ABSTRACT

LI, LINGCHEN. The Effects of Salt and Acid Concentration on the Dilute Solution Viscometry of Chitosan. (Under the direction of Dr. Samuel Hudson and Dr. Wendy Krause).

The intrinsic viscosities of two chitosan samples with different degree of acetylation and molecular weight were measured under different solvent and temperature conditions. Flow time was tested using Ubbelholde viscometer and intrinsic viscosities were determined using Huggins and Kraemer equations. The values of Huggins k' and Kraemer k'' helped estimate the solvent qualities. The influencing factors on intrinsic viscosity were discussed and it is concluded that the increase of temperature and ionic strength results in a decrease in intrinsic viscosity, while chitosans with larger molecular weight have higher intrinsic viscosities. Multiple parameters related to chain stiffness, including the relative stiffness parameter B, the empirical function $DA/(pH \cdot \mu)$ and temperature-relevant $d \ln[\eta]/d(1/T)$, were calculated and compared. The influencing factors on each of the three stiffness parameters vary and are not always consistent.

The Effects of Salt and Acid Concentration on the Dilute Solution Viscometry of Chitosan

by
Lingchen Li

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APPROVED BY:

_____	_____
Samuel Hudson	Wendy Krause
Committee Co-chair	Committee Co-chair

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Xiangwu Zhang

DEDICATION

To the greatest parents in the world and my dear boyfriend.

BIOGRAPHY

Lingchen Li was born in a small city called Bengbu in the eastern part of China. As an important transportation junction, the city witnessed the flourishing of textile industry and Lingchen was interested in textiles at an early age.

With the good wish to become a textile engineer, Lingchen Li went to Donghua University as an undergraduate in 2008 to major in textiles. Donghua University has the best textile college in China, and it was great pleasure and honor to study here. Lingchen's undergraduate project was related to green composites and she gained valuable experience in designing experimental, writing thesis and developing projects.

As a senior, Lingchen attended the 3+X program and came to North Carolina State University to go on with graduate education. Here she made a lot of new friends and was able to contact with the most advanced technology in textile field. The most important skills she was taught here were teamwork and divergent thinking. Lingchen majored in Textile Chemistry and studied on the solution properties of chitosans. After two year's learning, she was ready to be a good graduate student now and an eligible chemist in the future.

ACKNOWLEDGMENTS

During the writing of the thesis Dr. Samuel Hudson offered his help generously and provided excellent suggestions and references. I appreciate a lot for his kindness.

Thank Dr. Wendy Krause to be my committee co-chair even though she was very busy with her students' final exams. She also offered me great advice in designing experimental.

Thank Dr. Xiangwu Zhang for his time in busy schedule to be my committee member.

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1. **INTRODUCTION**

Chitin is widely distributed in nature, mainly in the exoskeleton of crustaceans and some fungi. It's a linear polysaccharide comprised by β-(1→4)-N-acetyl-2-amino-2-deoxi-D-glucose [1]. As the most important derivation of chitin, chitosan is produced from deacetylation of chitin under alkali sodium hydroxide or applying deacetylase to enzymatic hydrolysis [2]. The degree of acetylation (DA) and distribution of acetyl groups along chain determine the chemical structures of chitosan. The chemical structures of chitin and chitosan are in Figure 1.1. Chitosan has been widely used, especially in pharmaceutical and biomedical field. Table 1.1 implicated the main properties and applications of chitosan.

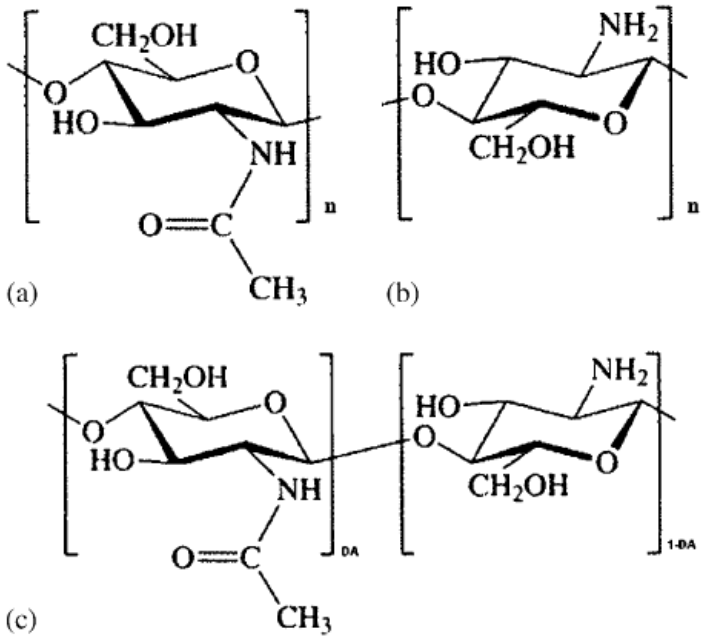

Figure 1.1. Chemical structure of (a) chitin; (b) chitosan; (c) partially acetylated chitosan [3]

Table 1.1. Principal properties of chitosan in relation to its use in biomedical applications [3]

Potential Biomedical applications	Principal characteristics
Surgical sutures Dental implants Artificial skin Rebuilding of bone Corneal contact lenses Time release drugs for animals and humans Encapsulating material	Biocompatible Biodegradable Renewable Film forming Hydrating agent Nontoxic, biological tolerance Hydrolyzed by lyzosyme Wound healing properties Efficient against bacteria, viruses, fungi

Both chitin and chitosan are semi-crystalline in the solid state [4]. When the DA is about 50%, chitosan is soluble in aqueous acid and becomes a polyelectrolyte. Chitosan is the only pseudo-natural cationic polymer and has many unique applications such as flocculants and depollution [3]. The solubility of chitosan also depends on distribution of acetyl groups, molecular weight and ionic concentration [3, 5, 6, 7].

Molecular weight and its distribution is another important characteristic which may be calculated by high-performance liquid chromatography or gel permeation chromatography [8, 9]. Weight-average molecular weight can be determined by light scattering [10]. A simple way is viscometry using the Mark-Houwink relation. The values of K and α vary with different solvents and some parameters are given in Table 1.2.

Table 1.2. Mark-Houwink parameters for chitosan in various solvents [3]

Solvent	K (mL/g)	a	T (°C)
0.1 M AcOH/0.2 M NaCl	1.81×10^{-3}	0.93	25
0.1 M AcOH/0.02 M NaCl	3.04×10^{-3}	1.26	25
0.2 M AcOH/0.1 M AcONa/4 M urea	8.93×10^{-2}	0.71	25
0.3 M AcOH/0.2 M AcONa (DA = 0.02)	8.2×10^{-2}	0.76	25
0.3 M AcOH/0.2 M AcONa (0<DA<0.03)	7.9×10^{-2}	0.796	25
0.02 M acetate buffer/0.1 M NaCl	8.43×10^{-2}	0.92	25

Since chitosan is soluble as a polyelectrolyte in acid, ion concentration is important in determining properties. The intrinsic persistence length L_p is related to chain stiffness. At a given ion concentration, the actual persistence length L_t is [9]

$$L_t=L_p+L_e \quad (1)$$

L_e is the electrostatic contribution which could be calculated following Odijk's work [11]. It has been proved that chitosan chains are semi-flexible and L_p values for several polysaccharides were calculated by Rinaudo and other groups [3]. From L_p, we're able to predict chain dimensions and intrinsic viscosity [12].

The behavior of chitosan solution is very complicated, varying with many factors including molecular weight distribution and degree of acetylation. Rinaudo and her group have done a lot of work in characterizing chitosans in aqueous solutions, including some other polysaccharide systems. Chen and his group also demonstrated some influencing factors for the intrinsic viscosity and conformation of chitosans. This thesis concerns the dependence of intrinsic viscosity on molecular weight, temperature and solvent properties

such as ionic strength. Some conformation-relevant parameters were also evaluated and compared with previous literature.

2. LITERATURE REVIEW

Polysaccharides are important sources in living organisms. They appear in all kinds of plants to form as cell walls, structural components, strengthening substances and they can be used for energy storage. According to different monosaccharides that build them, polysaccharides present different physical and chemical properties, i.e. linear or branched, acidic or neutral. Polysaccharides can form gel easily in solution, which is important in demonstrating chemical and physical properties.

For chitosans, degree of acetylation is one characteristic chemical factor that influences significantly on behaviors. Through detecting degree of acetylation, we are able to predict physical performance in solution.

2.1. Some Polysaccharides and Their Viscosities

One polysaccharide that exhibits biological activity is the pectin, including pectic substances. Pectins, consisting mainly of 1,4-linked α-D-galacturonic units [2], can be acidic or neutral, depending on whether or not the carboxyl groups are neutralized by common metal ions [13]. Pectins might be esterified in nature and have the ability to form gels if the degree of esterification (DE) is lower than 50%, so one application for pectate is in encapsulation after gelation [2]. According to various studies on all kinds of plants over the last two decades, pectic polysaccharides are active in many plants' immune system and a few other biological systems. Pectic polysaccharides are promising in health care for their biological activities [14].

There are several methods to determine the intrinsic viscosity of pectins. Several decades ago, Shubtsova et al. [13] introduced a low-molecular-weight electrolyte to help completely suppress ionization of pectin. Polyelectrolytes presented more complicated viscosity behavior. The (η_{sp}/c)-c curves were made to obtain $[\eta]_{\infty}$. Results indicated that the true values had nothing to do with the nature of replacing cations or the degree of replacement in carboxyl groups as long as the introduced electrolyte was HCl or NaCl. Fishman et al. [15] calculated the intrinsic viscosity of several pectin components using high-performance size-exclusion chromatography (HPSEC). It was a relative simple method however with complex calculations.

In the food industry, pectin solutions are stored and produced under different temperatures, indeed Muhidinov et al. [16] studied the variation of intrinsic viscosity under temperature range 20-60˚C. Pectin solutions were prepared in 0.17M NaCl, with pectins from apple, sunflower, orange and citrus. Flow time was measured under controlled temperatures at 20, 30, 40, 50 and 60˚C, then intrinsic viscosities were calculated using Huggins equation. Results suggested that intrinsic viscosity decreased almost linearly with increasing temperature for sunflower, orange and citrus pectins. However for apple pectin the slope was much sharper between 20 and 30˚C, then became smooth and in the end was comparable to those for other pectins between 40 and 60˚C (Figure 2.1). This might indicate a more intense structural change for apple pectin.

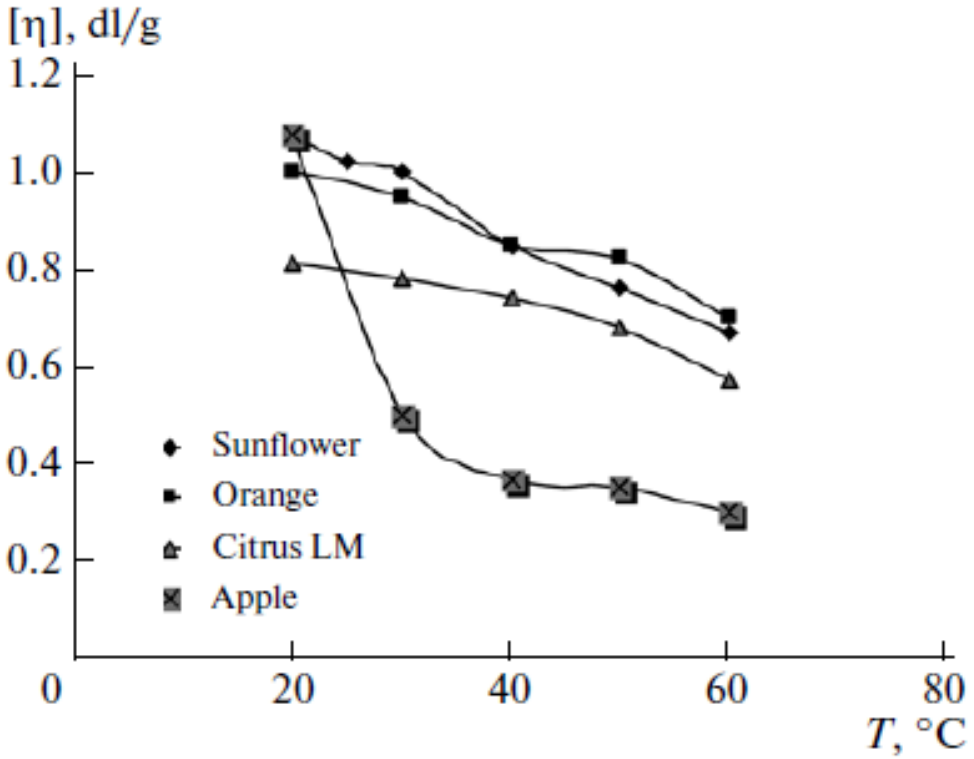

Figure 2.1. Dependence of intrinsic viscosity on solution temperature of pectins [16]

Gracesa covered several properties and development for alginates in his review [17]. Being a kind of polysaccharide extracted from seaweeds, alginates were unbranched (1-4)-linked glycuronans. Depending on different monomer arrangement orders, alginates could be homopolymeric or heteropolymeric (Figure 2.2, Figure 2.3). A characteristic property for alginates was the ability to form gels with the participation of divalent cations. The gels were thermostable over a large temperature scale, but would melt if the temperature is too high. The D-mannuronate to L-guluronate ratio (M:G ration) was crucial to diverse physical properties, e.g. the nature of gel. So there had been colorimetrical, chemically

stoichiometrical and spectroscopic ways to evaluate the M:G ratio. Alginates have numerous applications in the food and drink industry, medical and others (Table 2.1).

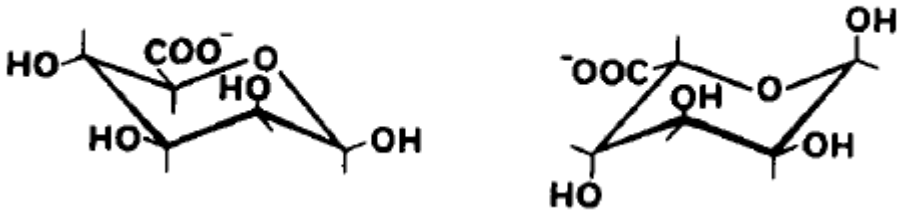

Figure 2.2. The component monosaccharides of alginate; D-mannuronate (left) and L-guluronate (right) [17]

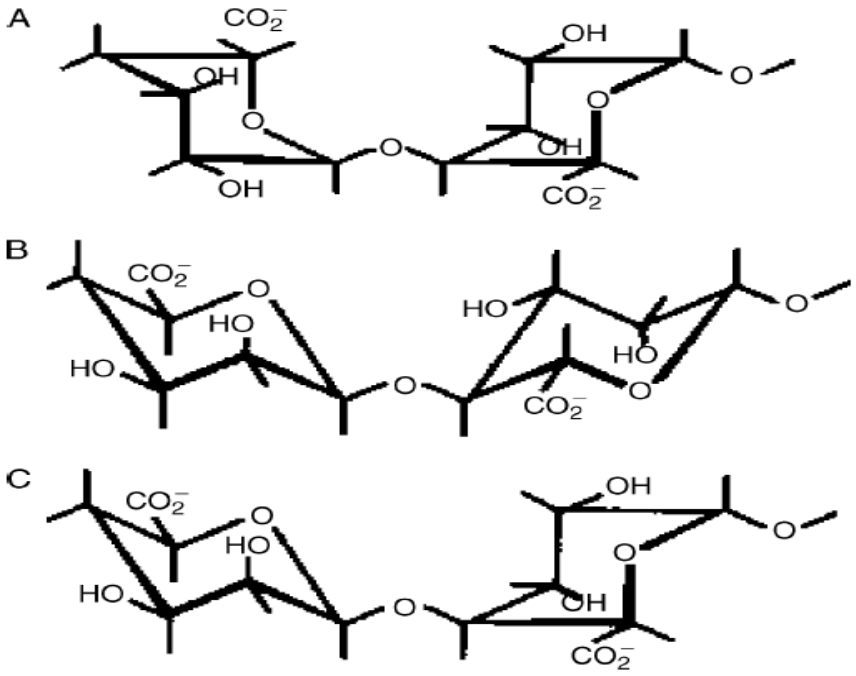

Figure 2.3. Chemical structure of repeat units of alginates:
(A) L-guluronic acid (G); (B) D-mannuronic acid (M); (C) alternating L-guluronic and D-mannuronic acids (GM) [2]

Table 2.1. Some major applications of alginates [17]

Area of application	*Function*	*Specific examples*
Food and drinks industry	Stabilizer	Foam stabilizer (beer).
		Phase-separation retardant (ice cream).
	Viscosifier	Suspension of fruit pulp.
		Thickener for sauces, milk shakes etc.
	Gelling agent	Reconstitution of foods (stoneless fruit, onion rings).
	Film	Coating of fish.
Pharmaceutical industry	Stabilizer	Emulsions in cosmetic preparations.
		Binder for tablets and lozenges.
	Gelling agent	Moulds for dental impressions.
	Film/fibres	Gastroenteric coatings for tablets.
		Haemostatic bandages.
	Therapeutic agents	Anti-acid and anti-ulcer compound.
Other uses	Viscosifier	Printing inks.
	Gelling agent	Enzyme/cell immobilization.

Sodium alginates are strong polyelectrolytes. Rinaudo et al. [18] considered viscosity of Na-alginates in dilute and semi-dilute solutions. The M:G ratio for all samples was around 1.3:1. Molecular weight was measured by size exclusion chromatography. Samples then were dissolved in solvents with NaCl concentration 0.05, 0.1, 0.2 and 0.5M. Intrinsic viscosities were determined through Ubbelholde viscometer at 25˚C. Results indicated that Huggins constant k' increased with higher concentration of NaCl. At the same time the parameters from Mark-Houwink equation was in good agreement with existing literature.

L.R.Harkness et al. [19] studied the intrinsic viscosity for sodium alginates in different $Na^{+}{}_{y}X^{y-}$. A dependence on X was determined. The average distance between alginate chain

ends was then estimated. When the concentration of sodium is low, the effect of X was negligible. However when there were enough anions, there was an effect. X might modify the formation of pairs between sodium and alginate, giving rise to observed viscosity change. Polymer chains were less extended when sodium increased, since the attraction between alginate ions and sodium would result in a self-curling system.

Hyaluronan, also known as hyaluronic acid, hyaluronate or HA, is a linear copolymer composed of N-acetylglucosamine and D-glucuronic acid [20] (Figure 2.4). HA is abundant in connective tissues and vitreous humour, since the hydrogen bonds within molecules can help build structure basis [21]. HA is a polyelectrolyte with negative charge in aqueous solutions, and it is also sensitive to hydration and cations, resulting in different three-dimensional structures [20, 21]. HA is widely used in biomedical applications, including tissues and biomaterials [21].

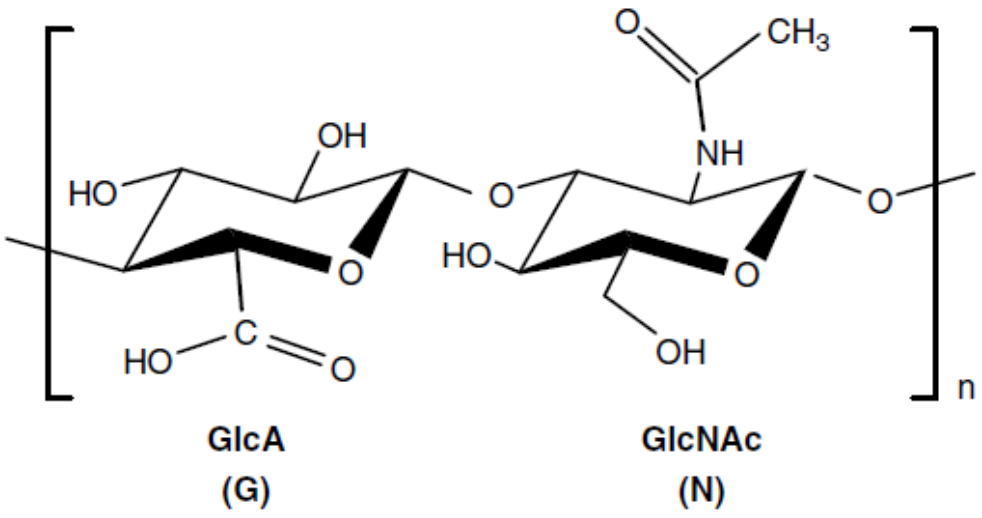

Figure 2.4. Chemical structure of the repeat unit in hyaluronan [2]

By determing intrinsic viscosity of HA, we can determine many other properties. Mendichi and his colleagues investigated the intrinsic viscosity, molar mass and gyration radius of hyaluronan [20]. Nine samples with a range of molecular weight were studied in 0.15M at 37˚C. Results indicated that the $[\eta] = f(M)$ power law curved obviously, suggesting wormlike chain structures. Deguine et al. used viscosity measurement as an indicator to determine free radical degradation for HA [22].

2.2. Solvent Properties of Polysaccharides

Marguerite Rinaudo and her colleagues made much progress in understanding natural polysaccharides. One of Rinaudo's articles discussed the non-covalent hydrophobic and ionic interactions of polysaccharides [23]. For polymers which have an amphiphilic character, hydrophobic interactions attribute a lot to some special properties, such as gelation. Two polysaccharide systems, neutral methylcelluloses and positively charged alkylated chitosans, were studied. Both samples showed reversible physical gelation under different temperature, salt concentration, etc. The gelation of alkylated chitosans was relevant to pH. Higher pH resulted in lower net charge, thus hydrophobic interactions dominated. For polysaccharides, the attraction between polyions and oppositely charge ions in the solution helped determine various properties. As long as chain confirmation was known, the activity coefficient of counterions without external salt in solution could be predicted. Ionic selectivity occurred to influence conformational stability, indeed, would determine mechanical properties of gels, if gelation existed. The importance of hydrogen bonds in polysaccharides was also discussed. They were significant in determining solubility and stiffness for polymers.

Rinaudo concerned more deeply, on different of types of gelation and mechanisms for polysaccharides in an earlier article [24]. The gels were classified into single polymer-based gels and two polymer-based gels. Single polymer-based gels, such as agarose, free of sulfate substituents, and curdlan or gellan, with κ-carrageenan and ι-carrageenan (Figure 2.5), performed a solution-gel transition when temperature increased or ionic charges decreased, resulting in thermoreversible gels. The gels were physically crosslinked, with lower modulus when temperature increased. Specific polysaccharides, for example pectin, would form gel when mixed with polyvalent metal ions, e.g. Ca^{2+}. They were called ionic crosslinked gels. Polymer chains with configuration of D-glacturonic and L-guluronic acid would form dimers with multivalent ions, causing aggregation. The concentration of Ca^{2+} dominated the modulus of the formed gels. Thermoforming gels attributed to hydrophobic interactions, as mentioned in the above passage, resulting in a phase separation. This gelation was the least rigid. Two polymer-based gels could be formed whether the original polysaccharides were gelling or non-gelling. The mechanism was to form an interpenetrating or a phase separated network.

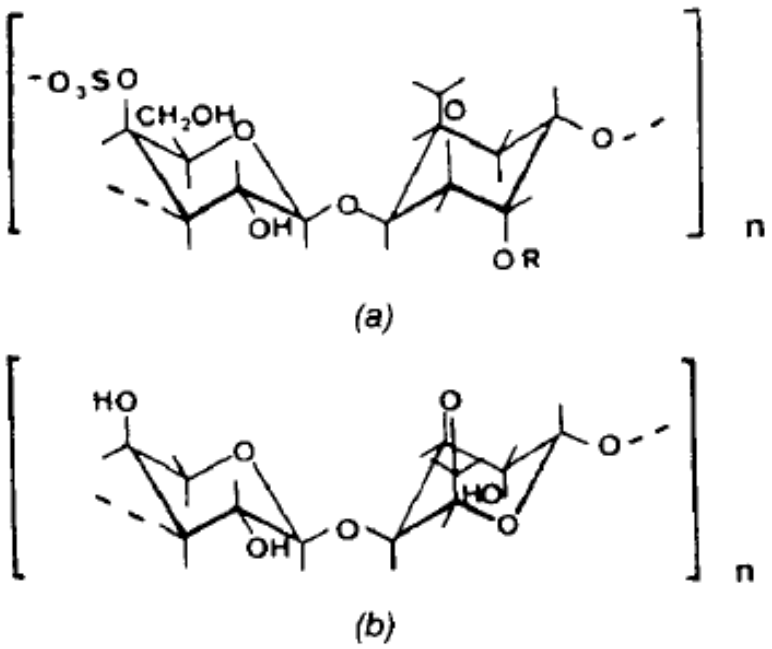

Figure 2.5. Chemical structure of some gelling polysaccharides (a) κ-carrageenan (R = H) and ι-carrageenan (R = SO_3^-) and (b) agarose [24]

Knowing the chemical structures of several polysaccharides are essential in predicting their physical properties, and this was demonstrated by Rinaudo [25]. In the experiment, GPC with three detectors was used to measure molecular weight distribution, the radius of gyration (R_g) and intrinsic viscosity. ^1H and ^{13}C NMR were used to characterize chemical structures. According to results for galactomannan, xanthan and gellan, both the Huggins constant and the intrinsic persistence length (L_p), which were calculated out from R_g, molecular weight and [η], were in agreement with earlier literatures. L_p, which related to stiffness, was one original physical property for polysaccharides.

2.3. Acetylation of Chitosan

The degree of acetylation (DA) is the most significant factor that influences the performance of chitosan. It indicates 'the molar percentage of monomeric units that have acetyl groups' [26] (Figure 2.6). In practical, DA usually varies from 0 to 60%, depending on processing parameters [27]. There are various ways to determining DA. Kasaai [28] classified them into spectroscopy, conventional and destructive ways (Table 2.2). Spectroscopy methods include infrared spectroscopic technique [26], ^{1}H NMR spectrometry [29] and UV spectrometry. The most conventional way is titration. A proper DA could be controlled at elevated temperature with alkalies [30]. Many mechanical, biological and physical properties are influenced by DA.

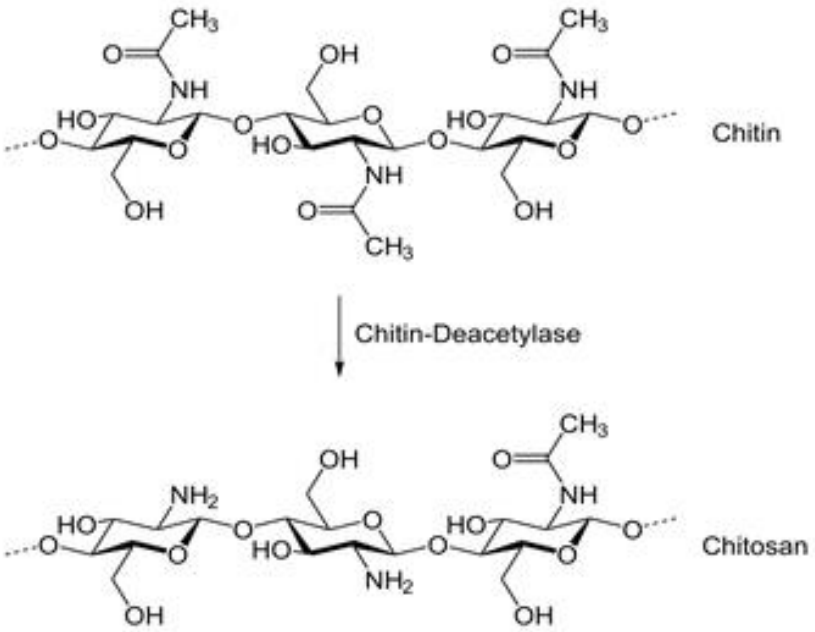

Figure 2.6. Deacetylation of chitosan [31]

Table 2.2. Different methods of the DA determination, their corresponding DA ranges, and their performances and limitations [28]

method	DA range	performances	limitations
conventional	applicable for soluble chitin/chitosan	Availability of the instruments is not a problem. Easy to use the instrument and easy to perform the method. Humidity is not an interference.	Applicable only for soluble chitin/chitosan samples. Proteins and mineral ions may induce interference peak(s) and result in unreliable results.
spectroscopy (^{13}C NMR, ^{15}N NMR)	0−100	Applicable for soluble and insoluble chitin/chitosan samples. Some information on chemical structure and sequence of comonomer units may be obtained from the spectra of chitin/chitosan samples. The more sensitive instrument generally results in the higher precision. Resolution, limit of detection, and accuracy of results are improved using cross-polarization and strong magnetic fields.	The impurities of chitin/chitosan (moisture, protein, pigments, and metal ions) may create interference peaks. These techniques are not sufficiently sensitive for low values of the DA. The availability of the instrument is a limitation due to the cost, special considerations, and sophistication, especially for the instruments having higher sensitivity and stronger magnetic fields.
spectroscopy (^{1}H NMR, IR, near-IR, UV)	soluble samples	^{1}H NMR and UV techniques are more precise and result in more accurate data in comparison to other methods.	IR and UV methods usually require carefully selected chitin/chitosan reference samples (with certain the DA values).
destructive	0−100	Entire range of the DA.	Two steps are required for analysis (decomposition of chitin/chitosan and analysis of the decomposed species). Long term is required to analyze the DA.

Franca et al. [32] examined the influence of N-acetylation on chitosan molecular structure. A molecular dynamics simulation was performed to examine the solubility and conformation of chitosan chains and nanoparticles. Results indicated that chain flexibility was inversely proportional to DA. With high DA, chains exhibited a stable two-folded helix structure.

Some biological properties of chitosan films influenced by degree of acetylation were studied by Chatelet et al. [33]. Five chitosan samples were supplied with different DA varying from 2.5 to 47%. Viscosity-average molecular weight and water content varied, too. Films were made through acid solution evaporation. DAs were measured by IR spectrometry. Cytocompatibility was evaluated by putting films into confluent cell cultures then cell development was observed for severay days. A blank culture was compared. Cell adhesion

and proliferation tests were made through a 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) test, utilizing a $3cm^2$ metallic ring with cells seeded on films. Results indicated that for film cytocompatibility towards keratinocytes and fibroblasts, DA wasn't significant. Nonetheless, DA was a key factor in cell adhesion and proliferation. Higher the DA, the lower the adhesion and proliferation. Different cell types would also influence test results.

Since cell adhesion was determined by DA, Freier et al. [27] investigated how to control cell adhesion by N-acetylation as well as degradation of chitosan films. Chitosans with selective DA were prepared using acetic anhydride, and another convention way is using alkaline hydrolysis to deacetylate chitosan. These two chemicals could help preserve original chemical compositions for chitosan. DA was determined by ^{1}H-NMR spectroscopy. Chick dorsal root ganglion (DRG) neurons were used to test cell compatibility and viability. According to test results, it turned out that degradation speeded up with higher DA, however faster degradation was gained at the cost of poor cell adhesion at very high DA. This article presented a valuable idea for tissue engineering in controlling biodegradation and cell compatibility.

2.4. Characterization of Chitosan Solutions

According to Berth et al. [34], there were a lot of discussion and contradiction on chitosan physical conformation in solutions compared to the comprehensive knowledge in chemical structure, which could be proved by determining DA and distribution of the acetyl group. In aqueous acid solution, chitosans form single-stranded stiff chains. However the

influence of varying DA is uncertain because of two effects. On one hand, more acetyl groups help enhance chain stiffness [35, 36, 37]. On the other hand, a lower DA suggests more amino groups, which will form highly charged polycations in acidic solutions. This was studied by Errinton et al. [38]. A third conclusion is the irrelevance of DA on chain stiffness by calculating persistence length Lp [39, 40].

Pedroni and his colleagues [41] tried to verify the uncertainty by directly visualizing polymer structure. Three samples with varying DA were uranyl stained then observed under transmission electron microscopy (TEM). It was proved that staining agents could help fix aggregate morphology under high vacuum. The TEM micrographs indicated a worm-like, single chain which was composed of blocks of almost fully deacetylated polysaccharides and full acetylated polysaccharides. The acetylated polysaccharides were micelle-like agglomerates. With a higher DA, both chain length and agglomerates' radius decreased. Indeed strings were compacted with a higher DA, because of less electrostatic repulsion among ammonium groups.

The Brugnerotto group [9] concerned the factors that influenced the physical properties of chitosan. NMR was used to examine DA and distribution of acetyl groups along the chain in order to determine chemical structures. GPC and an Ubbelohde capillary viscometer were used to analyze MWD and viscosities, respectively. The solvent was 0.2M AcONa/0.3M AcOH. Through computation, L_p was 110 Å for heterogeneous samples (DA<25%), and chain stiffness was not influenced by DA. At DA<60%, L_p increased slightly with DA. The chain was more flexible when temperature increased, which caused variation in viscosity.

Rinaudo and her colleagues [40] characterized polymer behaviors by using a multidetection instrument to determine MWD. Indeed the Mark-Houwink parameters in 0.2M AcONa/0.3M AcOH were calculated and compared to previous literature. The experiment was similar to Brugenerotte group and the calculated [η] and R_G indicated the solvent was bad, causing aggregation. L_p was irrelevant to DA, which was in agreement with the previous article (DA<25%).

3. EXPERIMENTAL

3.1. Preparation

Two chitosan samples were used in this article. Sample A with 5% DA is from the graduate work of Dr. Sanghoon Lim and was described in his PhD thesis with the number L3-56-3 (FPS-NCSU 2002). Sample B is commercial chitosan 00-ASSC-7676 with 15% DA (Vanson Inc, Redmond WA).

The samples were dried overnight at 80 ℃ in Fisher constant temperature oven then dissolved directly into 6 solvents with various acid and salt concentrations (see Table 4.2). Among them one batch (Sample A dissolving in HAc 0.3M/NaAc 0.3M solvent) was also made as a matched group, using serial dilution process. Dilutions are made by mixing a known volume of sample with a known volume of diluent [42] and the serial process is to use the previous solution as the mixed sample for next dilution production. In this way, dilutions are more accurate because systematic errors can be avoided in plotting intrinsic viscosity versus solute concentration. Usually each serial dilution is made by mixing the same volume of material with the same volume of diluent [42], however in order to make the data comparable to those solutions that were made individually by dissolving different amount of samples into each given solvent, different amounts of sample and solution were mixed in this article (Figure 3.1). Solutions were then centrifuged (IEC HN-SII Centrifuge) for 10 minutes and stored at 5 ℃.

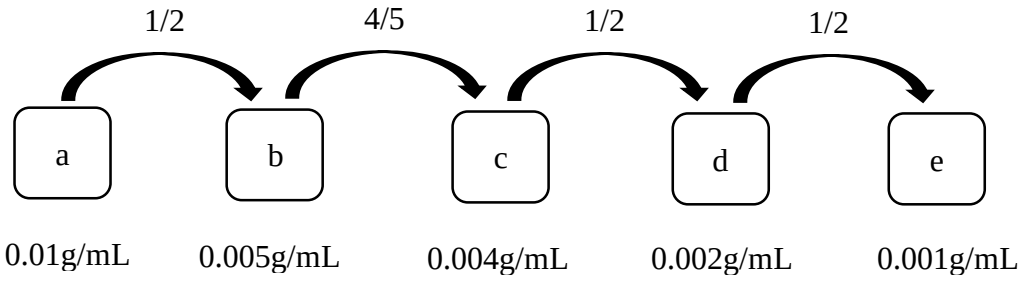

Figure 3.1. The serial dilution process for sample A dissolving in HAc 0.3M/NaAc 0.3M solvent (fraction represents the adding ratio of the previous solution)

3.2. Determination of Intrinsic Viscosity

Flow time was measured using an Ubbelohde capillary (Cannon-Fenske, No. 75) in Cannon CT-1000 constant temperature bath, which maintained temperature to be 30 ±0.1 and 40 ± 0.1˚C.

The samples were kept at room temperature for 24 h and placed in the constant temperature bath at 30 or 40 ℃ for 1 h before measuring flow time at the same temperature. Before formal measurement, each solution was made to flow through the capillary once for rinsing. And flow time for each sample was measured for 3 times. The relative viscosity η_{rel} equals to t/t_0, where t is the flow time of solution and t_0 is the solvent flow time.

4. RESULTS

The reduced viscosity for a polyelectrolyte is very complicated, varying as a function of molar mass, solvent quality, temperature and concentration [43]. Viscosity is usually higher than it would be in neutral solutions because there exists the electroviscous effect. The ion atmosphere is distorted by solvent stress in macroions' vicinity, giving rise to additional energy consumption [44]. At higher concentrations with more ions the thickness of ion atmosphere is small and this effect is neglectable. A polyelectrolyte with and without additional salt behaves as described above (Figure 4.1). In a salt-free solvent, the reduced viscosity increases dramatically to maximum as concentration is reduced because of the decreasing dissociation and coulomb repulsion of ionic groups and polymer coils extend [44]. Then at very low concentration the reduced viscosity decreases again because of the extreme low concentration of polymer coils even though they're highly expanded [43]. The solution is in a dilute state before reduced viscosity approaches the maximum value. By adding low-molecular salt, the coulomb repulsion force is shielded by external counter ions so the increasing of reduced viscosity is restrained [43]. By adding more salt, solutions behave like a neutral polymer and it's possible to extrapolate the reduced viscosity and determine intrinsic viscosity.

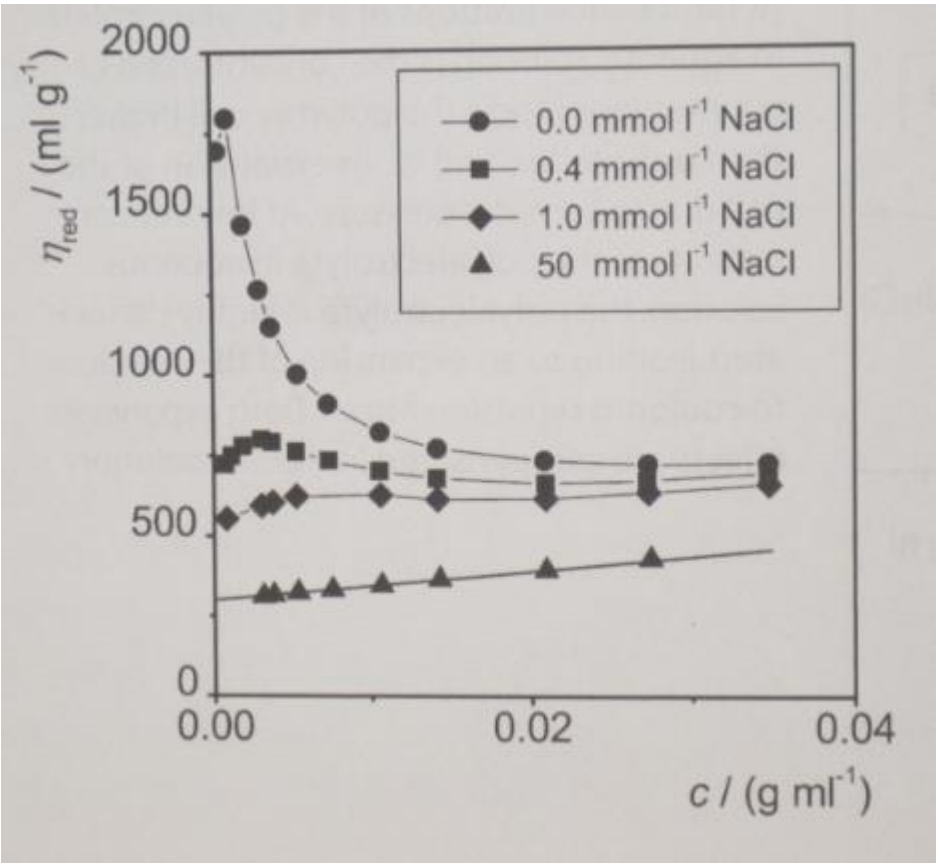

Figure 4.1. Reduced viscosity as a function of concentration for sodium pectinate in aqueous solution and different sodium chloride concentrations at 27 ℃ [43]

When chitosan is dissolved in acidic solvents, the polyelectrolyte solution have this equilibrium:

$$Chit\text{-}NH_2 + XH \leftrightarrow Chit\text{-}NH_3^+ + X^-$$

and the results are independent of X [40]. In this article HAc is the acid and different concentrations of NaAc salt was added to determine intrinsic viscosities. However since these are salt "impurities", the results can not reflect chain expansion in salt-free system.

Several viscosity terms are to be followed in Table 4.1, in which η is the solution viscosity and η_0 is the viscosity of a pure solvent.

Table 4.1. Some common viscosities and their definitions

Name	**Definitions**
Relative viscosity	$\eta_{rel} = \eta/\eta_0$
Specific viscosity	$\eta_{sp} = (\eta - \eta_0)/\eta_0 = \eta_{rel} - 1$
Reduced viscosity	$\eta_{red} = \eta_{sp}/C = (\eta_{rel} - 1)/C$
Inherent viscosity	$\eta_{inh} = (\ln \eta_{rel})/C$
Intrinsic viscosity	$[\eta] = (\eta_{red}/c)_{c\rightarrow 0} = (\eta_{inh})_{c\rightarrow 0}$

The intrinsic viscosity [η] was determined using Huggins (1) and Kraemer (2) equations [45, 46].

$$\eta_{sp}/C = [\eta] + k' [\eta]^2 C \quad (1)$$

$$(\ln \eta_{rel})/C = [\eta] + k'' [\eta]^2 C \quad (2)$$

k’ and k’’ are Huggins and Kraemer constants, respectively and C is the concentration of solution. By plotting η_{sp}/C and $(\ln \eta_{rel})/C$ against polymer concentration respectively and extrapolating to zero concentration, [η] values could be calculated. The experimental data and calculated results are listed in Table 4.2 and Table 4.3. Figure 4.2 demonstrated the Huggins and Kraemer plots. For both samples two solvents with highest salt concentration (0.3M) were also tested under 40 ℃. For sample A a serial dilution was compared in HAc 0.3M/NaAc 0.3M solvent to make sure that system error is sufficiently small. Notice that some datapoints were neglected because of deviation.

Table 4.2(a). Original flow time and reduced and inherent viscosities for Sample A

Sample	Solvent (HAc/NaAc)	Solvent Flow Time(s)	C(g/mL)	Flow Time(s)	η_{red} (mL/g)	η_{inh} (mL/g)
A	0.1M/0.01M	97.28	0.001	154.87	592.00	464.99
			0.002	203.85	547.75	369.90
			0.004	321.37	575.89	298.75
			0.005	389.55	600.88	277.48
			0.01	719.69	639.81	200.12
A	0.1M/0.1M	97.38	0.001	125.66	290.41	254.96
			0.002	158.71	314.90	244.23
			0.004	235.29	354.05	220.55
			0.005	283.32	381.89	213.59
			0.01	538.31	452.79	170.98
A	0.1M/0.3M	103.46	0.001	127.53	232.65	209.17
			0.002	156.39	255.80	206.58
			0.004	222.79	288.35	191.76
			0.005	269.10	320.20	191.18
			0.01	542.72	424.57	165.74
A	0.1M/0.3M	**40˚C**				
		85.67	0.001	104.03	214.31	194.18
			0.003	148.57	244.74	183.52
			0.005	202.80	273.44	172.34
			0.007	270.83	308.76	164.43
A	0.3M/0.01M	97.87	0.001	144.33	474.71	388.46
			0.002	201.11	527.43	360.11
			0.004	331.47	596.71	304.97
			0.005	392.41	601.90	277.73
			0.01	760.47	677.02	205.03
A	0.3M/0.1M	99.95	0.001	125.33	253.93	226.28
			0.002	154.61	273.44	218.12
			0.004	226.62	316.83	204.65
			0.005	271.63	343.53	199.95
			0.01	567.02	467.30	173.57
A	0.3M/0.3M	105.66	0.001	128.80	219.00	198.03
			0.002	154.83	232.68	191.05
			0.004	223.66	279.20	187.48
			0.005	263.36	298.50	182.66
			0.01	538.28	409.45	162.82

Table 4.2(a). Continued

A	0.3M/0.3M	**40˚C**				
		86.77	0.001	105.23	212.75	192.89
			0.003	151.85	250.06	185.49
			0.005	214.13	293.56	180.66
			0.007	276.43	312.25	165.53

Table 4.2(b). Original flow time and reduced and inherent viscosities for Sample B

Sample	Solvent (HAc/NaAc)	Solvent Flow Time(s)	C(g/mL)	Flow Time(s)	η_{red} (mL/g)	η_{inh} (mL/g)
B	0.1M/0.01M	97.28	0.001	152.94	572.16	452.45
			0.002	217.73	619.09	402.83
			0.004	345.08	636.82	316.55
			0.005	432.93	690.07	298.60
			0.01	965.28	892.27	229.48
B	0.1M/0.1M	97.38	0.001	126.70	301.05	263.17
			0.002	165.38	349.15	264.81
			0.004	273.37	451.82	258.05
			0.005	472.04	769.49	315.69
			0.01	1149.89	1080.82	246.88
B	0.1M/0.3M	103.46	0.001	139.39	347.32	298.11
			0.002	231.11	616.91	401.85
			0.004	303.06	482.31	268.69
			0.005	408.12	588.95	274.48
			0.01	1056.05	920.73	232.31
B	0.1M/0.3M	**40˚C**				
		85.67	0.001	110.97	295.32	258.76
			0.003	187.80	397.38	261.62
			0.005	285.47	466.44	240.73
			0.007	476.93	652.44	245.27
B	0.3M/0.01M	97.87	0.001	188.38	924.76	654.80
			0.002	292.37	993.68	547.20
			0.004	362.21	675.22	327.14
			0.005	446.23	711.88	303.44
			0.01	774.37	691.23	206.84
B	0.3M/0.1M	99.95	0.001	135.17	352.41	301.89
			0.002	178.34	392.16	289.52
			0.004	369.59	674.43	326.93
			0.005	396.15	592.70	275.43
			0.01	910.75	811.21	220.96
B	0.3M/0.3M	105.66	0.001	154.01	457.60	376.79
			0.002	194.90	422.30	306.13
			0.004	328.99	528.41	283.95
			0.005	386.97	532.48	259.62
			0.01	950.47	799.55	219.67

Table 4.2(b). Continued

B	0.3M/0.3M	**40˚C**				
		86.77	0.001	115.40	329.95	285.14
			0.003	211.63	479.66	297.19
			0.005	321.80	541.73	262.13
			0.007	503.43	685.98	251.17

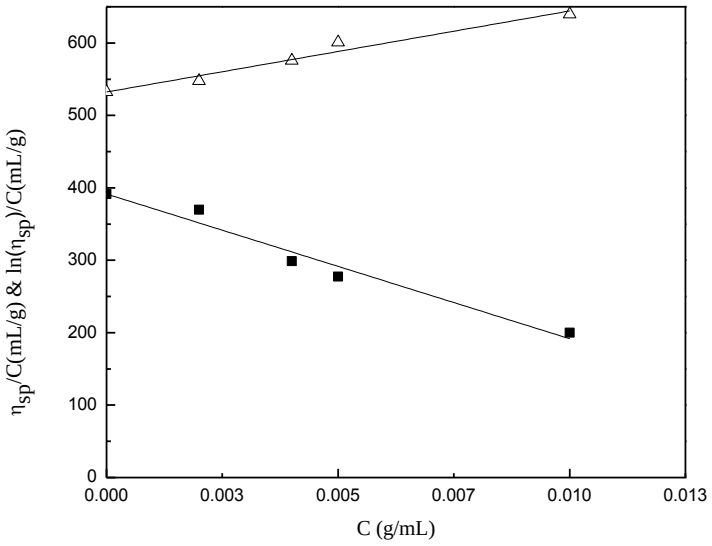

Figure 4.2.1. Kraemer and Huggins plots for sample A in HAc 0.1M/NaAc 0.01M solvent at 30 ℃ (Datapoint with solute concentration 0.001g/mL is neglected.)

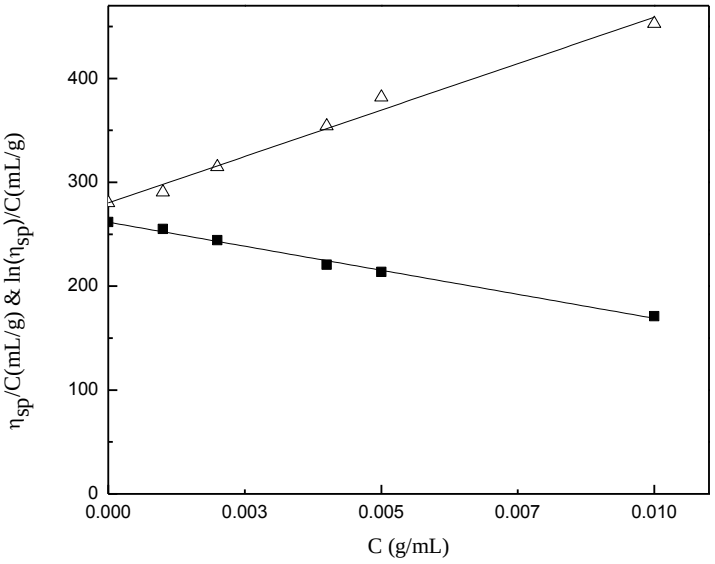

Figure 4.2.2. Kraemer and Huggins plots for sample A in HAc 0.1M/NaAc 0.1M solvent at 30 ℃

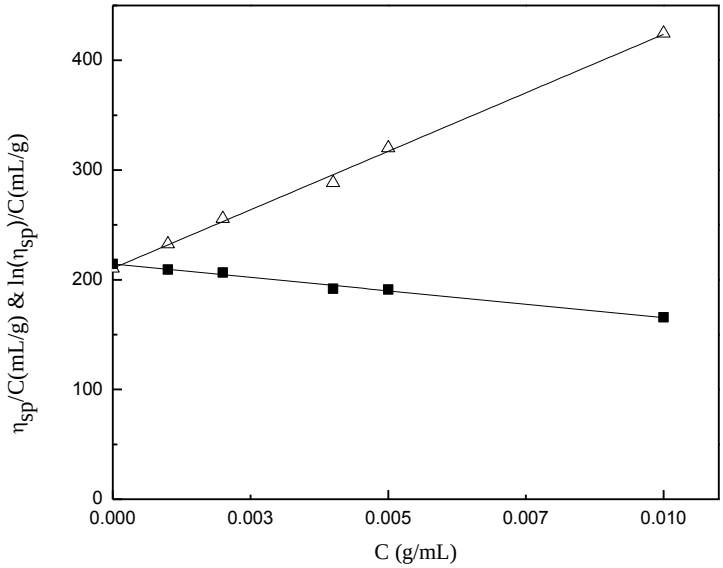

Figure 4.2.3(a). Kraemer and Huggins plots for sample A in HAc 0.1M/NaAc 0.3M solvent at 30 ℃

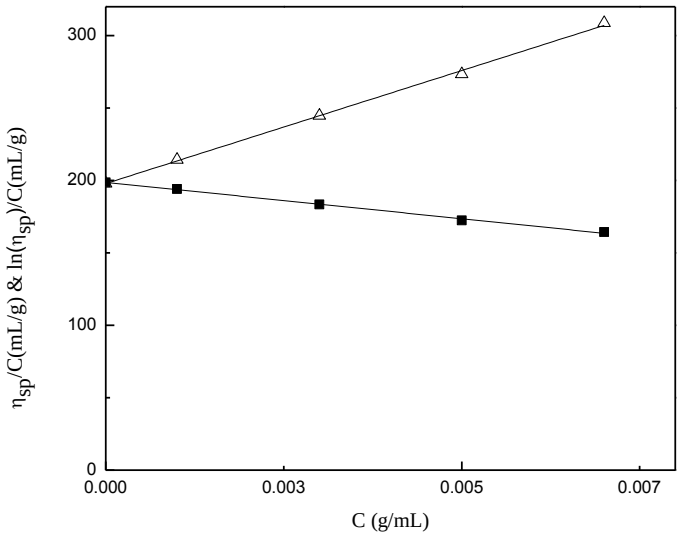

Figure 4.2.3(b). Kraemer and Huggins plots for sample A in HAc 0.1M/NaAc 0.3M solvent at 40 ℃

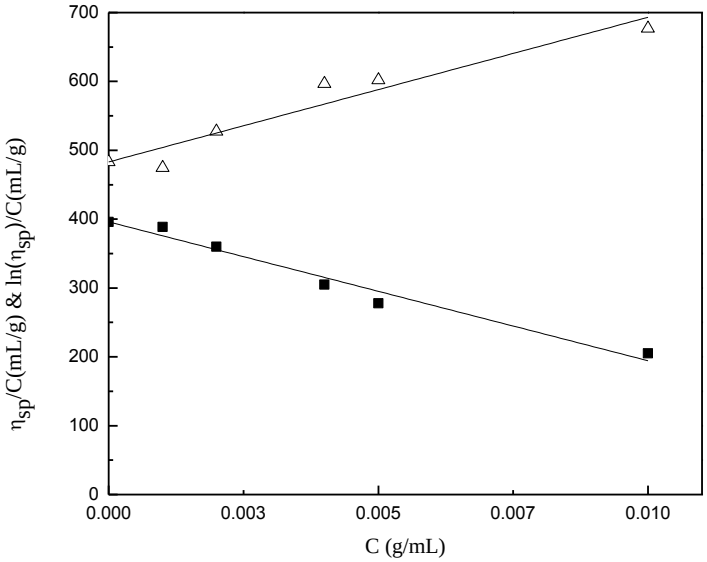

Figure 4.2.4. Kraemer and Huggins plots for sample A in HAc 0.3M/NaAc 0.01M solvent at 30 ℃

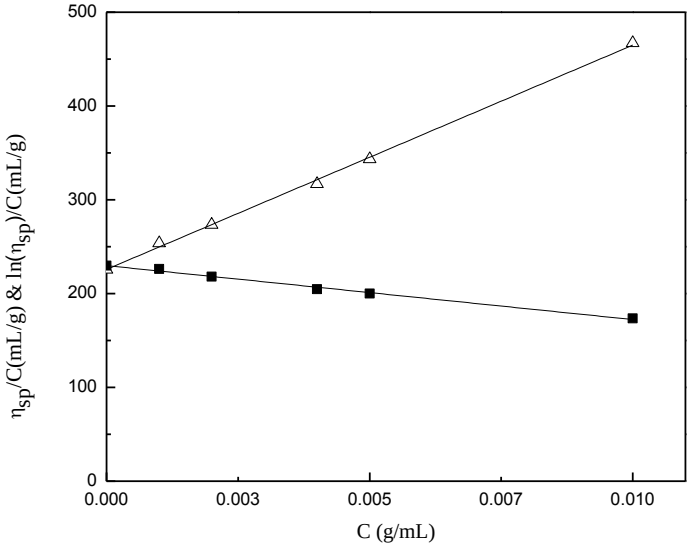

Figure 4.2.5. Kraemer and Huggins plots for sample A in HAc 0.3M/NaAc 0.1M solvent at 30 ℃

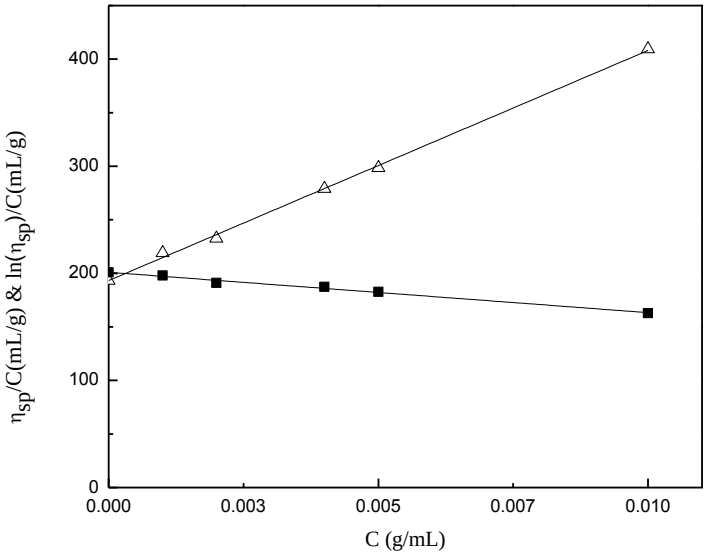

Figure 4.2.6(a). Kraemer and Huggins plots for sample A in HAc 0.3M/NaAc 0.3M solvent at 30 ℃

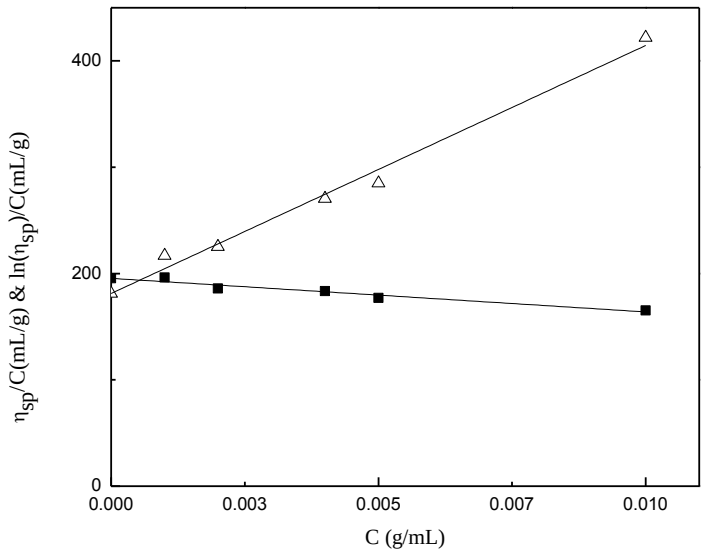

Figure 4.2.6(b). Kraemer and Huggins plots for sample A in HAc 0.3M/NaAc 0.3M solvent at 30 ℃ using serial dilution

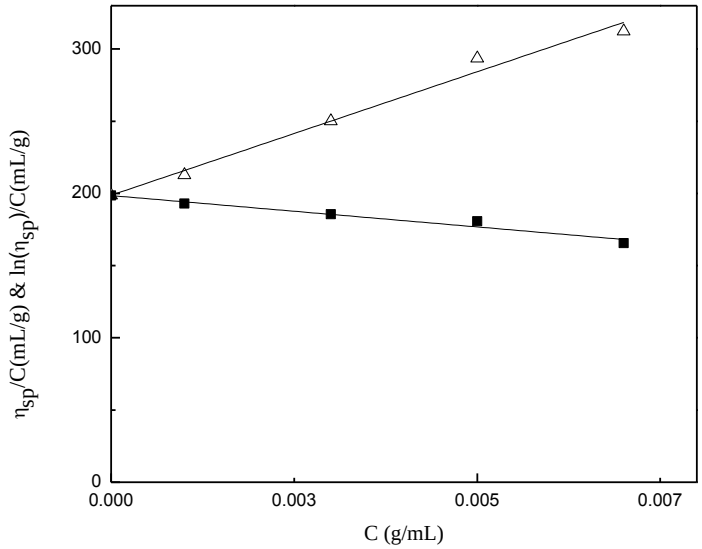

Figure 4.2.6(c). Kraemer and Huggins plots for sample A in HAc 0.3M/NaAc 0.3M solvent at 40 ℃

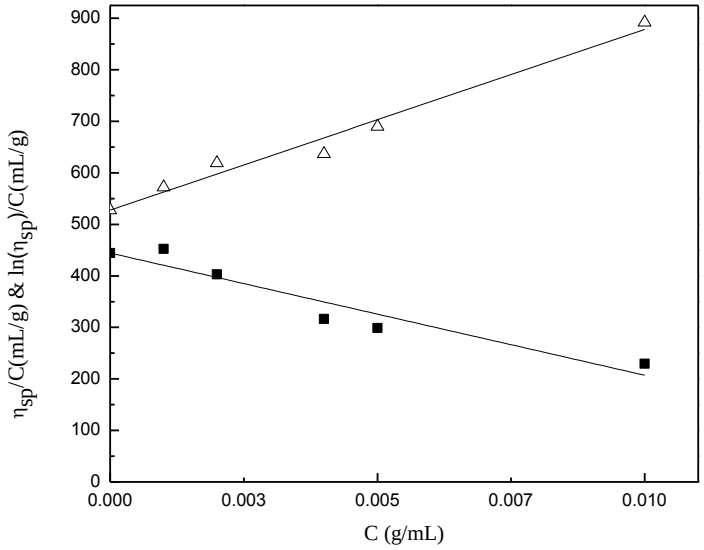

Figure 4.2.7. Kraemer and Huggins plots for sample B in HAc 0.1M/NaAc 0.01M solvent at 30 ℃

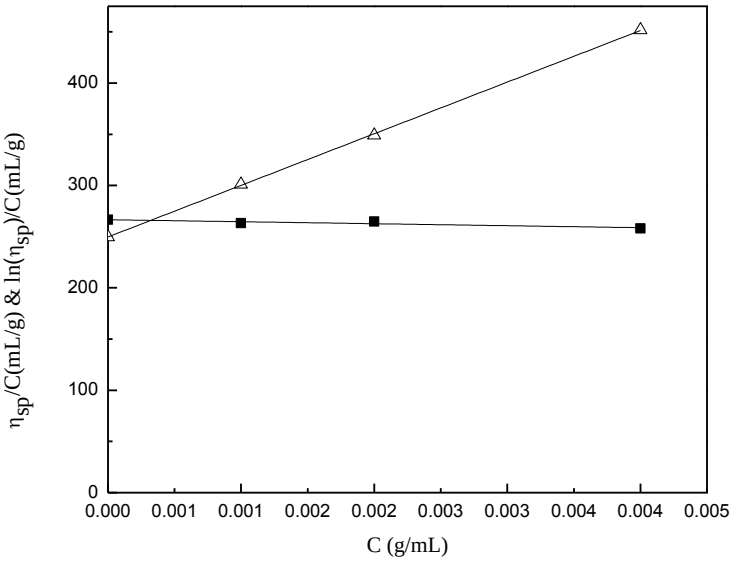

Figure 4.2.8. Kraemer and Huggins plots for sample B in HAc 0.1M/NaAc 0.1M solvent at 30 ℃ (Datapoints with solute concentration 0.005g/mL and 0.01g/mL are neglected.)

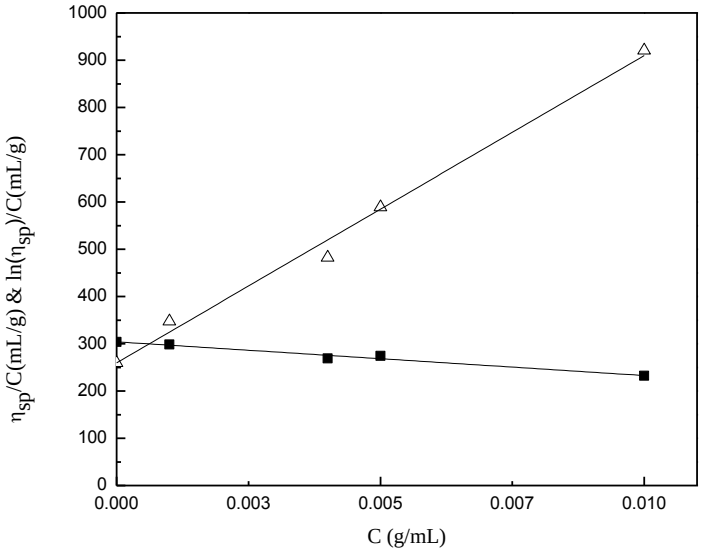

Figure 4.2.9(a). Kraemer and Huggins plots for sample B in HAc 0.1M/NaAc 0.3M solvent at 30 ℃ (Datapoints with solute concentration 0.002g/mL is neglected.)

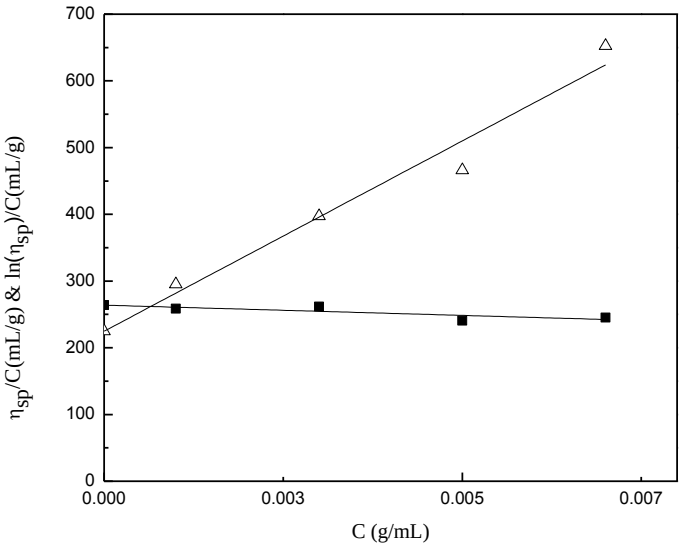

Figure 4.2.9(b). Kraemer and Huggins plots for sample B in HAc 0.1M/NaAc 0.3M solvent at 40 ℃

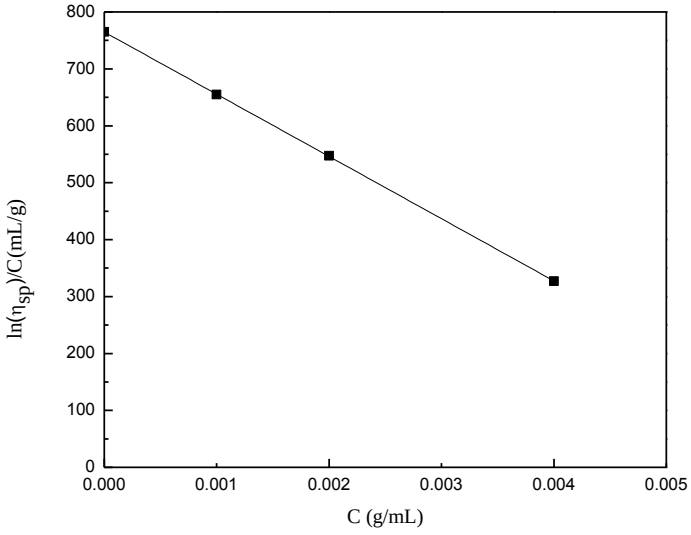

Figure 4.2.10. Kraemer plot for sample B in HAc 0.3M/NaAc 0.01M solvent at 30 ℃ (No effective data for Huggins plot and datapoints with solute concentration 0.005g/mL and 0.01g/mL are neglected.)

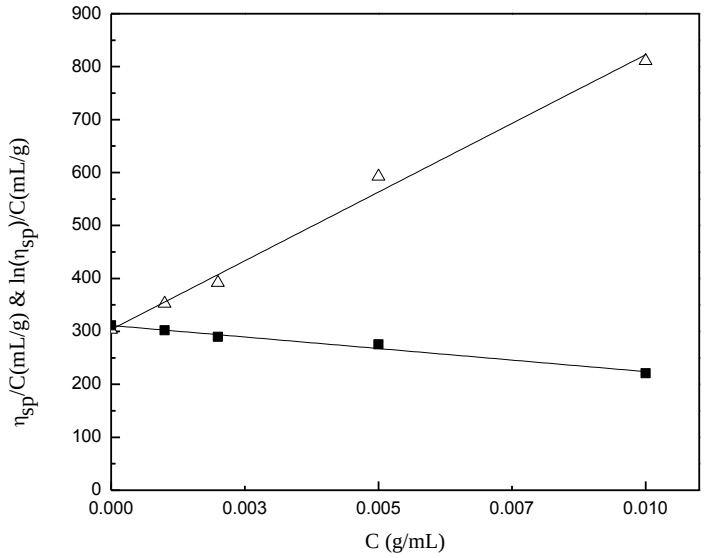

Figure 4.2.11. Kraemer and Huggins plots for sample B in HAc 0.3M/NaAc 0.1M solvent at 30 ℃ (Datapoints with solute concentration 0.004g/mL is neglected.)

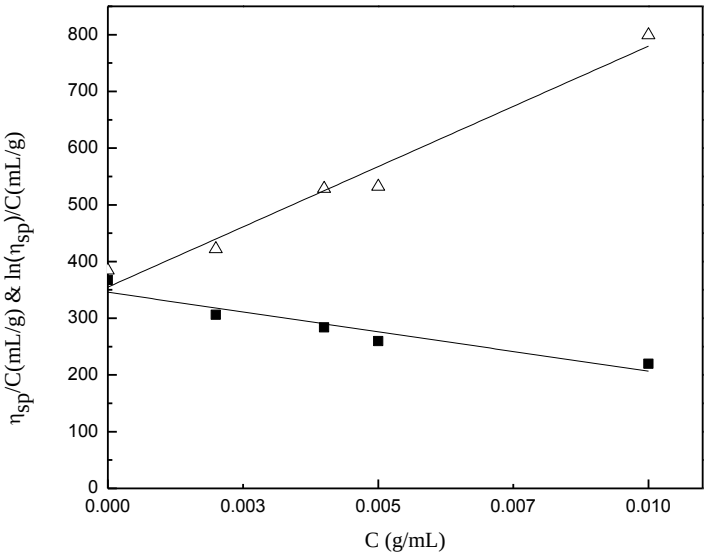

Figure 4.2.12(a). Kraemer and Huggins plots for sample B in HAc 0.3M/NaAc 0.3M solvent at 30 ℃ (Datapoints with solute concentration 0.001g/mL is neglected.)

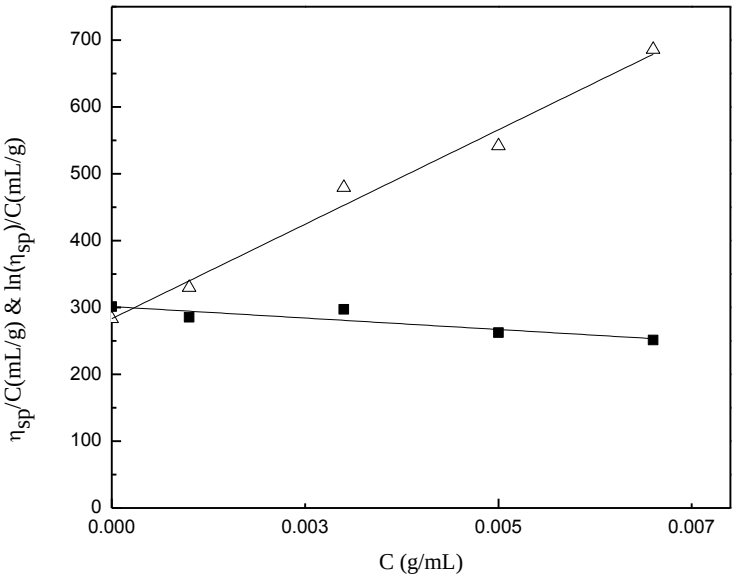

Figure 4.2.12(b). Kraemer and Huggins plots for sample B in HAc 0.3M/NaAc 0.3M solvent at 40 ℃

Table 4.3. Intrinsic Viscosity [η], Huggins Constant k' and Kraemer Constant k'' for two chitosan samples at temperature 30 and 40 ℃

Sample	**Solvent (HAc/NaAc)**	**[η](mL/g)**		**k'**		**k''**		**k' + k''**	
A	0.1M/0.01M	461.91		0.05		-0.09		-0.04	
	0.1M/0.1M	270.86		0.24		-0.13		0.11	
	0.1M/0.3M	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**
		212.44	198.4	0.47	0.40	-0.11	-0.13	0.36	0.27
	0.3M/0.01M	439.55		0.11		-0.10		0.01	
	0.3M/0.1M	227.79		0.46		-0.11		0.35	
	0.3M/0.3M	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**
		197.1	198.64	0.55	0.44	-0.10	-0.11	0.45	0.33
B	0.1M/0.01M	486.11		0.15		-0.10		0.05	
	0.1M/0.1M	258.14		0.76		-0.03		0.73	
	0.1M/0.3M	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**
		281.82	244.34	0.82	0.96	-0.09	-0.05	0.73	0.91
	0.3M/0.01M	764.83		-		-0.19		-	
	0.3M/0.1M	307.34		0.55		-0.09		0.46	
	0.3M/0.3M	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**	**30˚C**	**40˚C**
		375.94	292.31	0.28	0.66	-0.12	-0.08	0.16	0.58

5. DISCUSSION

5.1. Huggins k' and Kraemer k''

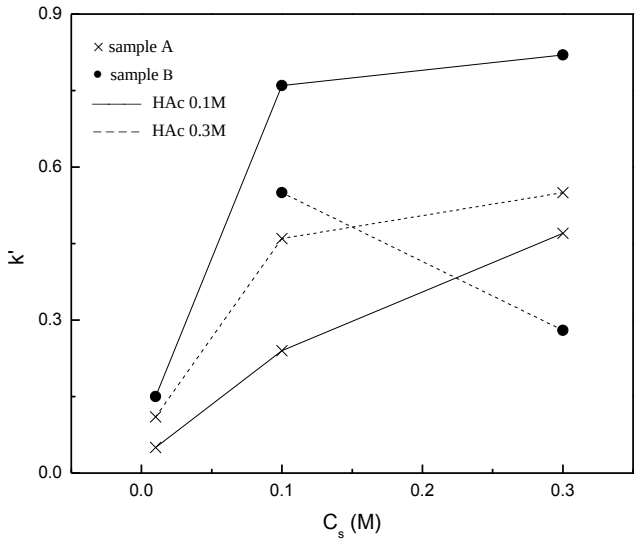

Figure 5.1. Effect of salt concentration on Huggins k'

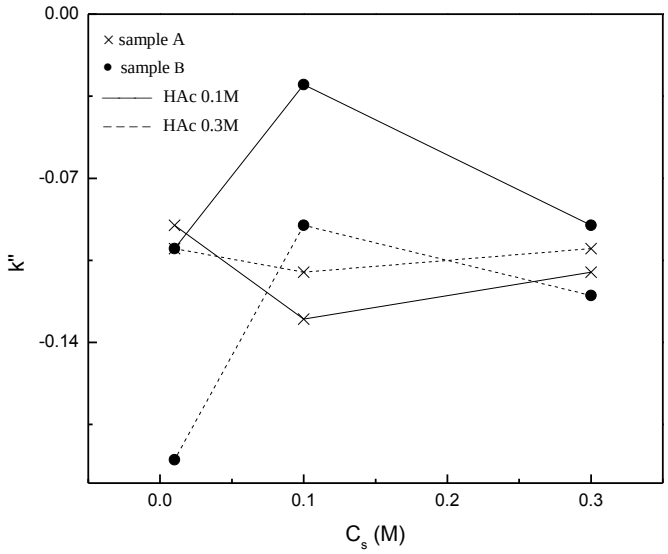

Figure 5.2. Effect of salt concentration on Kraemer k''

According to Flory, k' usually varies from 0.3 to 0.8 and is approximately constant for a series of polymer homologs in a given solvent [45]. In good solvent k' is expected to be 0.35 to 0.40 [45].The Huggins k' as a function of salt concentration is plotted in Figure 5.1. For sample A, the Huggins constant values vary from 0.05 to 0.55. Solvents with least salt (NaAc 0.01M) have very small k' value, indicating bad polymer solvation in a solvent which is too dilute. With the increase of salt concentration, the Huggins k' value also increases, representing better solvents. Higher temperature results in lower k', but the influence isn't that significant and this is in accordance with the influence of temperature on intrinsic viscosity. For chitosan sample B, temperature is a more important influencing factor. The Huggins k' values have tremendous variation in different solvents, ranging from 0.15 to 0.96. In HAc 0.3M/NaAc 0.01M solvent, k' isn't approachable since the salt concentration is too low to help polyelectrolytes behave like neutral solutions. However in the solvent with highest salt concentration (NaAc 0.3M) there's a "salt out" situation where k' decreases, since the solution behaves thermodynamically poor and polymer chains tend to entangle by themselves and precipitate out from solvent. Results demonstrate that in order to calculate intrinsic viscosity from Huggins equation, there are conditions to be met since the slope of Huggins plot must always be positive. Little or excess salt concentration might result in failure in extrapolating viscosities. Usually the Kraemer constant k'' is negative and smaller in magnitude than k' [45] and similar trend can be seen in figure 5.2.

We can extrapolate from Eq. (1) and (2) that k'+ k''= 0.5 theoretically [47]. The practical experimental data demonstrate varying results. When the sum-up value gets larger, the solution behaves better. As long as the value is over 0.35, it is considered a good solvent.

For sample A the most proximate sum-ups are 0.36 in 0.1M HAc/0.3M NaAc, 0.35 in HAc 0.3M/NaAc 0.1M and 0.45 in HAc 0.3M/NaAc 0.3M at 30 ℃. For sample B HAc 0.1M/NaAc 0.1M, HAc 0.1M/NaAc 0.3M and HAc 0.3M/NaAc 0.1M at 30 ℃ are all good solvents.

The values of k' and k'' provide us information in characterizing polymer-solvent interaction [47]. A larger Huggins k' value indicates a better solvent for polymer, while the polymer-solvent interactions are more favored than polymer-polymer interactions [48]. However, data from literature are not always approximate, sometimes even contradictory [47]. Some inevitable experimental errors attribute more on the value of k' and k'' than the intrinsic viscosity [49].

5.2. Influencing Factors of Intrinsic Viscosity

5.2.1. Effect of molecular weight on intrinsic viscosity

The viscosity average molecular weight might be determined by the Mark-Houwink equation,

$$[\eta] = KM^{\alpha} \quad (3)$$

where [η] is the intrinsic viscosity and K and α are Mark-Houwink constant and exponent respectively for a specific polymer in a particular solvent-temperature system [47]. The value of α is related to ionic strength, temperature and pH [50], varying from 0 to 2, equals to 0.5 in a θ solvent and 0.8 in a good solvent [45, 51]. α is also related to chain conformation, for if it's lower than 0.5, 0.5 to 0.8 and higher than 1.0, the shape is spherial, a random coil and rod-like, respectively [50]. The K and α constants for chitosans have been calculated by

many researchers for various solvents over decades. The value of K ranges from 3.0×10^{-5} to 5.6×10^{-1} and the value of α lies in between 0.58 to 1.26 [52]. Table 5.1 lists the Mark-Houwink parameters determined by Rinaudo group. We were able to estimate the molecular weight range and the results are listed in Table 5.2 and Figure 5.4.

Table 5.1. Mark-Houwink parameters for chitosans in HAc 0.3M/NaAc 0.2M [40]

DA (%)	**K (mL/g)**	**α**
2	0.082	0.76
11.5	0.076	0.76
21	0.074	0.76

Table 5.2. Estimated molecular weight according to Rinaudo's K and α

Sample	**K (mL/g)**	**α**	**Solvent (HAc/NaAc)**	**Molecular Weight**
A	0.082	0.76	0.1M/0.01M	8.6×10^4
			0.1M/0.1M	4.3×10^4
			0.1M/0.3M	3.1×10^4
			0.3M/0.01M	8.1×10^4
			0.3M/0.1M	3.4×10^4
			0.3M/0.3M	2.8×10^4
B	0.076	0.76	0.1M/0.01M	1.0×10^5
			0.1M/0.1M	4.4×10^4
			0.1M/0.3M	5.0×10^4
			0.3M/0.01M	1.8×10^5
			0.3M/0.1M	5.6×10^4
			0.3M/0.3M	7.3×10^4

The particular solvent in Rinaudo's article was HAc 0.3M/NaAc 0.2M. Usually there's a linear relationship between ionic strength and experimental viscosity: [53]

$$[\eta] = [\eta]_{\infty} + SC_s^{-1/2} \quad (4)$$

where the slope S is related to molecular stiffness [54] , which will be discussed later in this article. C_s is the molar salt concentration.

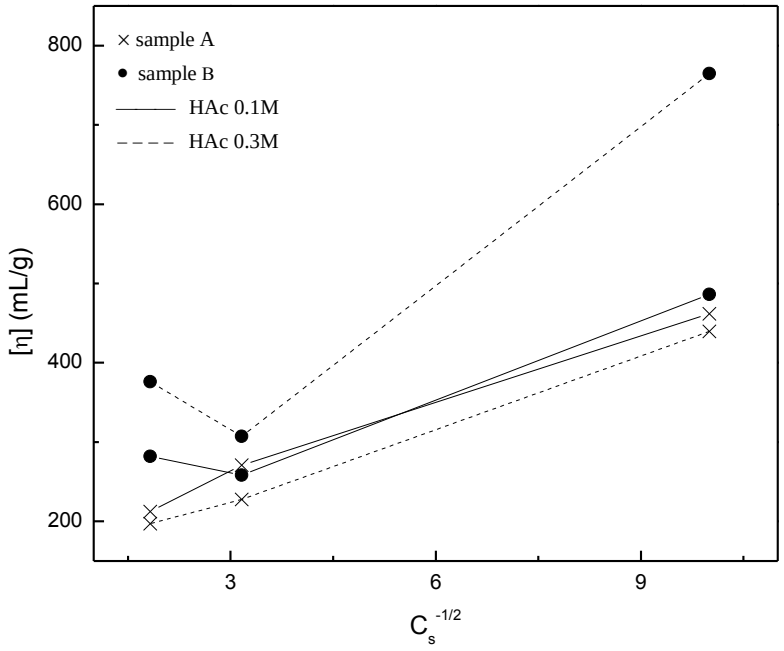

Figure 5.3. Plot of intrinsic viscosity as a function of salt concentration for both chitosans

From Figure 5.3, it's easy for us to calculate intrinsic viscosities in HAc 0.3M/NaAc 0.2M solvent by linear regression method for the two plots with 0.3M acid concentration. For sample B datapoints in 0.3M NaAc solvents are neglected since the salt concentration is too high, polymers might separate out from solvents according to previous discussion. Table 5.3 lists the results.

Table 5.3. Calculated molecular weight for solvent HAc 0.3M/NaAc 0.2M

Sample	**Deduced [η] in HAc 0.3M/NaAc 0.2M(mL/g)**	**Molecular Weight**
A	205.11	3.0×10^4
B	245.36	4.1×10^4

The hydrodynamic properties of chitosans are influenced by many factors including pH, salt concentration (ionic strength) and temperature [54]. We evaluated the molecular weight for both samples using Rinaudo's data, however factors such as pH and temperature weren't taken into consideration. Kasaai developed two equations in determination of K and α values for any chitosan-solvent system: [52]

$$\alpha = 0.6202 + 0.699x / (0.4806 + x) \quad (5)$$

$$\log (K \cdot 10^5) = -5.7676 \cdot \alpha + 5.9232 \quad (6)$$

with x equals DA/(pH·μ), where μ represents ionic strength. Results are listed in Table 5.7.

Table 5.4. Mark-Houwink constants K and α and molecular weight calculated from Kasaai's equations

Sample	Solvent (HAc/NaAc)	pH	μ (M)	α	K × 10^5(dL/g)	Molecular Weight
A	0.1M/0.01M	3.69	0.01	1.14	0.23	3.5×10^5
	0.1M/0.1M	4.64	0.1	0.75	40.53	1.3×10^5
	0.1M/0.3M	5.11	0.3	0.66	123.00	7.4×10^4
	0.3M/0.01M	3.22	0.01	1.15	0.19	3.3×10^5
	0.3M/0.1M	4.15	0.1	0.76	34.52	1.1×10^5
	0.3M/0.3M	4.61	0.3	0.67	115.90	6.7×10^4
B	0.1M/0.01M	3.69	0.01	1.25	0.06	3.8×10^5
	0.1M/0.1M	4.64	0.1	0.90	5.31	1.6×10^5
	0.1M/0.3M	5.11	0.3	0.74	46.15	1.3×10^5
	0.3M/0.01M	3.22	0.01	1.25	0.05	8.7×10^4
	0.3M/0.1M	4.15	0.1	0.92	4.13	1.9×10^5
	0.3M/0.3M	4.61	0.3	0.75	40.16	2.0×10^5

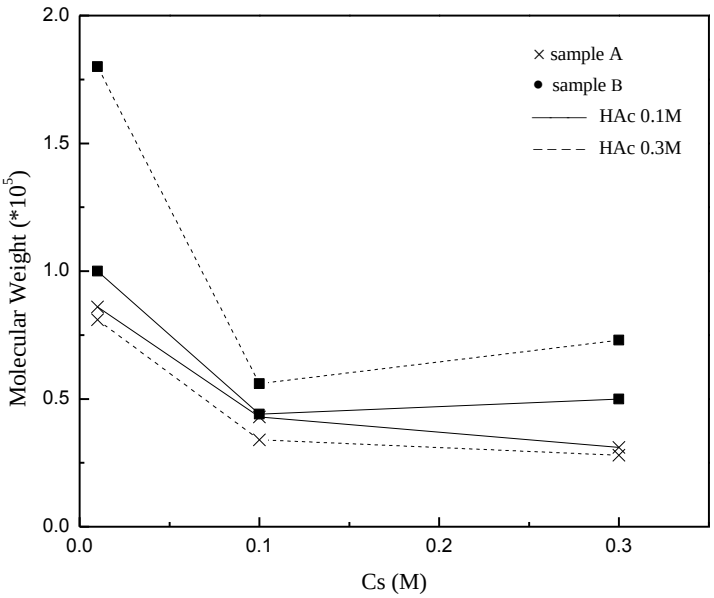

Figure 5.4. Estimated molecular weight under different salt concentration

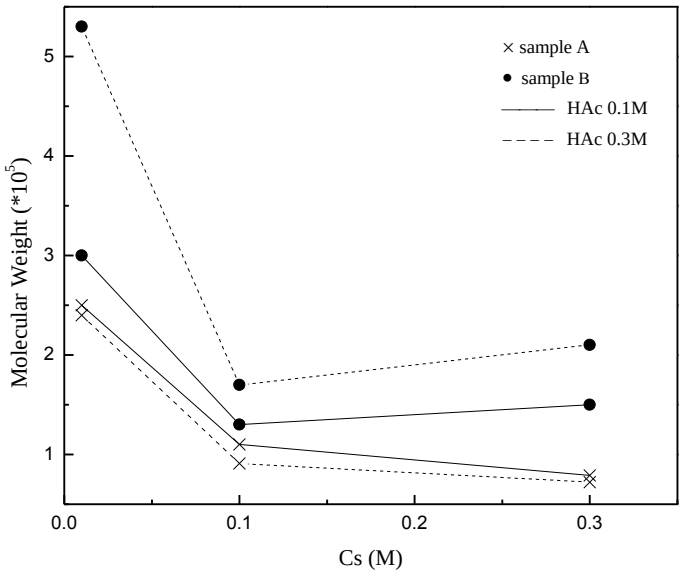

Figure 5.5. Calculated molecular weight under different salt concentration

Depending on Rinaudo's research, the molecular weight of sample A ranges from 2.8×10^4 to 8.6×10^4 and is approximately 3×10^4 (Figure 5.4). For sample B the molecular weight is between 4.4×10^4 and 1.8×10^5, and a more accurate value is 4.1×10^4 (Figure 5.4). While on the basis of Kasaai's demonstration, the molecular weight of sample A is between 6.7×10^4 and 3.5×10^5 and for sample B it is between 8.7×10^4 and 3.8×10^5 (Figure 5.5), and in solvents with same ionic strength, sample B has a higher molecular weight. It is manifest that the molecular weight of sample B is higher than that of sample A.

Based on Mark-Houwink equation, intrinsic viscosity is in proportional to the exponent of molecular weight. Table 4.3 shows in solutions with same salt concentration, the intrinsic viscosities of sample B are larger than those of sample A, demonstrating that the intrinsic viscosity of chitosans will increase with increasing molecular weight. Tsaih and Chen [55] had similar results after studying the influence of molecular weight on intrinsic viscosity for chitosans with same DA and at same ionic strength and pH solutions. They demonstrated that the volume fraction should be similar and molecular size was the only factor influencing molecular weight.

5.2.2. Effect of temperature on intrinsic viscosity

Table 5.5. Intrinsic viscosities at 30 and 40 ℃

Sample	Solvent (HAc/NaAc)	$[\eta]_{30}$ (mL/g)	$[\eta]_{40}$ (mL/g)	$[\eta]_{30} / [\eta]_{40}$	$-(d \ln[\eta] / dT)$
A	0.1M/0.3M	212.44	198.4	1.07	0.26
	0.3M/0.3M	197.1	198.64	0.99	0.04
B	0.1M/0.3M	281.82	244.34	1.15	0.36
	0.3M/0.3M	375.94	292.31	1.29	0.44

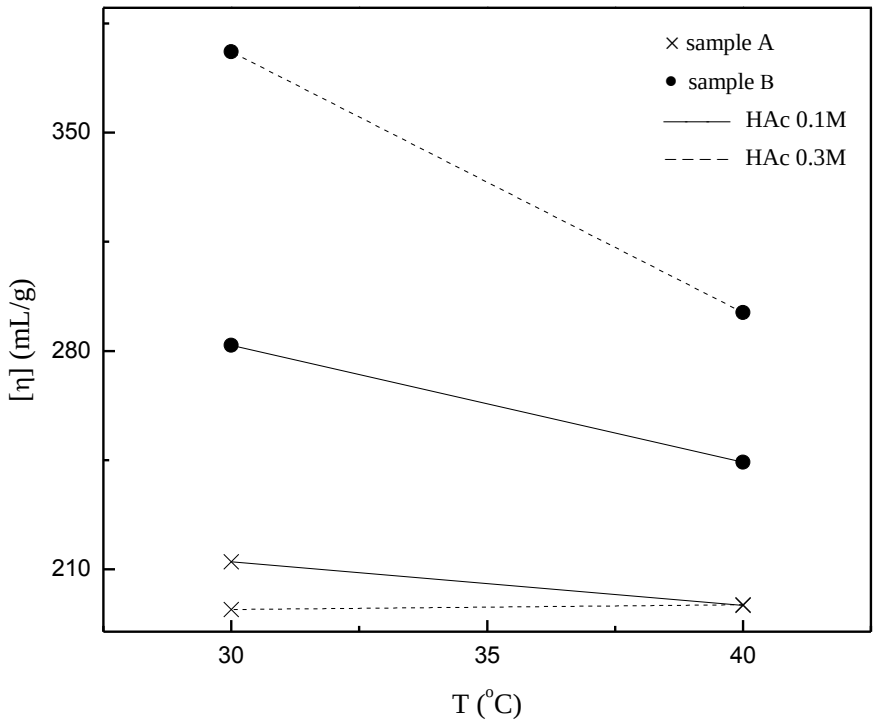

Figure 5.6. Intrinsic viscosity as a function of the temperature for both chitosans

In determining the intrinsic viscosity of polymers, it is important to keep a constant temperature. The intrinsic viscosity can increase, decrease or be independent of temperature, revealing different behavior [43]. The viscosities for chitosans A and B were measured at 30 and 40 ℃ in solvents with the strongest ionic strength (0.3M) and the results are listed in Table 5.5. Figure 5.6 indicates the effect of temperature on intrinsic viscosities for both samples.

For both samples in solvent HAc 0.1M/NaAc 0.3M and sample B in solvent HAc 0.3M/NaAc 0.3M, the temperature coefficients (d ln[η] / dT) are negative and relatively large, and intrinsic viscosities decrease rapidly with increasing temperature. Since the change of intrinsic viscosity might be caused by polymer-polymer (intramolecular) and polymer-solvent (intermolecular) interactions, we can conclude that when temperature increases, the intermolecular interactions become weaker while the intramolecular interactions get stronger [43]. At higher temperature, the coil expansion and the ratio of radius of gyration decreases, so does the average molecular weight, indeed chain is more flexible and intrinsic viscosity decreases [56, 57, 58]. Launay et al. [57] demonstrated that intrinsic viscosity was a function of volume fraction of solute and shape factor. Increasing in temperature might result in decrease in hydrogen-bonded hydration water [59], so volume fraction decreases.

Similar results were reported by Pogodina [60] and Chen [58] groups, and both of them noticed that the decrease of intrinsic viscosity was linear with increasing temperature. Chen and Tsaih [58] investigated intrinsic viscosity variation from 10 to 50 ℃ and there was a linear relationship, which manifested that in this temperature range there was no conformational transition for chitosan solutions.

For sample A in solvent HAc 0.3M/NaAc 0.3M, intrinsic viscosity didn't change much and the temperature coefficient is only 0.04, indicating a good solvent [61] and the influence of temperature in this circumstance is neglectable. From the figure it is also obvious that intrinsic viscosity is dependent on the solvent property, and sample B is more sensitive to acid concentration.

5.2.3. The effect of salt concentration on intrinsic viscosity

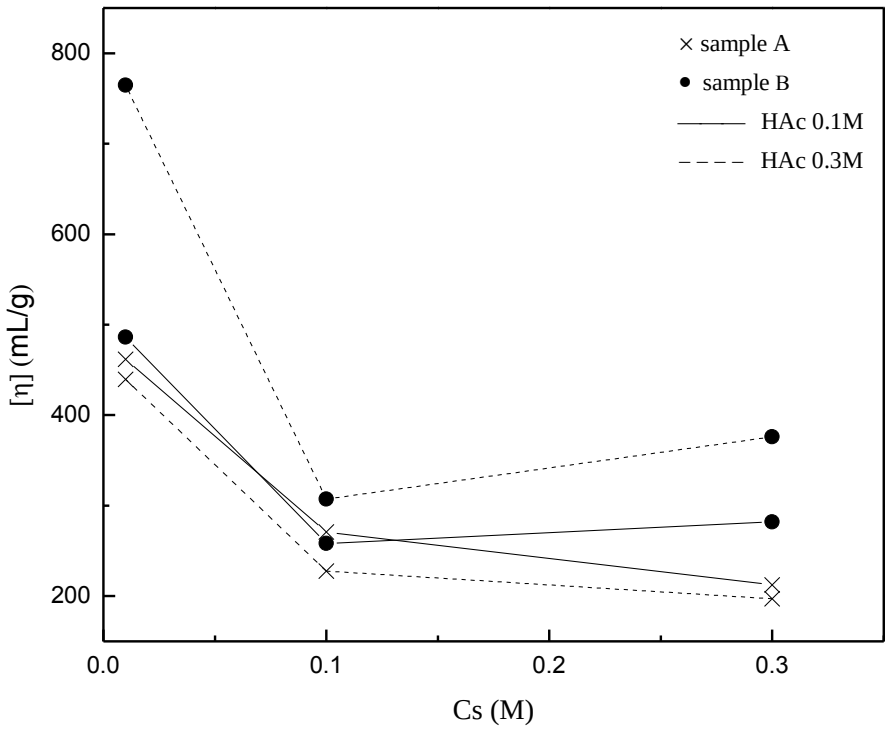

Figure 5.7. Intrinsic viscosities as a function of salt concentration (C_s) for both samples

From Table 4.3 and Figure 5.7, it is shown that in the same acidic solvents, the intrinsic viscosities of both samples have a decreasing tendency with increasing ionic strength. As a complicated polyelectrolyte, the stability of chitosan solutions is dependent on pH and external salt concentration of the solvent [12]. At low salt concentrations, the third electroviscous effect dominates. By reacting with ion-producing substance, the electrostatic repulsion between charges results in modification of the molecular free energy, indeed polymer chains remain extended [62]. However in solvents with high salt concentration, there are more counter-ions that screen the protonated amine group, resulting in molecule compactness, so intrinsic viscosity decreases [55]. Pogodina group [60] and Rodrigues-Sanchez group [63] reported similar results. However Chen et al. [64] demonstrated that the effect of ionic strength on the viscosities of chitosan-organic acid solutions was not significant, which might be attributed to the complete suppression of electrostatic repulsion by chloride ions in 0.3M HCl or a solvent with 0.5M NaCl.

Nevertheless for sample B, the intrinsic viscosities in solvents with the strongest ionic strength rebounded, in which case the solution might have very poor thermodynamical behavior and polymer chains are likely to self-entangled and even precipitate out from solvent.

We can also conclude that acid concentration is another influencing factor. For two samples the effects are opposite. And sample B is more sensitive on acid concentration.

5.2.4. Summary

From the analysis of molecular weight, it is demonstrated that sample B has a higher molecular weight than sample A. And higher molecular weight chitosans turn out to have higher intrinsic viscosities, which was manifested by Mark-Houwink equation. Increasing temperature results in a decrease intrinsic viscosity for most chitosans due to stronger polymer-solvent interaction and coil expansions. However a temperature-independent solution was also observed, showing a good solvent (HAc 0.3M/NaAc 0.3M) for chitosan sample A. The effect of ionic strength is negative, since molecules compact heavily because of the function of counter-ions in high salt concentration solutions.

Table 5.6 indicates the results of directly dissolved solutions (X) and serial diluted solutions (Y) for sample A in solvent HAc 0.1M/NaAc 0.3M. It is seen that data deviations are below 12% is 4.43% for final evaluated intrinsic viscosity, which could be neglected, while the results from serial dilutions are smaller than those from normally mixed solutions.

Table 5.6. Viscosities and deviation for directly dissolved (X) and serial diluted (Y) solution

Viscosities	C(g/mL)	X	Y	Deviation(%)
η_{red}	0.001	232.65	216.92	-6.76
	0.002	255.80	225.16	-11.98
	0.004	288.35	270.51	-6.19
	0.005	320.20	285.01	-10.99
	0.01	424.57	421.93	-0.62
η_{inh}	0.001	209.17	196.32	-6.14
	0.002	206.58	185.89	-10.02
	0.004	191.76	183.34	-4.39
	0.005	191.18	177.17	-7.32
	0.01	165.74	165.24	-0.31
[η] (mL/g)	--	197.10	188.36	- 4.43

5.3. Influence on Chain Stiffness

Table 5.7. Parameters relate to chain flexibility

Sample	Solvent	Stiffness parameter B	DA/(pH·μ)	*d* ln[η] / d (1/T)
A	0.1M/0.01M	-	1.36	-
	0.1M/0.1M	0.145	0.11	-
	0.1M/0.3M	-	0.03	651
	0.3M/0.01M	-	1.55	-
	0.3M/0.1M	0.182	0.12	-
	0.3M/0.3M	-	0.04	-74
B	0.1M/0.01M	-	4.07	-
	0.1M/0.1M	0.173	0.32	-
	0.1M/0.3M	-	0.10	1359
	0.3M/0.01M	-	4.66	-
	0.3M/0.1M	0.281	0.36	-
	0.3M/0.3M	-	0.11	2396

5.3.1. Relative stiffness parameter B

There are several ways in demonstrating the stiffness of a polymer chain. SmidsrØd and Haug [53] came up with the definition of stiffness parameter, B, which allowed for the comparison of stiffness in different polyelectrolytes. The empirical equation, [53]

$$S = B\,([\eta]_{0.1})^{\nu} \qquad (7)$$

relates B to salt tolerance, S, as mentioned in equation (4) where S is the slope of [η] vs $C_s^{-1/2}$. $[\eta]_{0.1}$ is the intrinsic viscosity at 0.1M ionic strength. The value of ν ranges from 1.2 for polyphosphate to 1.4 for DNA [73] and for chitosans the usual value equals to 1.217 [35]. In equations (4) and (7) the unit of [η] is dL/g. Stiffer chains result in lower B values [53].

Theoretically, the parameter B is a relative characteristic and the values of B for different polyelectrolytes are comparable regardless of DA and solvent [40], however most

of the authors didn't investigate B for different acid concentration. For each sample we got two B values, in 0.1 and 0.3M HAc solutions respectively. The results in Table 5.7 show that B parameters for sample A are 0.145 and 0.182, while for sample B are 0.173 and 0.281. Indeed the relative stiffness parameter B for sample B, which has a higher molecular weight, is greater than that of sample A. This is in accordance with the report of Tsiah and Chen [55], who demonstrated that for chitosans with molecular weights higher than or equal to 22.3×10^4 the value of B was between 0.143 and 0.152, whereas the parameter was between 0.110 and 0.138 for chitosans with 14.8×10^4 or less molecular weights. It is indicated that chitosans with higher molecular weight are more flexible, while lower molecular weight ones are stiffer. Trzciński et al. [65] investigated the effects of degree of acetylation and the type of counterions on B parameter and concluded that the value of B was independent of DA when DA < 0.21 and equal to 0.1 for the chitosans analyzed, and the effect of counter-ions was neglectable.

It is highlighted that B is only applicable to strong polyelectrolytes that have no conformational transition within the salt concentration range [53, 66]. And the compared polymers should have approximate charge density [35]. Kasaai [52] underlined that although there was evidence that higher molecular weight chitosans were more flexible, it was impropriate to say that the value of B increases with molecular weight since B parameter was comparable and should be independent of molecular weight.

5.3.2. pH-relevant parameter

Since the relative B parameter can't be used to evaluate chain stiffness for a specific polymer or in a characteristic solution (factors include molecular weight, μ, pH, T et al.), an empirical function, DA/(pH·μ), might be used to measure chain flexibility as a direct index, with the advantage of taking into consideration both the effects of solvent quality and chain structure [52]. Stiffer chains contribute to larger values of DA/(pH·μ).

Results are listed in Table 5.7. For each sample in solvents with the same acidity (DA and pH remain constant), the parameter value decreases sharply, indicating that higher ionic strength contributes to chain flexibility. For every specific solvent with the certain amount of acid and salt (pH and μ remain constant), the value of DA/(pH·μ) for sample B (DA=15%) is higher than that of sample A (DA=5%), making it clear that higher DA makes chitosan chains stiffer, since high degree of acetylation brings about more intra-molecular hydrogen bonds. For each sample in solvents with the same ionic strength (DA and μ remain constant), the function value in 0.3M acidic solvents is higher than in solvent with 0.1M acid, so it's turned out that less acid makes chains more flexible. This is because when pH is lower, the number of positive charges is larger, leading to chain expansion due to electrostatic repulsions [67]. Kasaai et al. also investigated the influence of DA/(pH·μ) value on Mark-Houwink parameters K and α and the validity of the prediction was proved through comparing with experimental data [52,67]. Results were written in equations (5) and (6).

5.3.3. Temperature-relevant parameter

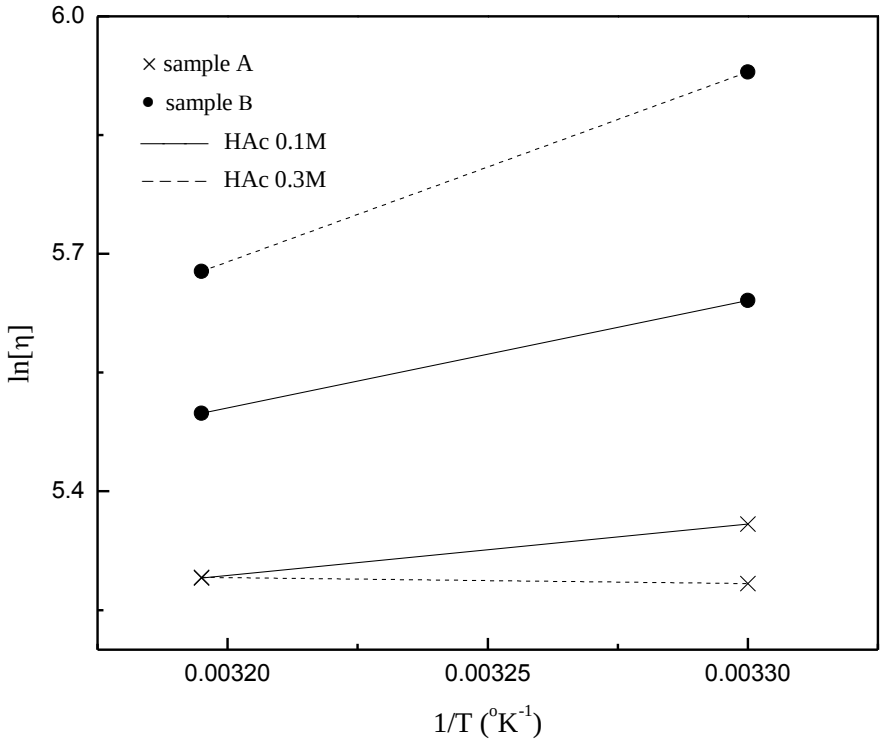

Figure 5.8. Plot of the natural logarithmic intrinsic viscosity (ln[η]) versus inverse of absolute temperature (1/T) for both chitosans

The effect of temperature on viscosity can be expressed by Arrhnius equation,

$$\eta = A\, e^{-Ea/RT} \tag{8}$$

with η the apparent viscosity, A the pre-exponential factor, Ea the activation energy, R the gas constant and T the absolute temperature in Kelvin. The values of Ea range between 2.09 $\times 10^7$ and 2.09 $\times 10^8$ J/(kg mol) and can help decide chain stiffness, where stiffer polymers have larger Ea [68]. The slope of d ln[η]/d (1/T), d ln ηrel/d (1/T) or d ln[η]/dT can be used as a measurement [52] because it relates to Ea [55] and larger slopes indicates stiffer polymers [69].

Table 5.7 and Figure 5.8 show that the values of d ln[η]/d (1/T) slope are from 651 to 2396, except for the temperature-independent data. Chen and Tsaih [58] reported the slope values in 0.01M HCl solvent were between 666 and 1334. Pogodina group [60] investigated that the slope was 488 in HOAc 0.33M/ NaCl 0.30M solvent. So it is manifest that these chitosans are relatively stiff [58] even though only two temperature variations was taken into consideration in this article.

In this article, the value of sample B is larger than that of sample A, indicating that sample B, which has a higher molecular weight, is stiffer. However there have been many contradictory results for the effect or molecular weight on temperature-relevant flexibility parameter d ln[η]/d (1/T) or d ln[η]/dT. Research by Trzciński group [65] and Pogodina group [60] demonstrated that the temperature coefficient was independent of molecular weight. However according to Chen group [58], d ln[η]/d (1/T) decreased with increasing molecular weight and high molecular weight chains were more flexible. Further discussion is needed to explain the contradiction. Since Bohdaneckýet al. [70] demonstrated that Mark-

Houwink exponents didn't change much when varying temperature, comparing with other particles at different temperature is accessible.

For sample A in solvent HAc 0.3M/NaAc 0.3M it is almost independent of temperature, so applying temperature-relevant parameter to evaluate chain stiffness is not suitable.

5.3.4. Summary

Multiple parameters are related to chain stiffness, among which the relative stiffness parameter B, the empirical function DA/(pH·μ) and temperature-relevant d ln[η]/d (1/T) were calculated and compared to literature. One big difference among them is that the empirical function DA/(pH·μ) is direct and settled, whereas the other two are relative characteristics.

It is reported that stiffer the chain, lower the value of B. Higher molecular weight polymers usually have greater values of B, thus they're more flexible than polymers with lower molecular weight. It should be noticed that B is not applicable to all polyelectrolytes and have some limits. From the the empirical function DA/(pH·μ), we can conclude that higher DA, more acid and less ionic strength contribute to chain stiffness. The influencing factors on each of the three stiffness parameters vary and are not always consistent, sometimes even conflicted. Since chitosan is a very complex polyelectrolyte, it is necessary to keep a proper experimental environment and choose suitable analyzing parameters.

6. CONCLUSION

6.1. Summary

In this thesis two chitosans were dissolved separately in six solvents with various acid and salt concentration. Flow time was measured at 30 and 40 ℃ and was related to intrinsic viscosity. Through analysis on Huggins k' and Kraemer k'', the qualities of solvents were estimated. The effects of molecular weight, temperature and salt concentration on intrinsic viscosity were evaluated and discussed. It is concluded that the influences of temperature and ionic strength were negative, while chitosans with larger molecular weight have higher intrinsic viscosities.

Parameters relate to chain stiffness were demonstrated and compared. The stiffness parameter B is a relative characteristic that allows for the comparison between different polyelectrolytes, and a stiffer chain brought about a lower B value. The pH-relevant function DA/(pH·μ) is empirical and help connect chain flexibility to solvent properties. The effect of temperature on chain stiffness has multiple expressions and the slope of d ln[η]/d (1/T) is an indicator of stiffness. The influencing factors on chain stiffness are uncertained and need to be analyzed differently.

6.2. Recommendations for Future Research

Although solvents with different concentrations of acid were applied, the influence of acid on viscosities and other chain conformations wasn't taken into consideration in this thesis. To use acid gradient concentration solvents is applicable.

There are several ways in demonstrating the stiffness of polymer chain. The model of worm-like chain was developed by Odijk to analyze local stiffness of polysaccharides [71, 72, 73] and it corresponds to persistence length, L_t. L_t is the sum-up of L_p(intrinsic persistence length) and L_e(electrostatic contribution) [12]. The value of L_p can be obtained experimentally using SEC or GPC and multiple detectors and is related to the radius of gelation [12,40]. In this article L_p is not calculated out due to equipment constraints, however this provides us a thread in comparing results in the future research.

In different solutions, chitosans can have different chain conformations. And there are some frequently used parameters to demonstrate conformation, which can be calculated from intrinsic viscosity data. The chain conformation models of chitosans is another wide field for us to research.

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