

Rheology and Thermal Studies of Stearoyl Chitosan Varying the Degree of Substitution

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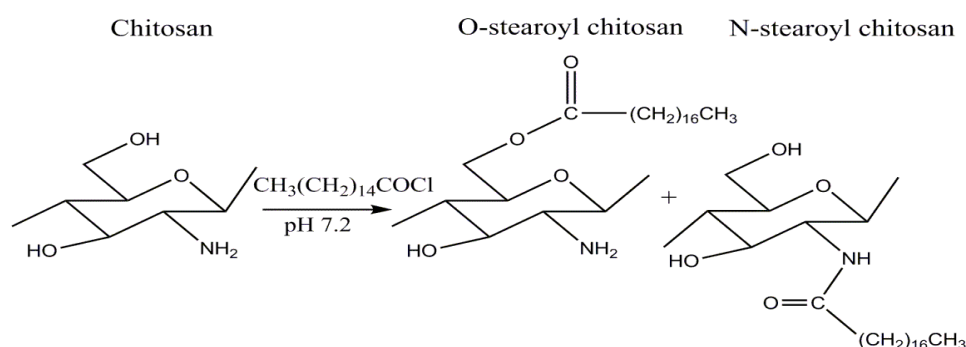
Abstract: Two compounds of stearoyl chitosan; degree of substitution (DS) 79% and DS 86%, were synthesized by using the starting material as chitosan and deacetylated chitosan respectively. Fourier transform infrared (FT-IR) and Nuclear Magnetic Resonance (¹H NMR) spectroscopy were used to confirm the chemical structure of prepared products. The thermal stability of stearoyl chitosan decreased with increased DS while the DO (degree of order) increased with increased DS. The 2% (w/v) stearoyl chitosan (DS 86%) solutions displayed shear thinning behavior. Also, the shear stress and viscosity values were higher for stearoyl chitosan dissolved in pyridine than in toluene at the same shear rate. Since 2% stearoyl chitosan dissolved in pyridine had high viscosity with shear thinning property, it can be suitable as rheology modifiers.

Keywords: acylation, deacetylation, rheology, stearoyl chitosan, thermal stability

I. Introduction

Chitosan, cationic copolymer of D-glucosamine and N-acetylglucosamine, is a natural linear biopolyaminosachcharide. It has an amphiphilic character due to the presence of hydrophilic amino groups and hydrophobic acetamide groups in its molecular structure. Due to its non-toxicity, biodegradability, biocompatibility, low immunogenicity, antimicrobial activity, physiochemical, and biological properties, it can be used in various fields. But the poor solubility in water and most common organic solvents limits its applications [1, 2].

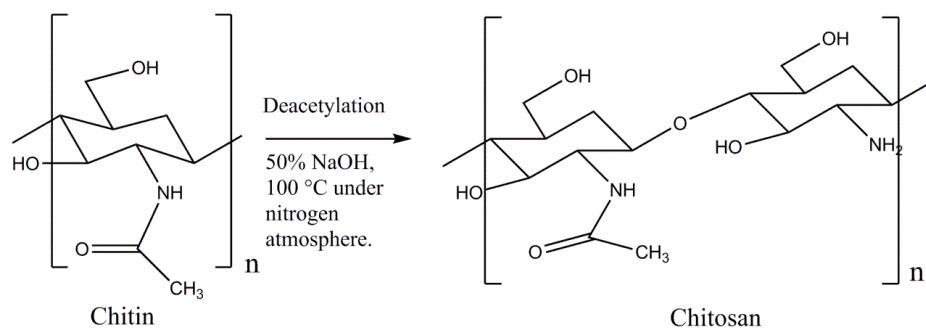
One of the chemical modification methods that increase organic solubility of chitosan is acylation. Stearoyl chitosan is one of the derivatives of acylated chitosan. The substitution reaction between stearoyl groups and hydrogen atoms of both hydroxyl and amino groups of chitosan results O-stearoyl chitosan and N-stearoyl chitosan, respectively (Scheme 1). But the main focus of this study was on N-stearoyl chitosan, which was the major product.



Scheme 1. The synthetic route for stearoyl chitosan.

Stearoyl chloride, which has C18; was selected for chitosan acylation due to its long hydrocarbon chain, which can lead to increase hydrophobicity of stearoyl chitosan. Thereby increase its organic solubility. By increasing the organic solubility results high viscous solutions. According to solubility studies, stearoyl chitosan can be dissolved in hexafluoroacetone [3].

The removal of acetyl groups from the molecular chain of chitin and leaving behind amino groups (-NH₂) that determines the reactivity of chitosan is known as the process of deacetylation (Scheme 1.1). DD (degree of deacetylation) is defined as the molar fraction of D-glucosamine residues in the chitosan chain [4, 5]. The quality and physicochemical characteristics of chitosan vary with DD. The DD value is used to differentiate chitin from chitosan, since the DD value of 75% or above is known as chitosan [6]. There are methods available to deacetylate chitin/chitosan. For example, using higher temperature or increasing the strength of NaOH solution can enhance the removal of acetyl groups from chitin/chitosan polymer. An example for deacetylation of chitosan is starting with DD 75% chitosan and ends up with DD 99.8% chitosan [7].



Scheme 2. The process of deacetylation.

Rheology, the science of deformation and flow of matter, investigates the response of materials to an applied stress or strain. There are two types of rheological behavioral patterns: viscous behavior and elastic behavior. The rheological behavior of polymer solutions can be explained using equation (1.1), which is the power law equation [8-11].

$$\tau = A\dot{\gamma}^n \quad (1.1)$$

where A is flow consistency index, which is derived from the curve logarithm shear stress versus logarithm shear rate and n is flow behavior index. τ is shear stress, $\dot{\gamma}$ is shear rate and η is shear viscosity. For a Newtonian fluid, $n=1$ and $A=\eta$. Most of the polymer solutions show non-Newtonian characteristics. Therefore, polymers exhibit shear thinning at higher shear rates. When the shear rate increases, disruptions occur faster than the chains can reentangle and the resultant decreasing entanglement allows molecules to flow with less resistance, which leads to decrease in viscosity [8-11].

This paper describes the preparation of two different degree substituted stearyl chitosan (DS 79% and DS 86%) by using the starting material as chitosan and deacetylated chitosan respectively. Spectroscopy methods such as FT-IR, and ^1H NMR were used to confirm the chemical structure of the palmitoyl chitosan substituents. TGA studies were carried out to determine the thermal stability while XRD analysis was used to characterize the crystalline structure of palmitoyl chitosan. The rheological behavior of the solutions of stearyl chitosan with two different degree substituents was assessed to investigate its' applicability in various fields such as fabricating nanofibers by electrospinning process, using as viscosity inducing agents in pharmaceutical formulations, and as rheology modifiers in industrial applications.

II. Materials and Methods

2.1 Materials

Chitosan (MMW, Degree of deacetylation 75-85%) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Monochloroacetic acid was purchased from Fisher Chemical Co. (NJ, USA). Hexanoyl chloride, palmitoyl chloride, stearyl chloride, deuterated solvents (d-acetic acid, d-trifluoroacetic acid and deuterium oxide) and other chemicals used in this study were purchased from Fisher Scientific (PA, USA). All chemicals used were ACS grade.

2.2 Preparation of deacetylated chitosan with a DD value of 94%

Deacetylated chitosan was synthesized by 'sodium hydroxide deacetylation of chitosan [12-14]. Chitosan, which has a DD of 75-85% (5 g), was reacted with 50% sodium hydroxide solution (50 mL) at a 1:10 (W/V) ratio in a round bottom flask (250 mL) under nitrogen atmosphere for four hours at 100 °C. The filtered, neutral product was dried in an oven (Isotemp Oven, Fisher Scientific, PA, USA) for 24 hours at 40 °C, then in a vacuum oven (Isotemp Model 280A, Fisher Scientific, PA, USA) for 24 hours at 40 °C.

The DD of chitosan was determined using pH-metric titration. According to the procedure, the dried chitosan (0.2 g) was dissolved in a mixture of 20 mL 0.1 M hydrochloric acid and 25 mL deionized water [5]. After 30 minutes of continuous stirring, 25 mL of deionized water added and stirring was continued for another 30 minutes. Finally, the completely soluble chitosan solution was titrated with 0.1 M sodium hydroxide solution. The following formula was used to calculate DD,

$$\text{DD}(\%) = 2.03 \cdot \frac{V_2 - V_1}{m + 0.0042(V_2 - V_1)} \quad (2.1)$$

where m is the weight of sample, V_1 , V_2 are the volumes of 0.1M sodium hydroxide solution corresponding to the deflection points, the number 2.03 is the coefficient resulting from the molecular weight of chitin monomer unit and the number 0.0042 is the coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units [5].

2.3 Preparation of stearyl chitosan

Two different degree substituted stearyl chitosan compounds (DS 79%, and DS 86%) were prepared by the method described by Tien et al. [15]. DS 79% stearyl chitosan was prepared by starting chitosan (1 g) was dissolved in aqueous acetic acid (240 mL, 0.453 M) by stirring for 24 hours. By slow addition of 0.1 M NaOH with strong agitation, pH was adjusted to 7.2, yielding gel slurry, the volume of which was adjusted to about 300 mL. Then stearyl chloride (5.44 g) was added and the reaction volume diluted to 400 mL with distilled water.

After 7 hours of reaction time, preparation was neutralized (pH 6.8-7.0) and precipitated with acetone. The filtered precipitate was washed at 55 °C with an excess methanol and decanted. The washing was repeated three times to remove fatty acids. Finally, the product was dried with pure acetone to obtain the corresponding derivative powder of DS 79% stearyl chitosan compound.

DS 86% stearyl chitosan was prepared by starting deacetylated chitosan (1 g), which was dissolved in aqueous acetic acid (240 mL, 0.453 M) by stirring for 24 hours. The rest of the procedure was exactly the same as the method followed to synthesize DS 79% stearyl chitosan.

2.4 Characterization

FT-IR analysis was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Electron Co., WI, USA). The resolution was set at 4 cm⁻¹ with a total of 32 scans and the wave number range between 400 and 4000 cm⁻¹. The concentration of 2% (w/v) in d-trifluoroacetic acid was used to collect ¹H NMR spectrum of stearyl chitosan on Bruker AV600 spectrometer under a static magnetic field of 600 MHz. Data analysis was carried out using the Mnova NMR software.

2.5 Determination of DS

The DS of prepared stearyl chitosan products were calculated by FT-IR spectroscopy [16].

2.6 Thermogravimetric analysis (TGA)

The thermal gravimetric analysis of stearyl chitosan was carried out using a TGA/DTA High Temperature 115 thermogravimetric/differential analyzer (Pyris-Diamond, PerkinElmer, MA, USA). The constant heating rate was set to 10 °C/min while the temperature range set to 50 °C to 450 °C. Analysis was performed under argon atmosphere.

2.7 X-ray diffraction (XRD)

XRD analysis was carried out using an X-ray diffractometer (X'Pert Pro MPD, PANalytical B.V., The Netherlands), operating at 40 kV and 20 mA with a Cu-K α source. The diffraction intensity was measured in the range of 2 θ angles between 5 ° and 40 °, with a step size of 0.020 °, step time of 40 s and scanning speed of 0.0005 ° s⁻¹.

2.8 Solubility studies

Solubility studies were conducted to find suitable solvents to dissolve stearyl chitosan compounds with high viscosity, which can be used in various applications. In this study, stearyl chitosan with DS 86% was tried to dissolve in the concentration of 2% (w/v) in a series of solvents such as isopropanol, trifluoroacetic acid, dichloromethane, dimethylformamide, hexane, toluene, tetrahydrofuran, acetone, chloroform, pyridine, dimethylsulfoxide, trifluoroethanol, dimethylacetamide, and hexafluoroacetone sesquihydrate by sonicating for seven hours [2, 17-20].

2.9 Rheological behavior

Rheological measurements were performed on a rheometer (DHR2, TA Instruments, DE, USA). The parallel plates with 40 mm diameter were used to collect data while the plate gap was set to 1000 μ m. Data analysis was carried out using TRIOS_v3.0.2.3156 software.

The frequency studies were conducted for stearyl chitosan solutions. The solution viscosity of stearyl chitosan solutions was measured using flow sweep, where the shear rate was linearly increased from 10 to 200 s⁻¹ at 25.0 °C. At there, an equilibration time of 20.0 s and an averaging time of 10 s were maintained [2].

III. Results and Discussion

3.1 Determination of DS

The DS of the prepared stearyl chitosan products (stearyl chitosan starting from chitosan and stearyl chitosan starting from deacetylated chitosan) were calculated by FT-IR spectroscopy [15]. According to the calculated values, the stearyl chitosan starting from chitosan had DS 79% while stearyl chitosan starting from deacetylated chitosan had DS 86%.

3.2 Structure characterization

3.2.1 FT-IR data analysis

As shown in Fig.1, the broad band at 3330 cm^{-1} in chitosan due to inter- and intra- molecular hydrogen bonding of $-\text{NH}_2$ and $-\text{OH}$ stretching vibrations was decreased in intensity in stearyl chitosan derivatives. The two new peaks at 1720 and 1710 cm^{-1} were due to $\text{C}=\text{O}$ of $-\text{NCOR}$ group and $\text{C}=\text{O}$ of $-\text{OCOR}$ group of stearyl chitosan, which were resulted from the acylation reaction between stearyl chloride and $-\text{NH}_2$ and $-\text{OH}$ groups of chitosan [15, 21]. Presence of both 1720 and 1700 cm^{-1} peaks confirmed that the substitution took place at both $-\text{NH}_2$ and $-\text{OH}$ groups in stearyl chitosan starting from chitosan. Mostly N-substitution took place

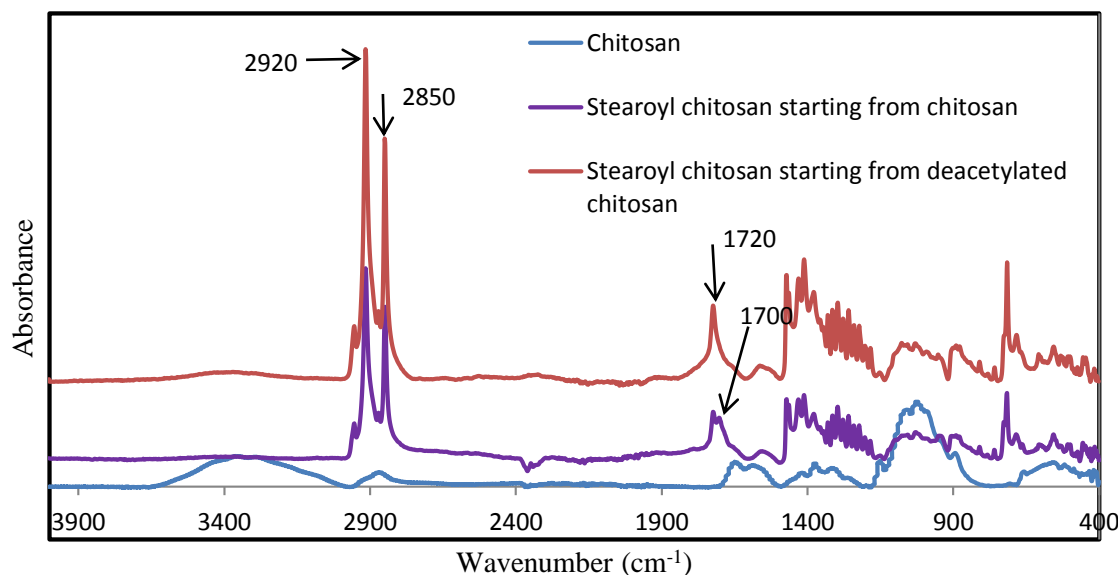


Figure 1: FT-IR spectra of chitosan and stearyl chitosan.

in stearyl chitosan starting from deacetylated chitosan due to presence only the intense peak at 1720 cm^{-1} . The peak intensity of 1720 cm^{-1} was higher in stearyl chitosan starting from deacetylated chitosan than that in stearyl chitosan starting from chitosan. This observation was confirmed that more substitution took place in stearyl chitosan starting from deacetylated chitosan, which has more N- active sites for acylation, and the activeness of amino group is stronger than that of hydroxyl group, the priority of $-\text{NH}_2$ group convert to $-\text{NCOR}$ was higher than converting $-\text{OH}$ to $-\text{OCOR}$.

The other two prominent peaks around 2920 and 2850 cm^{-1} were due to asymmetrical and symmetrical bending vibrations of methylene groups of long alkyl chain of stearyl chitosan [15]. The absorption intensity was higher in stearyl chitosan starting from deacetylated chitosan than that in stearyl chitosan starting from chitosan. This observation was confirmed that more substitution took place in stearyl chitosan starting from deacetylated chitosan. All of these observations were clearly confirmed that the formation of stearyl chitosan while more substitution took place in stearyl chitosan starting from deacetylated chitosan.

3.2.2 ^1H NMR data analysis

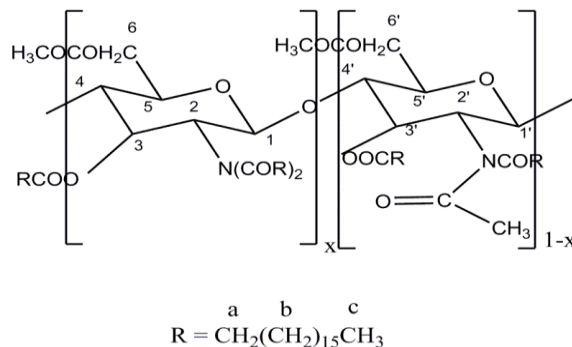


Figure 2: The protons corresponding to Fig. 3

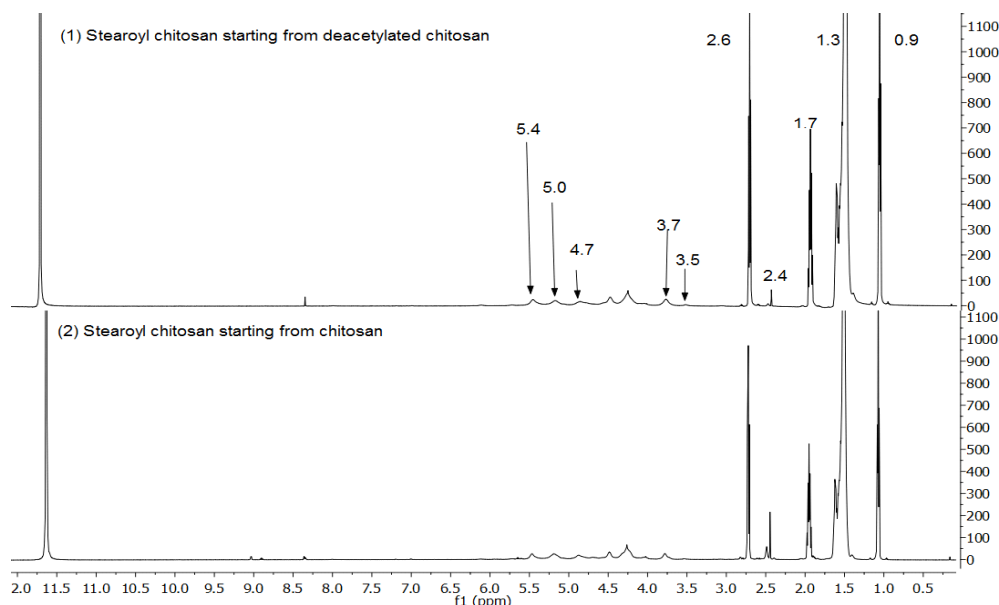


Figure 3: ^1H NMR spectra of stearyl chitosan.

The Fig. 3 illustrated the ^1H NMR spectrum of stearyl chitosan in deuterated trifluoroacetic acid (the d-TFA solvent resonance is at 11.5 ppm). The protons corresponding to Fig. 2 are shown in Fig. 3 [19]. As shown in Fig. 3, the resonances formed at 5.4, 5.0, 4.7 ppm were attributed to H1, H3 and H4 protons of chitosan, respectively. The resonances were at 3.5 and 3.7 ppm due to H6 and H5 of the ring protons (Scheme 5.7). The ^1H NMR data of Ma et al. [19] was confirmed by the presence of a multiplet peak at 2.6 ppm and the resonance at 2.4 ppm attributed to Ha. The broad multiplet peaks from 1.3 to 1.7 ppm were due to $-\text{CH}_2$ groups of stearyl chloride chain while a typical resonance at 0.9 ppm was due to $-\text{CH}_3$ of stearyl group.

3.3 Thermogravimetric analysis (TGA)

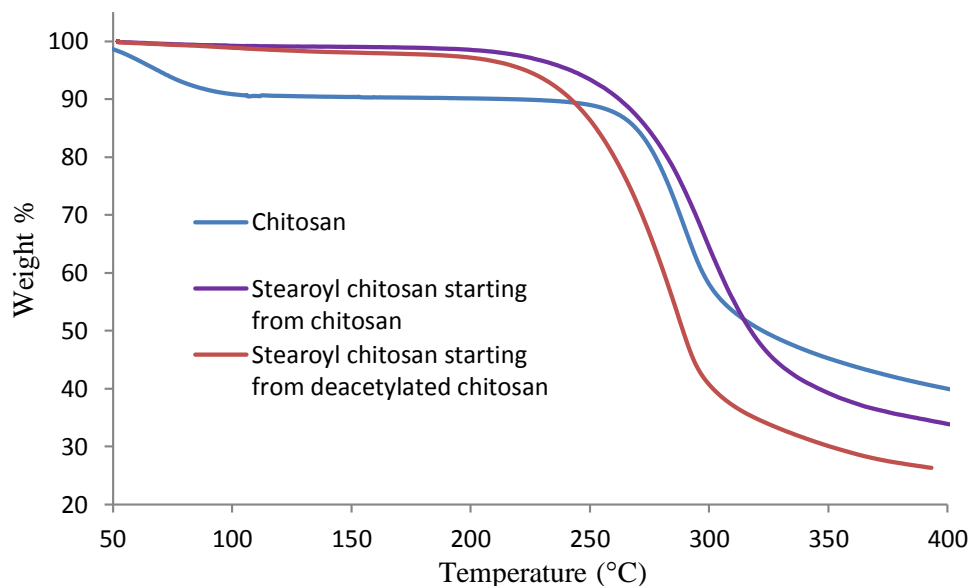


Figure 4: TGA curves for chitosan and stearyl chitosan.

As shown in Fig. 4, the onset of degradation occurred at 251.5 $^{\circ}\text{C}$, 242.24 $^{\circ}\text{C}$ and 226.6 $^{\circ}\text{C}$ for native chitosan, stearyl chitosan starting from chitosan and stearyl chitosan starting from deacetylated chitosan, respectively. Therefore, the thermal stability of stearyl chitosan was lower than that of parent chitosan. The thermal stability of stearyl chitosan starting from chitosan (DS 79%) was higher than that of stearyl chitosan starting from deacetylated chitosan (DS 86%). Hence, the thermal stability of stearyl chitosan decreased with increased DS. This could be due to less number of hydrogen bond formations in highly substituted stearyl chitosan [22].

3.4 X-ray diffraction (XRD)

In Fig. 5, there was an intense peak around 20° in chitosan while new sharp peaks were observed between 20° and 25° in stearyl chitosan. Among the two stearyl chitosan products, stearyl chitosan starting from deacetylated chitosan which has a DS of 86% showed the highest peak intensity. This suggested that stearyl chitosan had more crystalline and possibly more stable organization than that of chitosan [15].

The calculated DO values for chitosan, stearyl chitosan starting from chitosan (DS 79%) and stearyl chitosan starting from deacetylated chitosan (DS 86%) were 398 counts $(^\circ)^{-1}$, 1772 counts $(^\circ)^{-1}$ and 8797 counts $(^\circ)^{-1}$, respectively. Therefore, stearyl chitosan had a higher DO than chitosan. The DO of stearyl chitosan increased with increased DS. Hence, stearyl chitosan starting from deacetylated chitosan had more crystalline and stable organization.

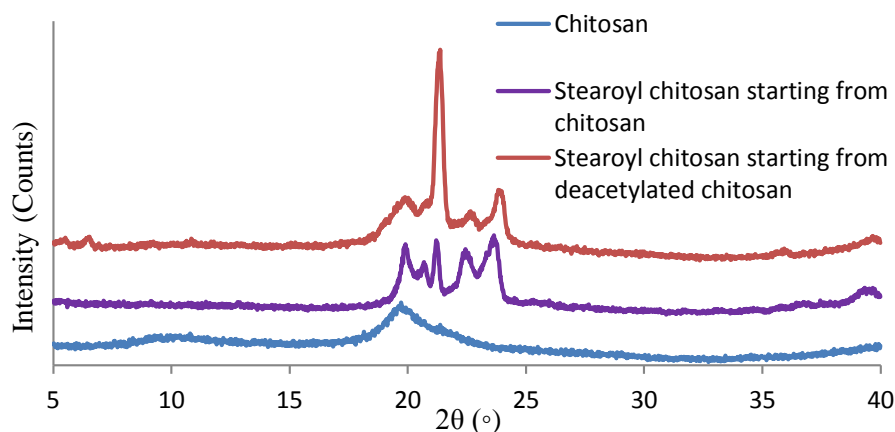


Figure 5: XRD for chitosan and stearyl chitosan.

3.5 Solubility studies and rheological behavior

According to the solubility studies, toluene and pyridine were the best solvents to dissolve stearyl chitosan with DS 86%, which had the highest degree of substitution. But, stearyl chitosan was dissolved only up to 2% concentration in toluene and pyridine. Therefore, the rheological properties were assessed in 2% (w/v) stearyl chitosan in toluene and pyridine.

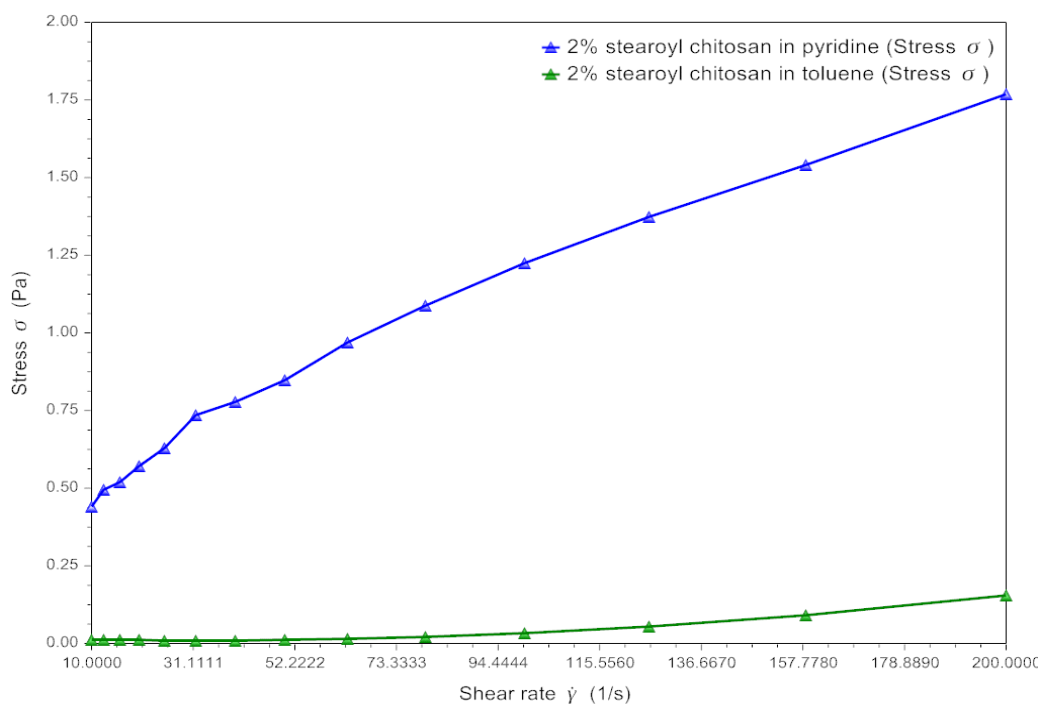


Figure 6: Influence of shear rate and different solvents on shear stress of 2% stearyl chitosan solutions.

In fig. 6, the shear stress of stearyl chitosan in pyridine and toluene did not increase in direct proportion to shear rate while the deviation was in the direction of shear thinning. Therefore, stearyl chitosan dissolved in

pyridine and stearyl chitosan dissolved in toluene exhibited shear thinning behavior. The shear stress value was higher for stearyl chitosan dissolved in pyridine than toluene at the same shear rate. This could be due to the increased viscosity of pyridine, which is 0.88 cP compared to the viscosity of toluene, which is 0.59 cP. Also it may be due to the formation of strong intermolecular interactions in stearyl chitosan dissolved in pyridine than that in toluene.

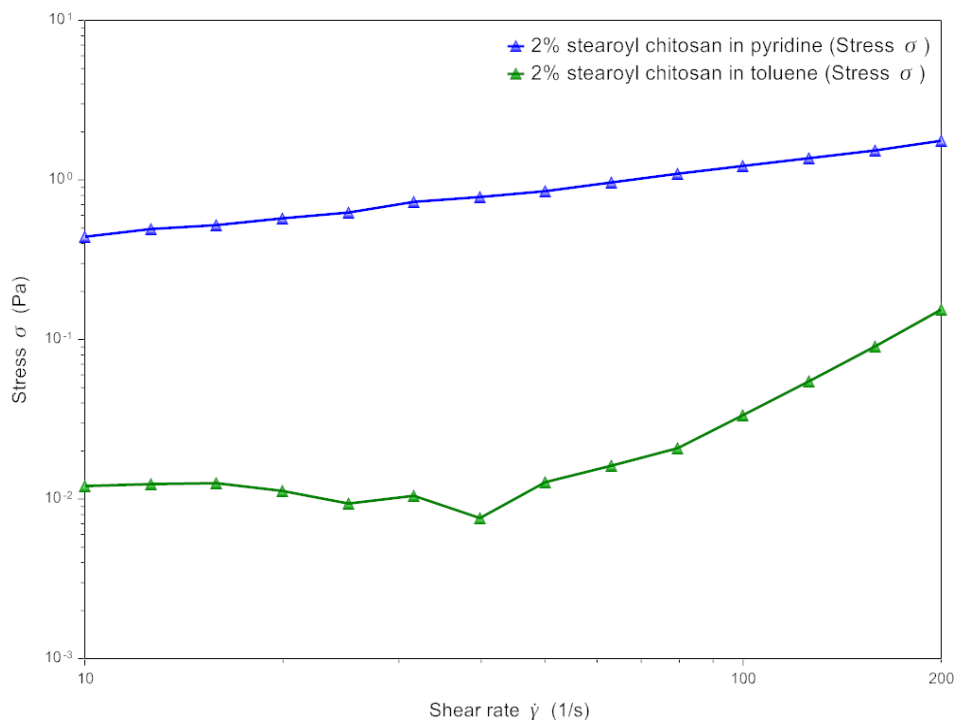


Figure 7: Power law plots (log shear stress versus log shear rate) of stearyl chitosan solutions.

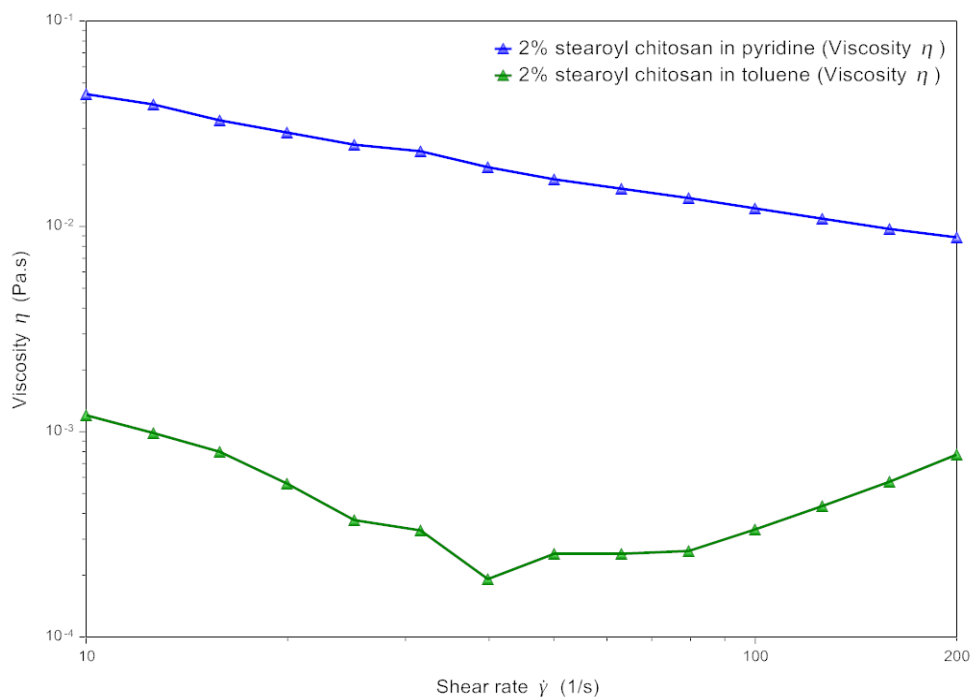


Figure 8: Viscosity as a function of shear rate for stearyl chitosan dissolved in pyridine and in toluene.

As shown in fig.6, the slopes of two plots were <1 . Therefore, stearyl chitosan in both pyridine and toluene showed shear thinning behavior. The viscosities of stearyl chitosan in pyridine and stearyl chitosan in toluene were decreased with increased shear rate (Fig. 8). Also the viscosities at shear rate 10 s^{-1} for 2% stearyl chitosan solutions were 0.044 Pa.s and 0.0012 Pa.s for in pyridine and in toluene solvents, respectively. Since

the conditions were maintained same for two solutions of stearyl chitosan, the higher solution viscosity for stearyl chitosan in pyridine was due to higher viscosity of pyridine than that of toluene.

IV. Conclusion

Two different degree substituted stearyl chitosan compounds were synthesized as DS 79% and DS 86%. According to TGA data, the thermal stability of stearyl chitosan was lower than that of parent chitosan. Also the thermal stability of stearyl chitosan decreased with increased DS. According to XRD data, crystallinity of stearyl chitosan increased with increased DS while stearyl chitosan had stable organization than chitosan. Solubility studies showed that the suitable solvents to dissolve stearyl chitosan with DS 86% are toluene and pyridine but only up to 2% (w/v) concentration. The 2% (w/v) stearyl chitosan (DS 86%) solutions displayed shear thinning behavior. The viscosities at shear rate 10 s^{-1} for 2% stearyl chitosan solutions were 0.044 Pa.s and 0.0012 Pa.s for in pyridine and in toluene solvents, respectively. Since 2% stearyl chitosan dissolved in pyridine had high viscosity with shear thinning property, it can be suitable as rheology modifiers.

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