

Article

Establishment of Mark–Houwink–Sakurada Equations for Chitin in Multiple Solvent Systems and Their Implications for Solution Conformation

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Abstract

Currently, only a limited number of Mark–Houwink–Sakurada (MHS) equations are available for chitin, and their applicability is constrained by the narrow range of suitable solvent systems. The Mark–Houwink–Sakurada (MHS) equation is a widely used and practical approach for estimating polymer molecular weight from intrinsic viscosity measurements, particularly when chromatographic techniques are not readily accessible. This study aimed to establish new MHS equations for chitin to facilitate reliable molecular weight determination across different solvents and temperatures. Chitin samples with varying molecular weights were prepared via H₂O₂ degradation, and their weight-average molecular weights (M_w) were determined by high-performance size-exclusion chromatography (HPSEC). Intrinsic viscosity ([η]) was measured using a capillary viscometer at 25 and 30 °C in three solvent systems: 5% LiCl/N,N-dimethylacetamide (LiCl/DMAc), 8% NaOH/4% urea, and 10% NaOH/0.3% tannic acid (*w/w*). Double-logarithmic plots of M_w versus [η] were constructed to derive the corresponding MHS equations. At identical molecular weights and temperatures, intrinsic viscosity followed the order: LiCl/DMAc > NaOH/urea > NaOH/tannic acid. Increasing temperature led to higher intrinsic viscosity and conformation parameter (*a*) values. Chitin dissolved in LiCl/DMAc and NaOH/urea exhibited rod-like conformations, with *a* values ranging from 0.79 to 0.97, whereas chitin in NaOH/tannic acid displayed random coil behavior (*a* = 0.56–0.69). These established MHS equations expand the solvent applicability for chitin molecular weight determination and provide insights into its solution conformation under different chemical environments.

Keywords: chitin; chitin dissolving solvent system; weight-average molecular weight; Mark–Houwink–Sakurada equation; conformation



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1. Introduction

Chitin is a linear polysaccharide composed of repeating units of 2-acetamido-2-deoxy- β -D-glucose linked by β -1,4-glycosidic bonds. It occurs mainly in three crystalline allomorphic forms: α -chitin, β -chitin, and γ -chitin [1,2]. Among them, α -chitin is the most abundant form and is predominantly found in the exoskeletons of crustaceans and insects. It crystallizes in a rhombic system with antiparallel chain packing, leading to extensive

inter- and intramolecular hydrogen bonding and a densely packed structure. In contrast, β -chitin, commonly present in squid pens and tubeworms, adopts a monoclinic crystal system with parallel chain arrangement. Although both α - and β -chitin are insoluble in aqueous and common organic solvents, the looser packing and reduced intermolecular hydrogen bonding of β -chitin confer higher solubility in certain solvents, enhanced chemical reactivity, and greater susceptibility to enzymatic degradation [3]. γ -Chitin is mainly found in fungi, yeasts, and insect pupae, and exhibits a mixed molecular arrangement consisting of alternating antiparallel and parallel chain layers. Kaya et al. [1] reported that the structure of γ -chitin more closely resembles that of α -chitin. Owing to its biodegradability, biocompatibility, low toxicity, and environmental friendliness, chitin has been widely applied in agriculture, food and nutrition, biomedicine, biochemistry, cosmetics, textiles, materials science, and wastewater treatment [4,5].

Chitin is a biopolymer naturally synthesized by crustaceans, and its molecular weight can vary substantially depending on environmental conditions, seasonal factors, and other biological influences. At present, chitin purification is predominantly performed using chemical methods, in which acids and bases are employed to remove calcium carbonate and proteins and thereby obtain purified chitin. However, such treatments often involve elevated temperatures or strong acidic and alkaline conditions, which can induce depolymerization and partial deacetylation. As a consequence, considerable batch-to-batch variations in molecular weight and degree of deacetylation are frequently observed. The molecular weight of a polymer plays a critical role in determining its solution viscosity, chain orientation, and entanglement behavior, as well as its mechanical properties, including tensile strength, impact resistance, and elongation at break. Moreover, molecular weight strongly influences the final performance of chitin-based materials, such as chemical resistance, optical transparency, and permeability [6]. Therefore, accurate determination of chitin molecular weight is essential for effective control of material properties and functional performance in both chitin and its derivatives.

Several analytical approaches have been employed for determining the molecular weight of chitin, including static light scattering (SLS), high-performance size exclusion chromatography (HPSEC), and viscosity-based methods relying on the Mark–Houwink–Sakurada (MHS) equation. Compared with chromatographic and light-scattering techniques, the viscosity-based MHS method offers practical advantages. Once established under defined conditions, molecular weight can be rapidly estimated from intrinsic viscosity using simple instrumentation, making it suitable for routine quality control. Therefore, expanding reliable MHS equations is of practical as well as scientific importance for chitin molecular weight evaluation. Regardless of the technique used, accurate molecular weight determination requires the complete dissolution of chitin while simultaneously avoiding polymer degradation, aggregation, or intermolecular association. However, the intrinsic insolubility of chitin in most common solvents poses a major challenge to reliable molecular weight analysis. To date, only a limited number of solvent systems have been reported to effectively dissolve chitin for molecular weight determination, including 2.77 M sodium hydroxide [7], 5% lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) [8,9], 8% sodium hydroxide/4% urea (*w/w*) [10], and the ionic liquid 1-ethyl-3-methylimidazolium acetate [6].

Among the available approaches, the viscosity method is widely regarded as the simplest, fastest, and most cost-effective technique for polymer molecular weight determination. In the case of chitin, a series of samples with known molecular weights are analyzed under defined solution conditions to measure their intrinsic viscosities ($[\eta]$). A double-logarithmic plot of $\log [\eta]$ versus $\log M$ is then constructed, and linear regression is

applied to derive the corresponding MHS equation, from which the constants K and a are obtained. The MHS relationship is expressed as:

$$[\eta] = KM^a$$

where K and a are empirical parameters, commonly referred to as MHS constants, which depend on the polymer species, solvent system, and experimental temperature. The exponent a represents the polymer conformation parameter and provides insight into the molecular configuration in solution. Specifically, values of $a < 0.50$ indicate a compact spherical conformation, $a > 0.80$ corresponds to an extended rod-like structure, and values between 0.50 and 0.80 are characteristic of random-coil conformations [8]. Once the intrinsic viscosity of an unknown chitin sample is measured under identical solution conditions, its viscosity-average molecular weight can be readily calculated using the established MHS equation.

To date, only a limited number of studies have reported MHS equations for chitin, and most of them are confined to specific solvent systems and narrow experimental conditions. Terbojevich et al. [9] first reported the dissolution of chitin in 5% LiCl/*N,N*-dimethylacetamide (LiCl/DMAc, *w/w*) and established an MHS equation at 25 ± 0.1 °C by measuring the intrinsic viscosities of five chitin samples with molecular weights ranging from 90 to 510 kDa using a capillary viscometer. The molecular weights were determined by static light scattering, yielding an MHS equation with $a = 0.69$ and $K = 2.4 \times 10^{-3}$ dL g⁻¹. Subsequently, Terbojevich et al. [11] expanded this dataset by incorporating nine additional chitin samples (120–1200 kDa) while maintaining the same solvent system and temperature. Analysis of the combined 14 samples resulted in an updated MHS equation with $a = 0.88$ and $K = 2.1 \times 10^{-4}$ dL g⁻¹.

Poirier and Charlet [8] further investigated chitin solutions prepared in 5% LiCl/DMAc at 30 ± 0.1 °C using 12 samples with molecular weights between 80 and 710 kDa, and reported a MHS equation with $a = 0.95$ and $K = 7.6 \times 10^{-5}$ dL g⁻¹. The discrepancy in the a value relative to that reported by Terbojevich et al. [11] was attributed to differences in molecular weight range and the use of samples with unspecified polydispersity indices. Beyond the LiCl/DMAc system, Einbu et al. [7] established an MHS equation for chitin dissolved in 2.77 M NaOH at 20 °C using six samples with molecular weights of 100–1200 kDa, obtaining $a = 0.68$ and $K = 1.0 \times 10^{-3}$ dL g⁻¹. In addition, Li et al. [10] reported an MHS relationship for crab shell chitin degraded into six fractions (188–1463 kDa) and dissolved in 8% NaOH/4% urea (*w/w*) at 25 ± 0.1 °C, yielding $a = 0.56$ and $K = 2.6 \times 10^{-3}$ dL g⁻¹ based on intrinsic viscosity measurements and molecular weights determined by SLS.

Based on the above literature, only a limited number of MHS equations have been reported for chitin, and the applicable solvent systems remain highly restricted, thereby limiting the broader application of the MHS approach for chitin molecular weight determination. Recently, a NaOH/tannic acid solvent system has been developed as a new dissolution medium for chitin [12], providing an opportunity to expand the solvent scope for MHS analysis. Accordingly, this study aimed to establish MHS equations for chitin in the NaOH/tannic acid system to enable reliable molecular weight determination under defined conditions. Chitin samples with different molecular weights were prepared via hydrogen peroxide degradation, and their weight-average molecular weights (M_w) were determined by HPSEC. Intrinsic viscosities of chitin dissolved in 10% NaOH/0.3% tannic acid were measured using a capillary viscometer at 25 and 30 °C. For comparison, intrinsic viscosity measurements were also conducted in two commonly used solvent systems, namely 5% LiCl/DMAc and 8% NaOH/4% urea. Double-logarithmic plots of M_w versus intrinsic viscosity were constructed to derive the corresponding MHS equations for each solvent system, thereby providing a practical framework for molecular weight evaluation

of chitin in both fundamental research and industrial applications. However, existing MHS equations for chitin are limited to a few solvent systems and specific experimental conditions, and no study has yet established an MHS relationship for the NaOH/tannic acid system. Moreover, systematic comparisons among different dissolution media under comparable conditions remain scarce. Therefore, this work addresses a methodological gap by expanding the solvent scope of MHS analysis and providing new insights into solvent-dependent solution behavior of chitin.

2. Materials and Methods

2.1. Materials and Chemicals

Shrimp shells of *Metapenaeus monoceros* were obtained from a shrimp processing plant in Keelung City, Taiwan. Commercial α -chitin (industrial and agricultural grade, C005) was purchased from Cheng-Li Industrial Co., Ltd. (New Taipei City, Taiwan). Sodium hydroxide, hydrochloric acid, potassium permanganate, tannic acid, hydrogen peroxide, and lithium chloride (all ACS reagent grade) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). N,N-Dimethylacetamide (DMAc, HPLC grade) was purchased from Acros Organics (Geel, Belgium). Oxalic acid (ACS reagent grade) was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA), and urea (ACS reagent grade) was purchased from Panreac (Barcelona, Spain).

2.2. Preparation of Chitin

Shrimp shells were thoroughly washed to remove viscera, tissue fluids, and adhering impurities, followed by air-drying for 3 days. The dried shells were then ground using a laboratory grinder and stored at 4 °C until further use. Chitin was purified from shrimp shell powder according to the method described by Tan et al. [5], with minor modifications. Shrimp shell-derived chitin is widely recognized as α -chitin, and the obtained product is therefore designated as α -chitin. Briefly, shrimp shell powder (60–80 mesh) was subjected to demineralization using 2 N HCl at a solid-to-liquid ratio of 1:15 (*w/v*) at room temperature for 2 h to remove calcium carbonate, until no further gas evolution was observed. The residue was subsequently washed with distilled water until neutral pH was reached. Deproteinization was then carried out by treating the sample with 2 N NaOH at a solid-to-liquid ratio of 1:10 (*w/v*) at 80 °C for 1 h, followed by repeated washing with distilled water to neutrality. To remove residual pigments, the material was oxidized with a 1% (*w/v*) potassium permanganate solution at room temperature for 1 h and subsequently treated with 1% (*w/v*) oxalic acid at 60 °C for 1 h to reduce excess potassium permanganate. Finally, the purified chitin was thoroughly washed with distilled water until neutral pH and dried at 50 °C to obtain α -chitin, which was designated as MMC.

2.3. Preparation of α -Chitin with Different Molecular Weights

Commercial α -chitin was washed with RO water until neutral pH to remove residual acids, bases, and other impurities, then dried at 50 °C and sieved to obtain chitin powder with a particle size of 100–150 mesh for subsequent experiments. A 10% hydrogen peroxide solution was prepared by diluting 35% H₂O₂ with deionized water. For degradation, a 1% (*w/w*) chitin suspension was prepared by mixing 0.5 g of α -chitin with 50 g of 10% H₂O₂ solution in a 100 mL glass vial. The vials were incubated in a thermostatic water bath at 60 °C for 0, 15, 60, 360, and 720 min to induce chitin degradation. After treatment, the samples were washed with deionized water to remove residual hydrogen peroxide, collected by filtration, and dried at 50 °C. The degraded α -chitin samples were designated D0, D15, D60, D360, and D720 according to the degradation time.

2.4. Determination of Degree of Deacetylation (DD)

Chitin powder was thoroughly mixed with KBr, finely ground, and pressed into transparent pellets. The pellets were analyzed using a Fourier transform infrared (FTIR) spectrometer (Nicolet iS5, Thermo Fisher Scientific, Madison, WI, USA) over the spectral range of 400–4000 cm^{-1} . Functional groups were identified based on the obtained spectra, and the degree of deacetylation (DD) of chitin was calculated according to the method described by Jiang et al. [4] using the following equation:

$$\text{DD (\%)} = 100 - \frac{A_{1655}}{A_{3450}} \times 115, \quad (1)$$

where A_{1655} is the absorbance of the Amide I band at 1655 cm^{-1} , and A_{3450} is the absorbance of the O-H band at 3450 cm^{-1} .

2.5. Determination of Chitin Molecular Weight by HPSEC

The molecular weight of chitin was determined by HPSEC following a modified method of Suenaga et al. [13]. The HPSEC system consisted of a pump (LC-10ATVP, Shimadzu Corp., Kyoto, Japan), a refractive index detector (RID-20A, Shimadzu Corp., Kyoto, Japan), a GPC column (Shodex GPC KD-806M, 7 μm , 8 $\times$ 300 mm, Showa Denko K.K., Tokyo, Japan), and a guard column (Shodex GPC KD-G 4A, 8 μm , 4.6 $\times$ 10 mm, Showa Denko K.K., Tokyo, Japan). Pullulan standards with molecular weights of 642, 194, 107, 47.1, 22.0, and 9.6 kDa (Shodex Standard P-82, Showa Denko K.K., Tokyo, Japan) were used to establish the calibration curve. The molecular weights of chitin were calculated based on the pullulan calibration curve and are therefore expressed as pullulan-equivalent molecular weights. For analysis, each standard or chitin sample (1%, w/w) was dissolved in 5% LiCl/DMAc by stirring for 2 days. The resulting solutions were filtered through a 0.45 μm nylon syringe filter prior to injection. The mobile phase flow rate was set at 0.5 mL/min, and the column temperature was maintained at 50 $^{\circ}\text{C}$.

2.5.1. Preparation of the Mobile Phase

Prior to preparing the 5% LiCl/DMAc solution, the required amount of LiCl was dried in an oven at 105 $^{\circ}\text{C}$ for 8 h. The dried LiCl was then added to DMAc in a 100 mL glass vial and stirred at room temperature using a magnetic stirrer for 8 h until completely dissolved. The resulting solution was filtered under vacuum through a 0.45 μm nylon membrane prior to use.

2.5.2. Sample Preparation and HPSEC Analysis

The prepared chitin (MMC) was dried in an oven at 105 $^{\circ}\text{C}$ for 8 h prior to dissolution. A 0.1% (w/w) chitin solution was then prepared in 5% LiCl/DMAc and stirred at 25 $^{\circ}\text{C}$ for 2–12 days. Before HPSEC analysis, the solution was filtered through a 0.45 μm nylon syringe filter.

2.5.3. Determination of Molecular Weight of Chitin with Different Degradation Times

Five α -chitin samples with different degradation times (D0, D15, D60, D360, and D720) were dried in an oven at 105 $^{\circ}\text{C}$ for 8 h prior to dissolution. Each sample was then dissolved in 5% LiCl/DMAc to prepare a 0.1% (w/w) chitin solution and stirred at 25 $^{\circ}\text{C}$ for 2 days. Before HPSEC analysis, the solutions were filtered through a 0.45 μm nylon syringe filter.

2.6. Establishment of the Mark–Houwink–Sakurada Equation

2.6.1. Preparation of Chitin Solutions in 5% LiCl/DMAc

Chitin samples with different molecular weights (D0, D15, D60, D360, and D720) were dried in an oven at 105 °C for 8 h prior to dissolution. Each sample was then dissolved in 5% LiCl/DMAc to prepare a 0.07 g/dL α -chitin solution and continuously stirred at 25 °C using a magnetic stirrer for 48 h until complete dissolution. The solutions were filtered through a 0.45 μ m nylon syringe filter, and the filtrates were subsequently diluted to four concentrations (0.07, 0.0525, 0.035, and 0.0175 g/dL) for subsequent measurements.

2.6.2. Preparation of Chitin Solutions in 8% NaOH/4% Urea or 10% NaOH/0.3% Tannic Acid

Chitin samples with different molecular weights (D0, D15, D60, D360, and D720) were suspended in either 8% NaOH/4% urea or 10% NaOH/0.3% tannic acid to prepare a 0.07 g/dL α -chitin suspension. The suspensions were frozen at −80 °C for 2.5 h, thawed at 25 °C for 1 h, and subsequently stirred at room temperature using a magnetic stirrer for 5 min. This freeze–thaw cycle was repeated three times. After the cycles, the solutions were filtered through a 0.45 μ m nylon syringe filter and then diluted with freshly prepared 8% NaOH/4% urea or 10% NaOH/0.3% tannic acid to four concentrations (0.07, 0.0525, 0.035, and 0.0175 g/dL) for further analysis.

2.6.3. Determination of Reduced and Intrinsic Viscosity

The viscosities of chitin solutions prepared in Sections 2.6.1 and 2.6.2 were measured at 25 ± 0.1 °C and 30 ± 0.1 °C using a capillary viscometer (Cannon–Fenske No. 75–100, Ramin Corporation, Magnolia, PA, USA). A 10 mL aliquot of each solution was introduced into the viscometer, which was positioned in the viewing window of a thermostatic water bath (Tanson TMV 40, Askim, Sweden). An additional water bath (Firstek B403, Taipei, Taiwan) was employed to enhance temperature stability. After sufficient thermal equilibration, the flow time between the upper and lower timing marks was recorded. Each measurement was performed in triplicate, and the mean value was used for subsequent calculations.

$$\text{Relative viscosity, } \eta_r = \frac{\eta}{\eta_0} = \frac{t}{t_0} \quad (2)$$

$$\text{Specific viscosity, } \eta_{sp} = \eta_r - 1 = \frac{\eta - \eta_0}{\eta_0} = \frac{t - t_0}{t_0} \quad (3)$$

$$\text{Reduced viscosity, } \eta_{red} = \frac{\eta_{sp}}{C} \quad (4)$$

$$\text{Inherent viscosity, } \eta_{inh} = \frac{\ln \eta_r}{C} \quad (5)$$

$$\text{Intrinsic viscosity, } [\eta] = \left(\frac{\eta_{sp}}{C} \right)_{C \rightarrow 0} = \left(\frac{\ln \eta_r}{C} \right)_{C \rightarrow 0} \quad (6)$$

where t_0 and t are the flow times of the solvent and the chitin solution, respectively, and C is the chitin concentration.

2.6.4. Calculation of Mark–Houwink–Sakurada Viscosity Parameters

The intrinsic viscosities determined in Section 2.6.3 and the corresponding molecular weights obtained in Section 2.5.3 were used to construct double-logarithmic plots of $\log [\eta]$ versus $\log M$. Linear regression analysis was performed according to the equation

$y = ax + b$ (i.e., $\log [\eta] = a \log M + \log K$), where a represents the slope (a) and K is equal to 10^b . The Mark–Houwink–Sakurada viscosity constants a and K were thus obtained.

$$[\eta] = KM^a \quad (7)$$

where $[\eta]$ is the intrinsic viscosity, M is the molecular weight, K is the Mark–Houwink–Sakurada parameter, and a is the Mark–Houwink–Sakurada exponent.

2.7. Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was applied to evaluate differences among experimental groups. When significant effects were detected, Duncan's New Multiple Range Test (DMRT) was used for post hoc comparisons. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SPSS for Windows (version 12.0; IBM Corporation, Armonk, NY, USA).

3. Results & Discussion

3.1. Preparation of Chitin Solution in 5% LiCl/DMAc

The self-prepared α -chitin (MMC) exhibited a degree of deacetylation (DD) of 26.63%, confirming its identity as chitin. The 5% LiCl/DMAc solvent system is widely used for chitin dissolution; however, successful dissolution requires strict control of moisture content. Both LiCl and chitin samples must be dried at 105 °C for at least 8 h prior to use, as LiCl is highly hygroscopic and the LiCl/DMAc system is particularly sensitive to trace amounts of water, which can markedly reduce dissolution efficiency [14].

Figure 1 presents the molecular weights of chitin dissolved in 5% LiCl/DMAc at 25 °C for durations ranging from 2 to 12 days, as determined by HPSEC. No significant differences in molecular weight were observed among samples dissolved for different periods, indicating that prolonged dissolution did not induce molecular degradation or aggregation. These results demonstrate that a 0.1% chitin solution can be fully dissolved in 5% LiCl/DMAc within 2 days under the tested conditions. Consistently, the HPSEC chromatograms shown in Figure 2 revealed comparable peak intensities and elution profiles for chitin samples dissolved for different durations, further confirming the absence of polymerization or degradation during stirring for up to 12 days.

Previous studies have rarely reported detailed dissolution conditions for chitin in 5% LiCl/DMAc, particularly with respect to dissolution time and temperature. Most reports provide only general descriptions, such as stirring at room temperature until complete dissolution [9,15], dissolving at 50 °C for 1 day [16], dissolving at room temperature for 120 h [17], or dissolving at room temperature for 7 days [18]. During the present study, it was further observed that chitin/5% LiCl/DMAc solutions prepared under lower ambient temperatures in winter (approximately 10–20 °C, without air conditioning) frequently exhibited colloidal precipitation or incomplete dissolution. In contrast, solutions prepared at higher ambient temperatures in summer (approximately 25 °C, with air conditioning) were consistently homogeneous and fully dissolved. Based on these observations, a dissolution condition of 25 °C for 2 days was selected and applied in all subsequent experiments.

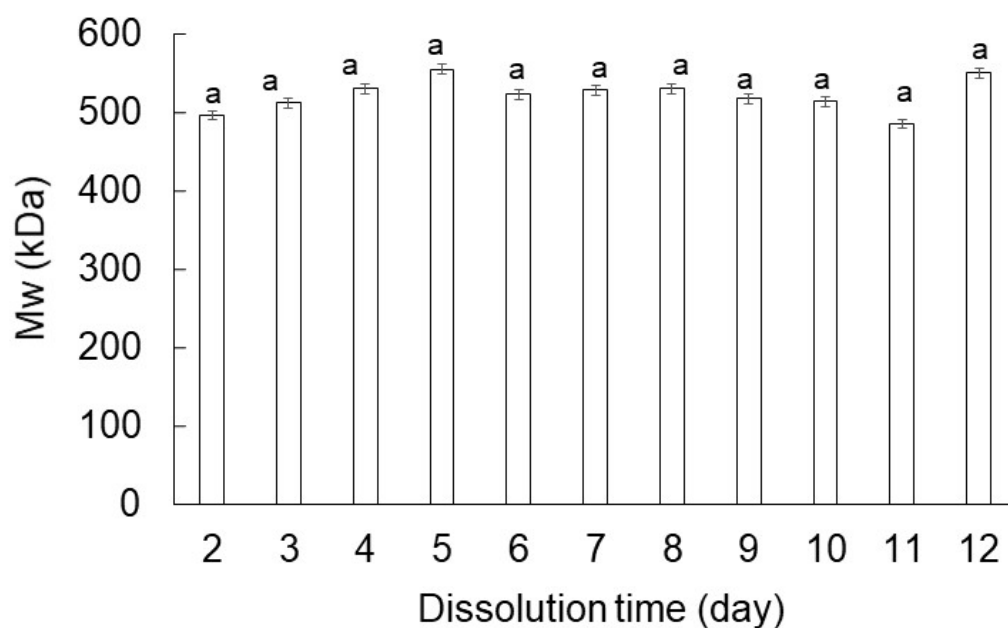


Figure 1. Molecular weight of chitin (MMC) which was dissolved in 5% LiCl/DMAc at 25 °C after different time and determined by HPSEC. MMC: The α -chitin was prepared with *Metapenaeus Monoceros* shells. All data were expressed as mean value $\pm$ standard deviation. Difference letters on the column indicate significant differences between the means obtained by Tukey's test ($p < 0.05$).

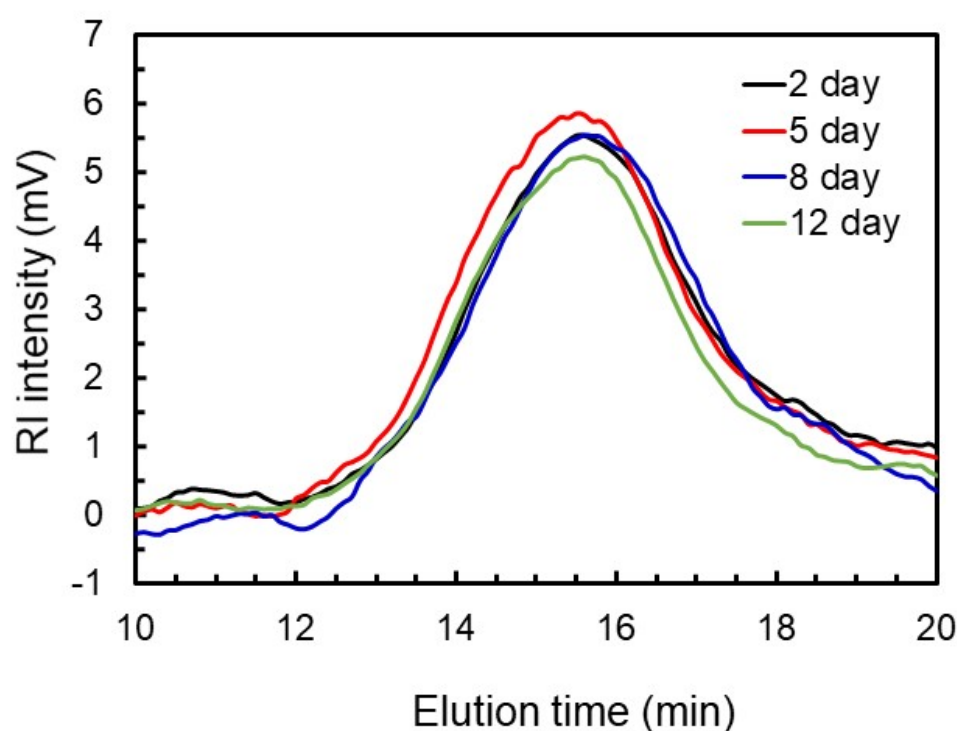


Figure 2. HPSEC elution patterns of chitin (MMC) which was dissolved in 5% LiCl/DMAc at 25 °C after different times. MMC: The α -chitin was prepared with *Metapenaeus Monoceros* shells.

3.2. Chitin with Different Degradation Times

Table 1 summarizes the degree of deacetylation (DD) of chitin subjected to heterogeneous degradation in 10% hydrogen peroxide at 60 °C for different durations. The undegraded sample (D0) exhibited a DD of 39.5%, whereas the DD values of all degraded samples ranged from 29.1% to 29.9%. These results indicate that the hydrogen peroxide

degradation process led to a moderate decrease in DD but did not alter the chemical identity of chitin, as all samples remained within the characteristic DD range of chitin.

Table 1. The degree of deacetylation (DD) and molecular weight of chitin at different degradation times.

Sample Code	Degradation Time (min)	DD (%)	Mw (kDa) *	Mn (kDa)	Mw/Mn
D0 **	0	39.5 ± 1.5	976.97 ± 73.80	206.32 ± 51.61	4.74
D15	15	29.8 ± 1.4	414.80 ± 9.71	58.54 ± 5.42	7.09
D60	60	29.9 ± 1.8	286.85 ± 2.27	42.47 ± 6.49	6.76
D360	360	29.1 ± 0.8	172.07 ± 1.10	23.73 ± 1.41	7.25
D720	720	29.8 ± 2.0	157.86 ± 1.69	21.48 ± 0.79	7.35

All data were expressed as mean value ± standard deviation ($n = 3$). * Mw: Weight-average molecular weight; Mn: Number-average molecular weight; Mw/Mn: Polydispersity index. ** D0–D720 represent the products of commercial α -chitin after degradation for 0–720 min, respectively.

Following and modifying the method described by Chang et al. [19], the molecular weights of chitin samples with different degradation times were determined by HPSEC, and the corresponding data are also presented in Table 1. Both the weight-average molecular weight (Mw) and number-average molecular weight (Mn) decreased progressively with increasing degradation time, confirming effective depolymerization of chitin chains. The polydispersity index (PDI) values ranged from 4.74 to 7.35, reflecting the inherently broad molecular weight distribution of chitin and the heterogeneous nature of the degradation process.

Building on the molecular weight parameters summarized in Table 1, Figure 3 presents the HPSEC chromatograms of chitin samples obtained at different degradation times. As degradation time increased, the main chitin peak systematically shifted toward longer retention times, consistent with a decrease in molecular size/molecular weight under size-exclusion separation. In addition, the early-eluting region corresponding to the high-molecular-weight fraction diminished and migrated to longer retention times, further supporting progressive depolymerization with extended hydrogen peroxide treatment.

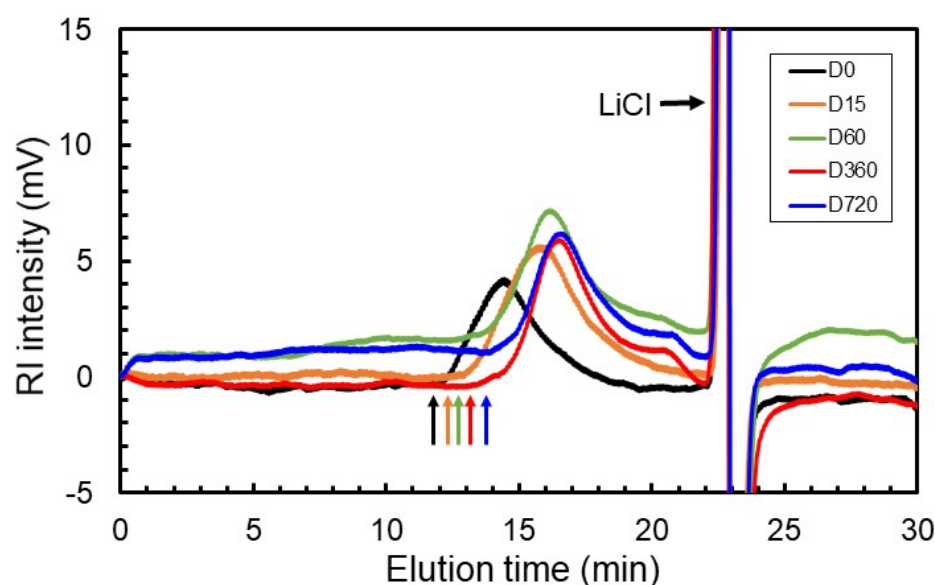


Figure 3. HPSEC elution patterns of chitin at different degradation times. D0–D720 represent the products of commercial α -chitin after degradation for 0–720 min, respectively. The arrows in the figure indicate the earliest location of the RI signal in these chitin samples (D0: black; D15: orange; D60: green; D360: red; D720: blue).

Notably, chromatograms of chitin degraded with 10% hydrogen peroxide exhibited a pronounced shoulder and/or tailing around ~20 min, accompanied by relatively high polydispersity indices (Table 1). This behavior suggests that the degradation did not occur as a uniform shift in the entire molecular weight distribution, but rather generated an increased proportion of low-molecular-weight species while retaining a broader population of higher-molecular-weight chains. Such non-uniform chain scission is consistent with heterogeneous oxidative degradation, in which different chain populations may experience different extents of cleavage, leading to broadened distributions and the emergence of secondary features (shoulder/tailing) in the SEC profiles, as observed in Figure 3.

3.3. Intrinsic Viscosity

Intrinsic viscosity is a fundamental solution property of polymers and is influenced by molecular weight, solvent environment, and temperature. Table 2 summarizes the intrinsic viscosities of chitin samples with different molecular weights dissolved in 5% LiCl/DMAc, 8% NaOH/4% urea, and 10% NaOH/0.3% tannic acid at 25 and 30 °C. As shown in Table 2, the intrinsic viscosity of chitin varied systematically with molecular weight, solvent system, and measurement temperature. Under identical solvent and temperature conditions, intrinsic viscosity decreased with decreasing molecular weight, reflecting the shorter chain length and reduced hydrodynamic volume of lower-molecular-weight chitin. In contrast, chitin samples with higher molecular weights exhibited larger intrinsic viscosities due to their longer polymer chains and greater chain expansion in solution.

Table 2. The intrinsic viscosities (dL/g) of different molecular weight chitins in different solvents under 25 and 30 °C.

Sample	25 ± 0.1 °C			30 ± 0.1 °C		
	5% LiCl/DMAc	8% NaOH/4% Urea	10% NaOH/0.3% Tannic Acid	5% LiCl/DMAc	8% NaOH/4% Urea	10% NaOH/0.3% Tannic Acid
D0 *	14.98 ± 1.53	6.80 ± 0.99	0.91 ± 0.20	17.92 ± 1.12	8.35 ± 0.40	1.48 ± 0.04
D15	8.23 ± 0.91	2.79 ± 0.14	0.55 ± 0.13	9.41 ± 0.19	3.05 ± 0.11	0.85 ± 0.02
D60	5.59 ± 0.87	2.05 ± 0.27	0.45 ± 0.04	6.76 ± 1.55	2.17 ± 0.07	0.64 ± 0.07
D360	3.86 ± 0.08	1.39 ± 0.15	0.35 ± 0.05	4.39 ± 1.08	1.47 ± 0.03	0.45 ± 0.05
D720	3.62 ± 0.16	1.34 ± 0.42	0.32 ± 0.03	4.01 ± 1.12	1.41 ± 0.05	0.42 ± 0.04

* D0–D720 represent the products of commercial α -chitin after degradation for 0–720 min, respectively.

At a given molecular weight and within the same solvent system, the intrinsic viscosity measured at 30 °C was consistently higher than that measured at 25 °C. This temperature-dependent increase in intrinsic viscosity suggests an expansion of the hydrodynamic volume of chitin chains in solution. In the LiCl/DMAc system, elevated temperature has been reported to enhance hydrophobic interactions within polymer chains [20], which may facilitate stronger solvation of chitin by DMAc molecules. Such enhanced polymer–solvent interactions can promote chain expansion, resulting in increased intrinsic viscosity.

Similarly, in the NaOH/urea and NaOH/tannic acid solvent systems, increasing temperature is known to influence the balance between polymer–polymer and polymer–solvent interactions. Enhanced hydrophobic interactions at higher temperatures may weaken specific interactions between chitin and urea or tannic acid, thereby allowing the chitin chains to adopt a more extended conformation in solution. This conformational expansion increases the effective hydrodynamic volume of the polymer chains and consequently leads to higher intrinsic viscosity values [20–23].

At the same molecular weight and temperature, the intrinsic viscosity of chitin followed the order: 5% LiCl/DMAc > 8% NaOH/4% urea > 10% NaOH/0.3% tannic acid. This

trend indicates that differences in intrinsic viscosity arise from distinct dissolution mechanisms and polymer–solvent interactions in the three solvent systems. In the LiCl/DMAc system, LiCl is known to interact strongly with DMAc, forming LiCl–DMAc complexes as a result of weak salt dissociation in the aprotic solvent [24]. Upon the introduction of chitin, LiCl can interact with the hydroxyl and acetamide groups of chitin, effectively disrupting intermolecular hydrogen bonding and promoting chain swelling and dissolution. Importantly, intramolecular hydrogen bonding within the chitin backbone is largely preserved, resulting in a relatively rigid and extended chain conformation in solution [25]. In addition, it has been proposed that Li^+ ions can associate with carbonyl groups of chitin, imparting partial positive charges to the polymer chains and rendering chitin a polyelectrolyte-like species in LiCl/DMAc solutions [17]. Electrostatic repulsion between similarly charged chitin chains may further enhance chain expansion, leading to an increased hydrodynamic volume and, consequently, higher intrinsic viscosity values compared with those observed in alkaline solvent systems.

In the NaOH/urea system, chitin dissolution is generally attributed to the combined action of alkaline treatment and freeze–thaw processing. Sodium hydroxide disrupts hydrogen bonding within and between chitin chains, facilitating the penetration of water molecules into the polymer matrix. During freeze–thaw cycles, the crystallization and volumetric expansion of water at low temperatures further promote chain separation and enhance solvent accessibility. Subsequently, urea molecules can form hydrogen bonds with hydroxyl groups of chitin, stabilizing the dissolved chains in solution. According to previous studies, chitin dissolved in NaOH/urea systems is suggested to adopt a relatively extended chain conformation under these conditions [26]. The dissolution mechanism in the NaOH/tannic acid system shares similarities with that of the NaOH/urea system; however, the interaction between tannic acid and chitin introduces additional effects. Tannic acid is capable of forming multiple hydrogen bonds with chitin chains, which may induce partial folding or coiling of the polymer around tannic acid molecules. Such interactions can weaken intermolecular hydrogen bonding and hydrophobic interactions among chitin chains, thereby stabilizing dissolution. Owing to the high density of hydroxyl groups in tannic acid, these interactions are expected to be more pronounced for higher-molecular-weight chitin, leading to a reduced hydrodynamic volume and, consequently, lower intrinsic viscosity values compared with those observed in the NaOH/urea system [12].

In both NaOH/urea and NaOH/tannic acid systems, chitin chains are not expected to experience significant electrostatic repulsion, in contrast to the LiCl/DMAc system. Moreover, the presence of intramolecular hydrogen bonding and solvent-mediated chain interactions may favor more compact chain conformations, resulting in smaller hydrodynamic volumes. Collectively, these factors account for the lower intrinsic viscosities observed in alkaline solvent systems relative to those measured in LiCl/DMAc.

3.4. MHS Equation

The intrinsic viscosities of chitin samples with different molecular weights measured in various solvent systems at 25 and 30 °C (Table 2), together with the corresponding weight-average molecular weights (M_w) determined by HPSEC, were plotted on double-logarithmic coordinates to generate Figure 4. Linear regression analysis was then performed to determine the MHS parameters a and K , which are summarized in Table 3.

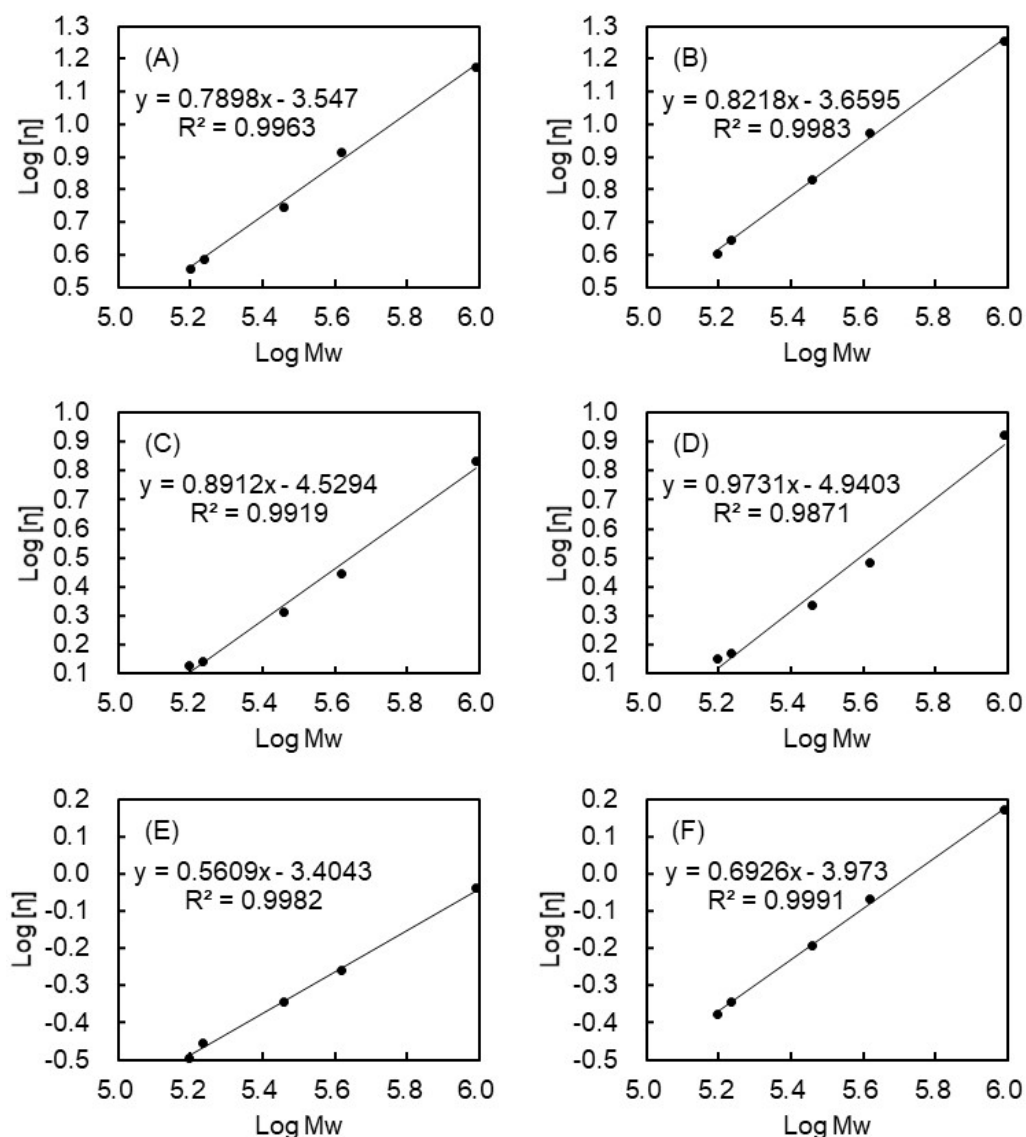


Figure 4. Plots of $\log Mw$ versus $\log [\eta]$ of chitin in (A) 5% LiCl/DMAc at $25 \pm 0.1^\circ\text{C}$; (B) 5% LiCl/DMAc at $30 \pm 0.1^\circ\text{C}$; (C) 8% NaOH/4% Urea at $25 \pm 0.1^\circ\text{C}$; (D) 8% NaOH/4% Urea at $30 \pm 0.1^\circ\text{C}$; (E) 10% NaOH/0.3% Tannic acid at $25 \pm 0.1^\circ\text{C}$; (F) 10% NaOH/0.3% Tannic acid at $30 \pm 0.1^\circ\text{C}$.

Table 3. Mark–Houwink–Sakurada equation constant (a , K) for chitin.

Solvent	Temp. ($^\circ\text{C}$)	Mw (kDa)	a	K (dL/g)
5% LiCl/DMAc	25	159–977	0.79	2.84×10^{-4}
5% LiCl/DMAc	30	159–977	0.82	2.19×10^{-4}
8% NaOH/4% Urea	25	159–977	0.89	2.96×10^{-5}
8% NaOH/4% Urea	30	159–977	0.97	1.15×10^{-5}
10% NaOH/0.3% Tannic acid	25	159–977	0.56	3.94×10^{-4}
10% NaOH/0.3% Tannic acid	30	159–977	0.69	1.06×10^{-4}

As summarized in Table 3, in the 5% LiCl/DMAc solvent system, the MHS parameters a and K were 0.79 and $2.84 \times 10^{-4} \text{ dL g}^{-1}$ at 25°C , and 0.82 and $2.19 \times 10^{-4} \text{ dL g}^{-1}$ at 30°C , respectively. Terbojevich et al. [9] previously reported an a value of 0.69 for chitin dissolved in 5% LiCl/DMAc at $25 \pm 0.1^\circ\text{C}$; however, that study was based on a limited number of samples with a relatively narrow molecular weight range (90–510 kDa).

In a subsequent investigation, Terbojevich et al. [11] expanded both the sample number and molecular weight range (120–1200 kDa), yielding a higher a value of 0.88. At 30 °C, Poirier and Charlet [8] reported an a value of 0.95 for α -chitin in 5% LiCl/DMAc and suggested that higher a values reflect a rigid, rod-like molecular conformation with good draining ability, analogous to that of cellulose. Supporting this interpretation, McCormick et al. [27] reported an a value of 1.19 for cellulose dissolved in 9% LiCl/DMAc. The a values obtained in the present study (0.79 at 25 °C and 0.82 at 30 °C) are closer to those reported by Terbojevich et al. [11], which is likely attributable to the comparable molecular weight ranges employed (159–977 kDa in this study). Collectively, the present results, together with those reported in the literature, are consistent with a predominantly rod-like chain conformation of chitin in the 5% LiCl/DMAc solvent system.

As shown in Table 3, in the 8% NaOH/4% urea solvent system, the MHS parameters a and K were 0.89 and $2.96 \times 10^{-5} \text{ dL g}^{-1}$ at 25 °C, and 0.97 and $1.15 \times 10^{-5} \text{ dL g}^{-1}$ at 30 °C, respectively. These relatively high a values indicate that α -chitin adopts a highly extended chain conformation in 8% NaOH/4% urea at both temperatures.

Li et al. [10] reported markedly lower a and K values ($a = 0.56$, $K = 2.6 \times 10^{-3}$) for chitin dissolved in 8% NaOH/4% urea at 25 ± 0.1 °C, suggesting a random-coil conformation. They further reported a slope (b) value of 0.47 for the double-logarithmic relationship between radius of gyration (R_g) and molecular weight, which is also characteristic of random-coil polymers ($R_g = CM^b$). The discrepancy between these results and the present study may be attributed to differences in molecular weight range, solution preparation conditions, and characterization techniques. In particular, Fang et al. [28] subsequently demonstrated, using combined dynamic and static light scattering analyses, that chitin dissolved in 11% NaOH/4% urea exhibits an extended wormlike chain conformation, as evidenced by structure-sensitive parameters (ρ) and persistence length (L_p). The results obtained in the present study are in closer agreement with those reported by Fang et al. [28].

In contrast, in the 10% NaOH/0.3% tannic acid solvent system, the a and K values of chitin were 0.56 and $3.94 \times 10^{-4} \text{ dL g}^{-1}$ at 25 °C, and 0.69 and $1.06 \times 10^{-4} \text{ dL g}^{-1}$ at 30 °C, respectively (Table 3). These values are characteristic of random-coil polymer conformations at both temperatures. The lower a values observed in this solvent system may be attributed to strong hydrogen-bonding interactions between tannic acid and chitin chains, which can promote partial folding or wrapping of the polymer around tannic acid molecules. Such interactions are expected to reduce the effective hydrodynamic volume of chitin chains in solution, leading to a more compact, random-coil-like conformation.

A comparative analysis of the three solvent systems further highlights the decisive role of solvent–polymer interactions in governing chitin chain conformation. In LiCl/DMAc and NaOH/urea systems, effective disruption of intermolecular hydrogen bonding and favorable solvation of the polymer backbone appear to promote extended or rod-like conformations, as reflected by relatively high a values. Similar solvent-induced conformational expansion has been reported for other polysaccharides, including cellulose in LiCl/DMAc systems [29–31]. In contrast, the lower a values observed in the NaOH/tannic acid system suggest that specific interactions between tannic acid and chitin chains may restrict conformational expansion, leading to reduced hydrodynamic volume and random-coil behavior. These findings underscore that the MHS exponent a not only reflects molecular weight dependence but also provides insight into solvent quality and chain stiffness in solution.

Future studies could further elucidate the conformational characteristics of chitin in different solvent environments using complementary techniques such as dynamic and static light scattering to directly evaluate persistence length and chain flexibility. Additionally, systematic variation in solvent composition and temperature may help establish a more comprehensive structure–property relationship framework for chitin solutions, thereby

enhancing the predictive capability and broader applicability of MHS-based molecular weight determination.

3.5. Effect of Temperature on Intrinsic Viscosity and Chitin Conformation

Tables 2 and 3 show that both the intrinsic viscosity and the MHS exponent (a) of α -chitin in 5% LiCl/DMAc, 8% NaOH/4% urea, and 10% NaOH/0.3% tannic acid increased with increasing temperature. This trend suggests a temperature-induced expansion of the hydrodynamic volume of chitin chains across all solvent systems. In the LiCl/DMAc system, elevated temperature has been reported to enhance hydrophobic interactions within polymer chains [20], which may promote stronger polymer–solvent interactions between chitin and DMAc. Such enhanced solvation can facilitate chain expansion, leading to increased intrinsic viscosity. When the temperature was increased from 25 °C to 30 °C, the a value of chitin increased slightly from 0.79 to 0.82 (Table 3), indicating that the chitin chains retained a predominantly rod-like and extended conformation within this temperature range. Accordingly, the MHS equations for chitin in 5% LiCl/DMAc were determined as $[\eta] = 2.84 \times 10^{-4} \text{ M}^{0.79}$ at 25 °C and $[\eta] = 2.19 \times 10^{-4} \text{ M}^{0.82}$ at 30 °C. In 8% NaOH/4% urea, the corresponding equations were $[\eta] = 2.96 \times 10^{-5} \text{ M}^{0.89}$ at 25 °C and $[\eta] = 1.15 \times 10^{-5} \text{ M}^{0.97}$ at 30 °C. For the NaOH/tannic acid system, the MHS equations were $[\eta] = 3.94 \times 10^{-4} \text{ M}^{0.56}$ at 25 °C and $[\eta] = 1.06 \times 10^{-4} \text{ M}^{0.69}$ at 30 °C, consistent with random-coil behavior.

In the NaOH/urea and NaOH/tannic acid solvent systems, increasing temperature is also expected to enhance hydrophobic interactions along the chitin chains, which may weaken specific interactions between chitin and urea or tannic acid. As a result, the chitin chains can adopt a more extended conformation in solution, leading to an increase in hydrodynamic volume and, consequently, higher intrinsic viscosity values [20–23].

For chitin dissolved in the NaOH/urea system, increasing the temperature from 25 to 30 °C resulted in an increase in the a value from 0.89 to 0.97. This change suggests a modest temperature-induced expansion of the chitin chains in solution. Nevertheless, the a values remained within a relatively narrow range (0.89–0.97), indicating that the chitin chains predominantly retained a highly extended, rod-like-type conformation over the investigated temperature range.

For chitin dissolved in the NaOH/tannic acid system, increasing the temperature from 25 to 30 °C resulted in an increase in the a value from 0.56 to 0.69. This change indicates a modest temperature-induced expansion of the chitin chains in solution. However, the a values remained within the range of 0.56–0.69, which is characteristic of random-coil conformations, suggesting that chitin predominantly retained a random-coil-type chain conformation under these conditions.

4. Conclusions

At 25 °C, a 0.1% chitin solution could be completely dissolved in 5% LiCl/DMAc (w/w) by stirring at 100 rpm for 2 days, and chitin remained stable in this solvent system for up to 12 days without detectable aggregation or degradation. At an identical molecular weight and temperature, the intrinsic viscosity of chitin followed the order: 5% LiCl/DMAc > 8% NaOH/4% urea > 10% NaOH/0.3% tannic acid, reflecting solvent-dependent differences in polymer–solvent interactions and chain conformation.

An increase in temperature from 25 to 30 °C led to higher intrinsic viscosity and Mark–Houwink–Sakurada (MHS) exponent (a) values in all solvent systems, indicating modest chain expansion while preserving the overall conformation regime characteristic of each solvent. In the LiCl/DMAc system, chitin exhibited a highly extended, rod-like-type conformation, whereas chitin dissolved in NaOH/urea also displayed a predominantly

extended chain conformation. In contrast, chitin in the NaOH/tannic acid system adopted a random coil-type conformation over the investigated temperature range.

This study establishes solvent- and temperature-specific MHS equations for chitin across three widely used solvent systems, providing a practical and reliable framework for molecular weight determination and offering insights into solvent-dependent chain conformation relevant to both fundamental studies and industrial applications.

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Abbreviations

The following abbreviations are used in this manuscript:

<i>a</i>	Mark–Houwink–Sakurada exponent
ANOVA	Analysis of variance
DD	Degree of deacetylation
DMRT	Duncan’s New Multiple Range Test
FTIR	Fourier transform infrared
GPC	Gel Permeation Chromatography
HPSEC	High-performance size exclusion chromatography
<i>K</i>	Mark–Houwink–Sakurada parameter
LiCl/DMAc	LiCl/N,N-dimethylacetamide
MHS	Mark–Houwink–Sakurada
<i>M_n</i>	Number-average molecular weight
<i>M_w</i>	Weight-average molecular weights
PDI	Polydispersity index
SLS	Static light scattering
SPSS	Statistical Product and Service Solutions

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