment influenced its flocculation activity. Overall, highly acetylated aloe glucomannan holds promise as a versatile and stable flocculant for industrial applications, warranting further exploration in solid-liquid separation processes.

#### 4. Conclusions

This study investigated the isolation and flocculating properties of highly acetylated aloe glucomannan, a significant polysaccharide component found in *Aloe vera*. The fractionation process successfully enriched the glucomannan content by 3.5 times and the O-acetyl content by 3.3 times compared to aloe gel. Characterization techniques, including FTIR, <sup>1</sup>H NMR, gel permeation chromatography, and thermogravimetric analysis, confirmed the presence of highly O-acetylated glucomannan with an average molecular weight of approximately 780 kDa.

The evaluation of flocculating properties revealed an optimal concentration for maximum flocculation efficiency at each pH level. Additionally, metal ions showed varying effects on the flocculating activity of the acemannan fractionate, with Fe<sup>3+</sup> enhancing overall flocculation efficiency despite its impact on initial sedimentation rates. Moreover, heat treatment had no significant effect on aggregation efficiency but significantly impacted the sedimentation rate, with a substantial decrease observed at high temperatures (above 80 °C).

The results suggest that highly acetylated aloe glucomannan has promising potential as an edible flocculant. Its unique properties and stability under heat treatment make it an attractive candidate for use

in processes that require solid-liquid separation and aggregation control. However, further research is warranted to fully understand the underlying mechanisms governing the flocculation process of aloe polysaccharides and to explore their applications in diverse industrial settings.

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