

## Rheological behavior and thermal stability of palmitoyl chitosan varying the degree of substitution

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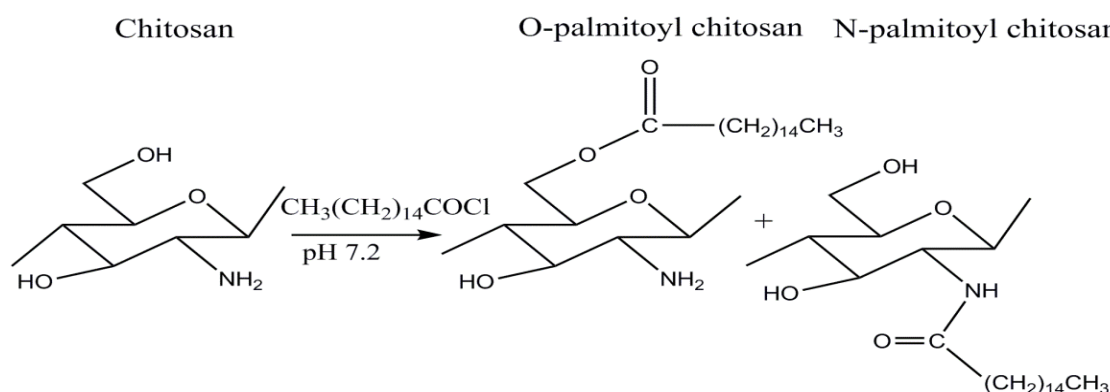
**ABSTRACT:** Degree of substitution (DS) 42%, 44% and 81% of palmitoyl chitosan were prepared by reacting chitosan and palmitoyl chloride at pH 7.2 by varying the reaction time. The chemical structure of prepared compounds were confirmed by using spectroscopy methods such as Fourier transform infrared (FT-IR), and <sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR) spectroscopy. DS was determined by FT-IR spectroscopy. Both thermal stability and crystallinity of palmitoyl chitosan increased with increased DS. The palmitoyl chitosan solutions displayed shear thinning behavior while the viscosity of three different degree substituted 2% (w/v) palmitoyl chitosan solutions increased with increased DS. Since DS 81% palmitoyl chitosan solution had high viscosity with shear thinning property, it can be suitable for pharmaceutical formulations and as rheology modifiers. For the first time, physicochemical characteristics and rheological properties were studied by varying the DS of palmitoyl chitosan.

**KEYWORDS** - acylation, crystallinity, degree of substitution, palmitoyl chitosan, rheology

### I. INTRODUCTION

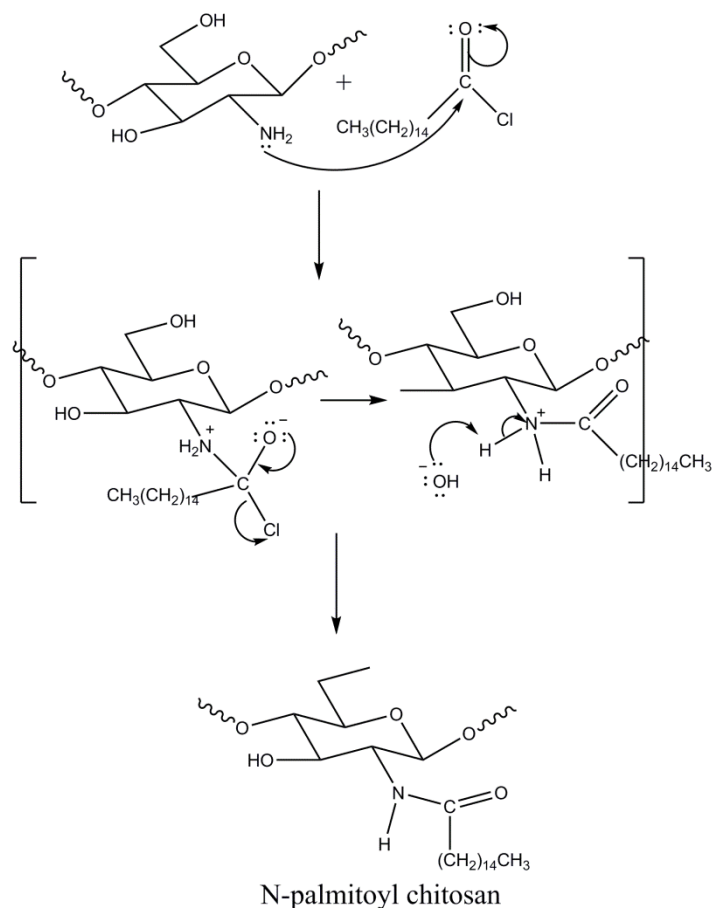
Chitosan, a natural linear biopolyaminosaccharide, is obtained by alkaline deacetylation of chitin, which is the main component of protective cuticles of crustaceans such as shrimps, crabs, prawns, lobsters, and cell walls of some fungi [1]. It is a cationic copolymer of D-glucosamine and N-acetylglucosamine. Due to its non-toxicity, biocompatibility, biodegradability, antimicrobial activity, physicochemical and biological properties and low immunogenicity, chitosan can be utilized in a number of fields such as biotechnology, biomedical applications (wound healing, drug delivery systems, gene delivery, tissue engineering and antimicrobial agents), food industry (dietary ingredient, food preservative, edible films and coatings and food emulsions), waste water treatment, textiles, cosmetics, and agriculture [2-10]. However, the poor solubility in water and most common organic solvents limits its applications [11].

Acylation is one of the chemical modification methods that increase the organic solubility of chitosan. Chitosan acylation by fatty acid chlorides generates hydrophobic derivatives through reactions on hydroxyl and amine groups of chitosan by the incorporation of long alkyl chains [12]. The inter- and intra- molecular hydrogen bondings of chitosan are replaced by hydrophobic interactions, which are believed to enhance its



**Scheme 1.** The synthetic route for palmitoyl chitosan.

solubility in non-polar organic solvents [13]. Among the acylated chitosan derivatives, hexanoyl, octanoyl, myristoyl, palmitoyl and stearoyl chitosan are important [14, 15]. In this study, main focus was on palmitoyl chitosan.



**Scheme 2.** Nucleophilic acyl substitution ( $S_N2$  type) occurring in N-palmitoyl chitosan.

As shown in Scheme 1 and 2, the nucleophilic acyl substitution reaction ( $S_N2$  type) between palmitoyl groups and hydrogen atoms of both hydroxyl and amino groups of chitosan results palmitoyl chitosan. Among the products, N-palmitoyl chitosan (major product), O-palmitoyl chitosan and N,O-palmitoyl chitosan are important.

According to the studies of Tien et al. [14] on the drug release kinetics of acylated chitosan showed that the longer release time was belonged to palmitoyl chitosan, which has a long acyl chain. The pharmaceutical formulations based on palmitoyl chitosan with DS between 40% and 50% can be used in oral administration while pharmaceutical formulations with DS higher than 50%, can be used as implants [14].

This paper describes the preparation of three different degree substituted palmitoyl chitosan (DS 42%, DS 44% and DS 81%) by reacting chitosan and palmitoyl chloride at pH 7.2 by varying the reaction time. Spectroscopy methods such as FT-IR, and  $^1\text{H}$  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 palmitoyl chitosan with three different degree substituents was assessed to investigate its' applicability in various fields such as 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). Palmitoyl chloride, deuterated trifluoroacetic acid and other chemicals used in this study were purchased from Fisher Scientific (PA, USA). All chemicals used were ACS grade.

### 2.2 Palmitoyl chitosan preparation

Three different degree substituted palmitoyl chitosan compounds (DS 42%, DS 44% and DS 81%) were prepared by the method described by Tien et al. [14]. When preparing DS 42% palmitoyl chitosan product, about 1 g of chitosan 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 (pH/Ion 510, Oakton Instruments, IL, USA), yielding gel slurry, the volume of which was adjusted to about 300 mL. Then palmitoyl chloride (30 mL,  $d=0.9$  g/mL) was added and the reaction volume diluted to 400 mL with distilled water.

After 5 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 42% palmitoyl chitosan compound.

Other two compounds were obtained following the same procedure, except for varying the reaction time of chitosan and palmitoyl chloride. The reaction time was varied for 7 hours and 10 hours to obtain DS 81% palmitoyl chitosan and DS 44% palmitoyl chitosan, respectively.

### 2.3 Characterization

FT-IR analysis was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Electron Co., WI, USA) using air. The resolution was set at 4  $\text{cm}^{-1}$  with a total of 32 scans and the wave number ranged between 400 and 4000  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR measurements were performed on Jeol ECX 400 NMR spectrometer under a static magnetic field of 400 MHz. Data analysis was carried out using Mnova NMR software.

### 2.4 Determination of DS

The DS of prepared palmitoyl chitosan products were calculated by FT-IR spectroscopy [14].

### 2.5 Thermogravimetric analysis (TGA)

The thermal gravimetric analysis and differential thermal analysis of three different degree substituted palmitoyl chitosan were 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.

### 2.6 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 $\alpha$  source. The diffraction intensity was measured in the range of  $2\theta$  angles between 5° and 40°, with a step size of 0.020°, step time of 40 s and scanning speed of 0.0005°  $\text{s}^{-1}$ .

### 2.7 Solubility studies

Solubility studies were carried out to find suitable solvents to dissolve three different degree substituted palmitoyl chitosan compounds with high viscosity, which can be used in various applications. Each substituents of palmitoyl chitosan was tried to dissolve in the concentration of 2% (w/v) in a series of solvents such as formic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, ethanol, isopropanol, hexafluoroisopropanol, dichloromethane, dimethylformamide, hexane, pyridine, toluene, tetrahydrofuran, acetone, chloroform, dimethylsulfoxide, trifluoroethanol, and dimethylacetamide by sonicating (FS30, Fisher Scientific) for seven hours [10, 12, 14-23].

### 2.8 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  $\mu\text{m}$ . Frequency studies (flow sweep, where the shear rate was linearly increased from 10 to 200  $\text{s}^{-1}$  at 25.0 °C) were carried out on palmitoyl chitosan solutions. At there, an equilibration time of 20.0 s and an averaging time of 10 s were maintained. All data were analyzed with TRIOS\_v3.0.2.3156 software.

## III. RESULTS AND DISCUSSION

### 3.1 Determination of DS

The DS of the prepared palmitoyl chitosan products were calculated by FT-IR spectroscopy using the following equation [14],

$$DS(\%) = \left( \frac{A_{1655}}{A_{3450}} - 0.12 \right) \times 100$$

where  $\frac{A_{1655}}{A_{3450}}$  is the ratio of absorbance at 1655  $\text{cm}^{-1}$  (due to amide I band) and the hydroxyl band at 3450  $\text{cm}^{-1}$  and the value 0.12 represents the acetyl groups specified in native chitosan.

	5 hours	7 hours	10 hours
DS <sub>1</sub>	37.06	103.3	28.72
DS <sub>2</sub>	41.41	50.25	44.08
DS <sub>3</sub>	51.56	89.28	56.80
DS <sub>4</sub>	39.44	80.26	44.47
Average DS	42.37	80.77	43.52

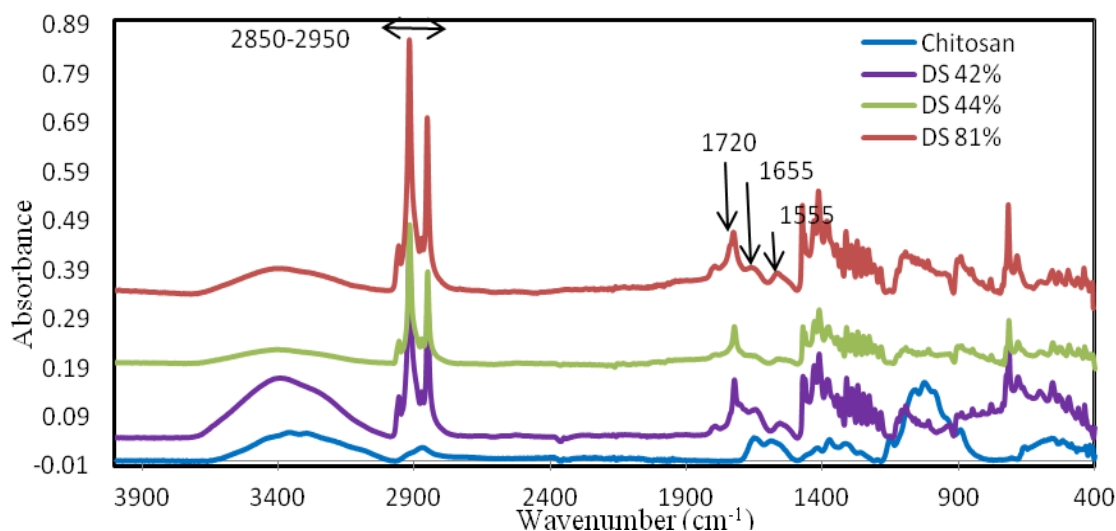
**Table 1.** Calculated DS values for palmitoyl chitosan.

According to the calculated values, the three different degree substituents of palmitoyl chitosan were DS 42%, DS 81% and DS 44%.

### 3.2 Structure characterization

#### 3.2.1 FT-IR data analysis

As shown in Fig.1, the broad absorption peak at 3000-3700 cm<sup>-1</sup> was due to OH and NH<sub>2</sub> stretchings of chitosan molecule. This broad absorption peak was not evident in palmitoyl chitosan due to palmitoyl groups were substituted into the hydroxyl and amine groups on chitosan molecules. By confirming the results of Tien et al. [14] and Xu et al.[24], chitosan showed two absorption peaks at 1655 cm<sup>-1</sup>(carbonyl stretching of amide I band) and 1570 cm<sup>-1</sup> (N-H bending vibrations of primary amine band). After N-palmitoylation, the peak at 1570 cm<sup>-1</sup> was disappeared while two prominent bands at 1655 cm<sup>-1</sup> (amide I band) and 1555 cm<sup>-1</sup> (amide II) were observed. O-palmitoylation of chitosan was confirmed by the presence of an intense peak at 1720 cm<sup>-1</sup>[14, 25]. Absorption intensity of the peaks was increased with increased DS of palmitoyl chitosan. Therefore, the hydrogens of both -NH<sub>2</sub> and -OH groups of chitosan molecules were substituted by palmitoyl groups resulting N-palmitoyl chitosan (major product) and O-palmitoyl chitosan.



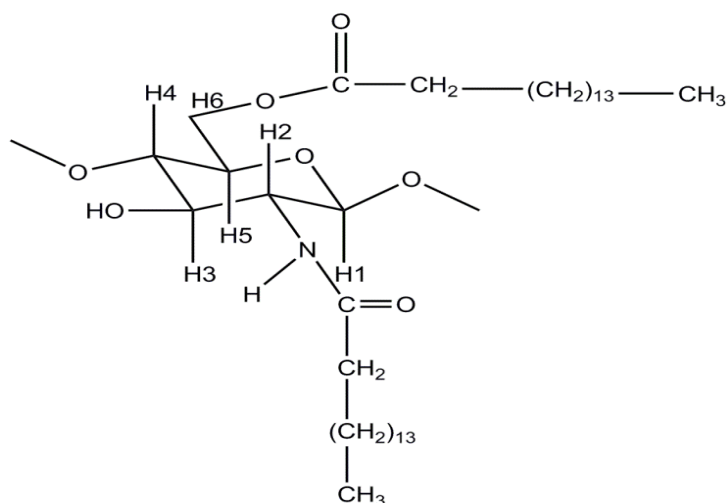
**Fig. 1.** FT-IR spectra of chitosan and three different degree substituted palmitoyl chitosan.

According to Tien et al.[14], the peaks in Fig.1 at 2850–2950 cm<sup>-1</sup> were due to –seven CH<sub>2</sub> groups of palmitoyl chitosan. Also the absorption intensity at 2850–2950 cm<sup>-1</sup> was increased with increased DS of palmitoyl chitosan. All of these observations clearly confirmed the formation of palmitoyl chitosan.

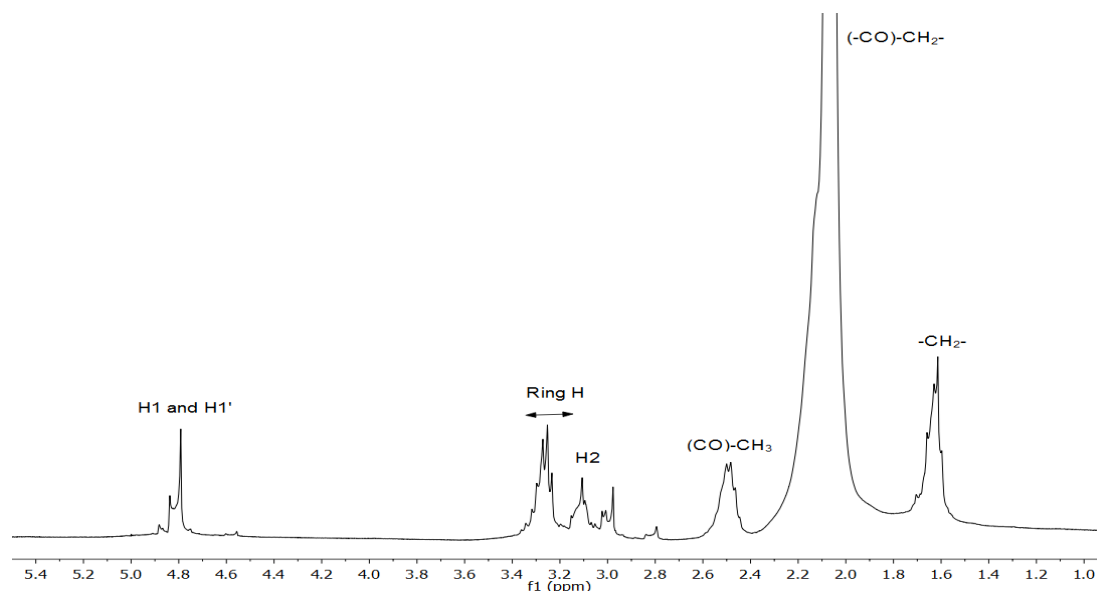
#### 3.2.2 <sup>1</sup>H NMR data analysis

The enlarged region from 0.9 to 5.5 ppm of the <sup>1</sup>H NMR spectrum of palmitoyl chitosan in deuterated trifluoroacetic acid (the d-TFA solvent resonance at 11.5 ppm) was presented in Fig. 3 (The protons corresponding to Fig. 3 are shown in Fig. 2). It was similar to that reported by Tien et al. [14] and Signini and Campana Filho [26]. The resonance at 2.0-2.1 ppm was due to N-acetyl protons of N-acetyl glucosamine while the resonances at 3.1-3.2 ppm were due to an H-2 proton of glucosamine or N-acetyl glucosamine residues. The resonances at 3.2-3.4 ppm assigned to ring protons (H3, 4, 5, 6, 6') while the peaks at 4.5 and 4.8 ppm were considered to the H-1 protons of the of glucosamine and N-acetyl glucosamine residues, respectively. The

chemical modification of chitosan by palmitoylation was confirmed by the peaks at 1.2 ppm [-CH<sub>2</sub>-] and 1.9 ppm [-CH<sub>2</sub>-(CO)] [14, 27].



**Fig. 2.** The protons corresponding to Fig. 3



**Fig. 3.** The enlarged region from 0.9 to 5.5 ppm of the <sup>1</sup>H NMR spectrum of palmitoyl chitosan.

### 3.3 Thermogravimetric (TGA) analysis

As shown in Fig. 4, the onset of degradation occurred ~ 251.5 °C, 218 °C, 220 °C and 235 °C for native chitosan, DS 42 % palmitoyl chitosan, DS 44 % palmitoyl chitosan and DS 81% palmitoyl chitosan, respectively. The thermal stability of palmitoyl chitosan was lower than that of parent chitosan. Therefore, acylation decreases the thermal stability of chitosan. This could be related to less number of hydrogen bond formations between palmitoyl chitosan molecules since the most of -OH and -NH<sub>2</sub> groups were substituted by palmitoyl groups. Also the thermal stability of palmitoyl chitosan increased with increased DS. This could be due to the increased number of hydrophobic interactions formed between palmitoyl chitosan molecules, which can enhance the stability.

### 3.4 X-ray diffraction (XRD)

According to Fig. 5, there was an intense peak around 20° in chitosan while new sharp peaks were observed between 20° and 25° in palmitoyl chitosan. The peak intensities were increased with increased DS of palmitoyl chitosan in XRD patterns. This suggested that palmitoyl chitosan had more crystalline and possibly more stable organization than that of chitosan [11]. Also the crystallinity of palmitoyl chitosan increased with increased DS.

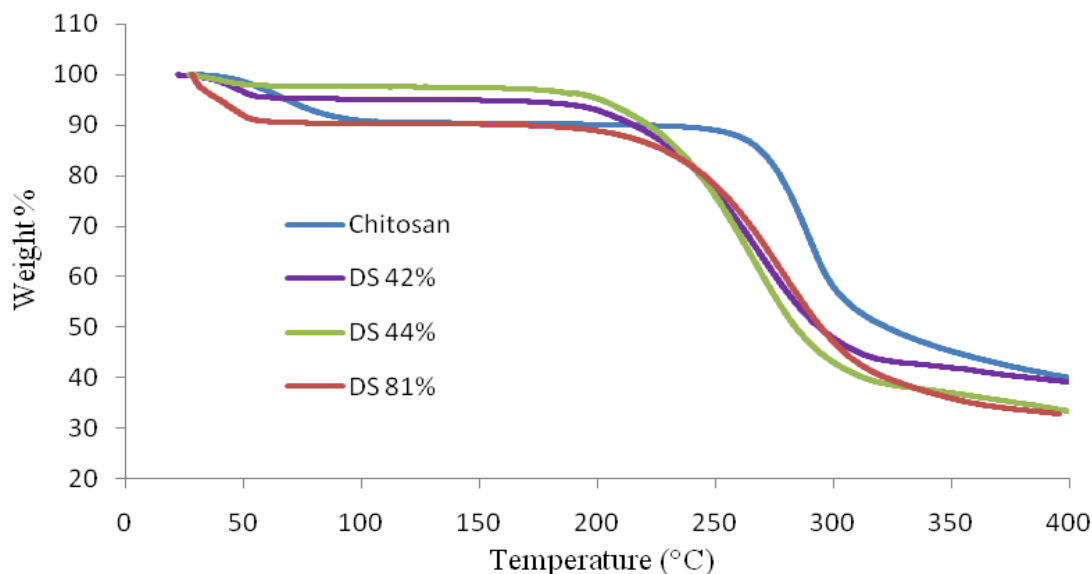


Fig. 4. TGA curves for chitosan and three different degree substituted palmitoyl chitosan.

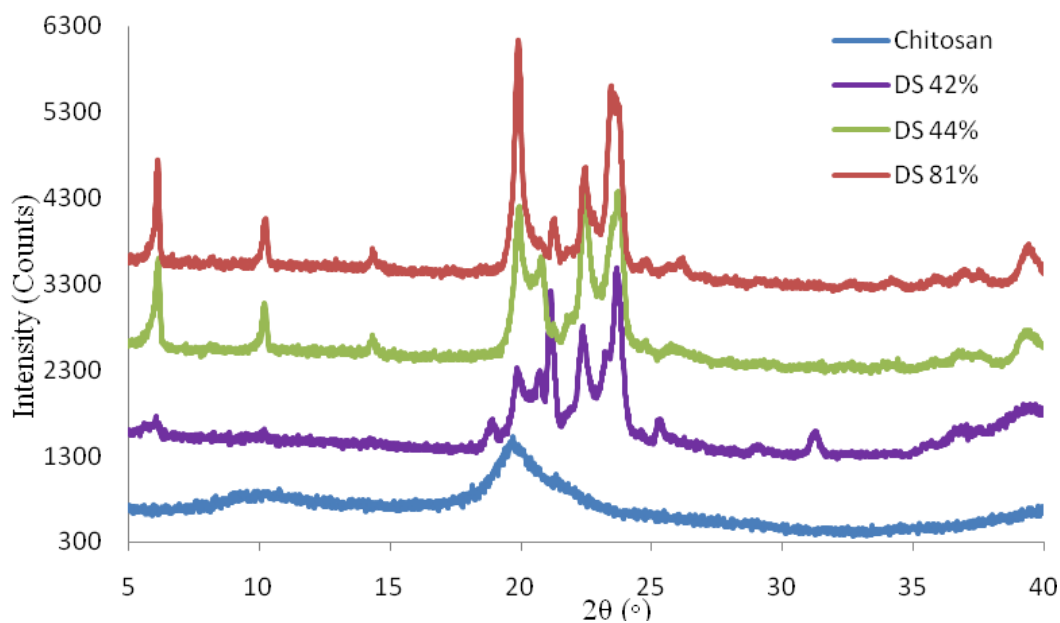


Fig. 5. XRD for chitosan and three different degree substituted palmitoyl chitosan.

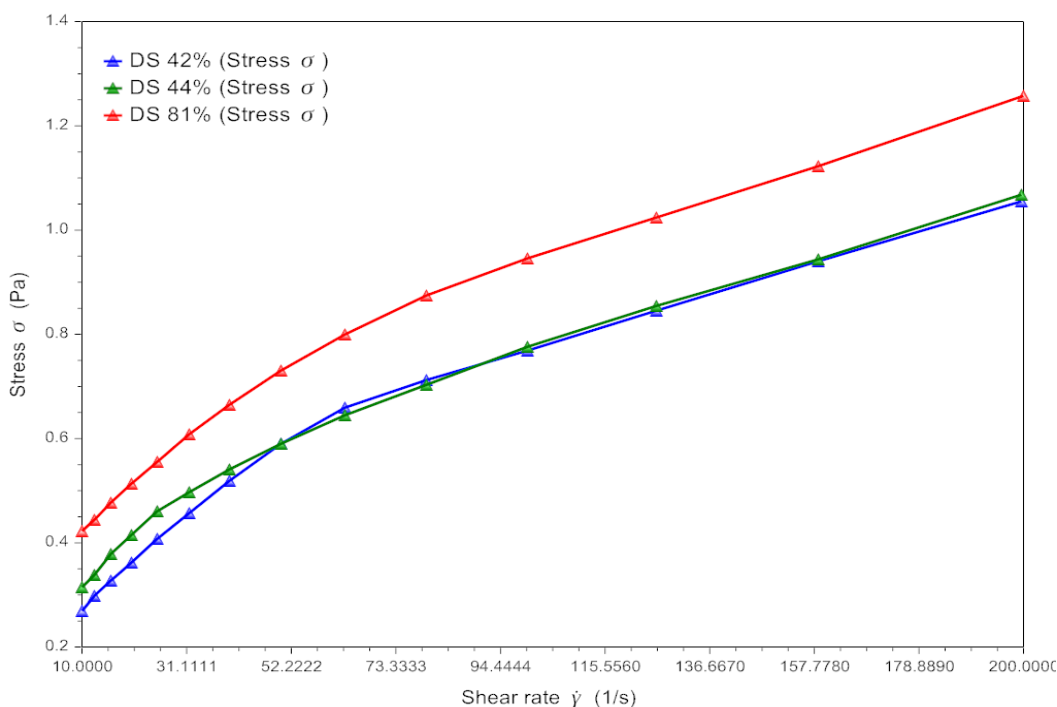
### 3.5 Solubility studies and rheological behavior

According to the solubility studies, pyridine was the best solvent to dissolve three different degree substituents of palmitoyl chitosan. Therefore, 2% (w/v) palmitoyl chitosan in pyridine of the three different degree substituents were used to analyze the rheological behavior.

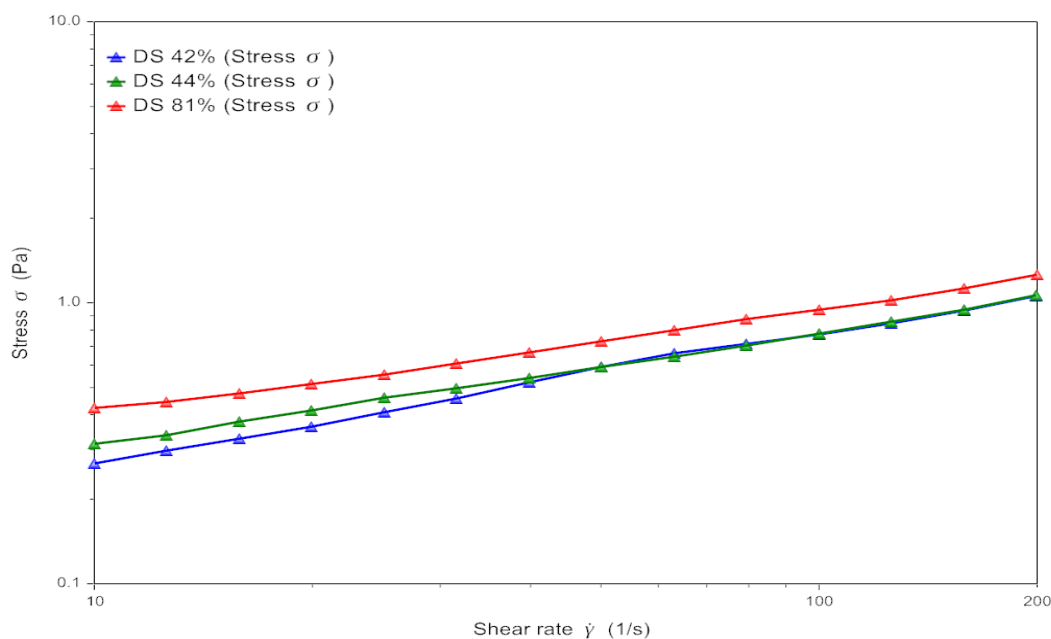
As shown in Fig. 6, the shear thinning behavior was displayed for all three different degree substituents of palmitoyl chitosan (DS 42%, DS 44% and DS 81%) solutions due to shear stress did not increase in direct proportion to shear rate and the deviation was in the direction of shear thinning.

The shear thinning behavior of three different degree substituted palmitoyl chitosan solutions in pyridine was confirmed by the power law plots in Fig. 7 by gaining a slope  $< 1$ . Therefore, DS 42%, DS 44% and DS 81% palmitoyl chitosan solutions in pyridine showed non-Newtonian rheological property.

As shown in Fig. 8, the solution viscosity of palmitoyl chitosan solutions decreased with increased shear rate in all three different degree substituted palmitoyl chitosan solutions. This behavior was prominent in shear thinning, non-Newtonian fluids.



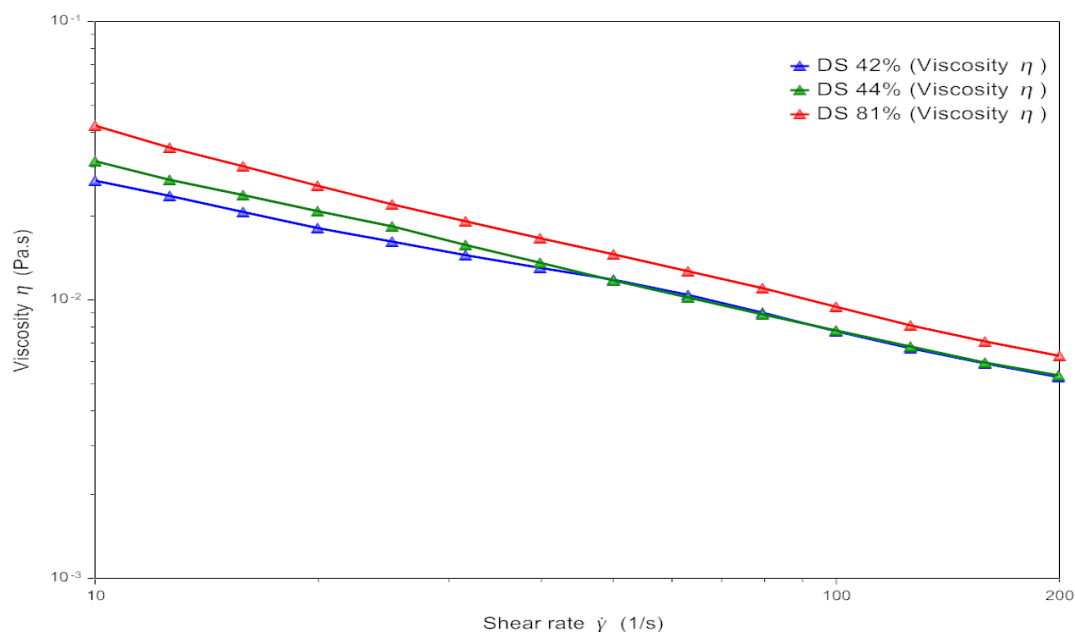
**Fig. 6.** Influence of shear rate on shear stress of three different degree substituted 2% palmitoyl chitosan solutions.



**Fig. 7.** Power law plots (log shear stress versus log shear rate) of three different degree substituted 2% palmitoyl chitosan solutions.

The viscosities at shear rate  $10 \text{ s}^{-1}$  for three different degree substituted 2% (w/v) palmitoyl chitosan solutions were 0.027 Pa.s, 0.032 Pa.s and 0.042 Pa.s for DS 42%, DS 44% and DS 81%, respectively. Therefore, the viscosity increased with increased DS for 2% solutions. As DS increases, the hydrophobic bond formation increases, which limit the mobility of molecules. This results increased viscosity with increased DS. Since DS 81% palmitoyl chitosan solution had a high viscosity with shear thinning property, it can be used as a viscosity inducing agent in pharmaceutical formulations.





**Fig. 8.** Dependence of solution viscosity on shear rate of three different degree substituted 2% palmitoyl chitosan solutions.

#### IV. CONCLUSION

The reaction between chitosan and palmitoyl chloride produced three different degree substituted palmitoyl chitosan compounds. According to TGA data, the onset of degradation for chitosan, DS 42 % palmitoyl chitosan, DS 44 % palmitoyl chitosan and DS 81% palmitoyl chitosan were 251.5 °C , 218 °C, 220 °C and 235 °C, respectively. Therefore, the thermal stability of palmitoyl chitosan is lower than chitosan while it is increased with increased DS. According to XRD data, palmitoyl chitosan had more crystalline areas and possibly more stable organization than that of chitosan. Also the crystallinity of palmitoyl chitosan was increased with increased DS. According to the solubility studies, pyridine was the best solvent to dissolve palmitoyl chitosan. The shear thinning behavior was displayed by all three different degree substituted palmitoyl chitosan solutions. The viscosity at shear rate 10 s<sup>-1</sup> for three different degree substituted 2% (w/v) palmitoyl chitosan solutions were 0.027 Pa.s, 0.032 Pa.s and 0.042 Pa.s for DS 42%, DS 44% and DS 81%, respectively. Since DS 81% palmitoyl chitosan had high viscosity with shear thinning property, it can be suitable for pharmaceutical formulations and as rheology modifiers.

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