

Urea-Induced Conformational Changes of Chitosan Molecules and the Shift of Break Point of Mark–Houwink Equation by Increasing Urea Concentration

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ABSTRACT: Chitosan solutions of the same 83% degree of deacetylation (DD) but different weight average molecular weights (M_w s) (78–914 kDa) in 0.01M HCl containing different concentrations of urea (0–6M) were prepared. Intrinsic viscosity ($[\eta]$) and weight average molecular weight (M_w) of chitosan were measured with a capillary viscometer and light scattering, respectively. Mark–Houwink exponent a was used as the parameter of conformational index. The Mark–Houwink exponent a increased with increasing concentrations of urea. When solutions contained 0, 2, 3, 4, and 6M urea, the value of a increased from 0.715 to 0.839, 0.894, 1.000, and 1.060, respectively. This indicates the occurrence of urea-induced conformational transitions of chitosans. The break point shifted from 223 kDa in solutions containing no urea to 280 kDa in 2M urea solutions, to 362 kDa in 4M urea solutions and further to 481 kDa in 6M urea solutions. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 75: 452–457, 2000

Key words: chitosan; urea-induced conformational change; Mark–Houwink equation; conformation

INTRODUCTION

Chitosan, a series of different deacetylated chitinous materials, is a random copolymer comprised primarily of repeat sugar units with the structure of a (1–4)-linked 2-amino-2-deoxy- β -D-glucopyranose and also comprised of some units exhibiting the repeat structure of a (1–4)-linked 2-acetamido-2-deoxy- β -D-glucopyranose. The characteristics of chitosan depend on the degree of deacetylation (DD), distribution of acetylglucosamine, chain length, and molecular weight (M_w) distribution.¹

Parameters frequently used to characterize the conformation of a polymer in solution include

Mark–Houwink exponents such as a , b , e , and v . These parameters are obtained from the slopes of the logarithmic plots of intrinsic viscosity, sedimentation coefficient, diffusion coefficient, and radius of gyration, respectively, versus logarithmic molecular weight of the polymer. Values of a between 0.5 and 0.8 indicate that the conformation of the polymer is a random coil. Values of a larger than 1.0 or smaller than 0.5 indicate that the polymer is in the shape of rods or spheres, respectively.^{2–4} Mark–Houwink exponent a as well as Mark–Houwink coefficient K are empirical parameters, depending on the polymer–solvent interaction and on the temperature. For a given polymer in a variety of solvent, a high value of a is usually but not always, associated with a lower value of K .⁵

Conformation of chitosan in solution depends on the degree of protonation (electrostatic repulsive force) and number of intra- and/or intermo-

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lecular hydrogen bonds formed.^{2,6-13} The equilibrium between electrostatic repulsion and restricted rotation by two types of intramolecular hydrogen bonds and β -1,4 glycosides of the glucopyranose ring in the chitosan chain maintain the conformation of chitosan. Intramolecular hydrogen bonds occurrences are between (1) the CH_2OH group and the carbonyl of the acetyl group of two neighboring repeat units and (2) between the OH group and the β deoxy of the two neighboring segments.¹³

Frangou et al.¹⁴ reported that increase urea concentration in a xanthan solution caused the temperature of the conformational transition to increase. This was attributed to the increase in urea concentration stabilizing the hydrogen bonds of xanthan molecules and, in turn, stabilizing its ordered structure, so the transition temperature increased. However, Southwick et al.¹⁵ reported that adding 4M urea to a xanthan solution resulted in ruining the intermolecular hydrogen bonds. The conformation of xanthan changed from order to disorder. Watase et al.¹⁶ reported that adding urea to agarose gel caused the exothermic peak in the differential scanning calorimetry thermogram to shift to lower temperature regions. This also was attributed to urea disrupting the hydrogen bonds of the agarose gel and resulted in a decrease in the melting point temperature. Sklyar et al.¹⁷ reported that chitosan solutions containing 4–8M urea resulted in a linear increase in relative viscosity with increasing urea concentration. This also was attributed to hydrogen bonds being disturbed by urea. Chen et al.¹⁸ reported that adding 4M urea to chitosan solutions resulted in an increase in intrinsic viscosity and persistence length and a decrease in chain flexibility. The changes were attributed to hydrogen bonds being hindered by urea.

According to Mark–Houwink equation, the logarithmic plots of $[\eta]$ versus M_w for linear polymers should be linear over the whole range of molar masses. However, a break⁵ may occur at a certain molar mass. Some molecules that have their M_w s reduced and others that have M_w s higher than a specific molar mass possess different conformations because of different polymer–solvent interactions. The point at which the break occurred on the regression line is called the break point. Tsaih and Chen¹⁹ reported that molecular weight-induced conformational changes occurred and that the break point was about 223 kDa. When the above-mentioned chitosans were dissolved in 0.01M HCl/4M urea solution, the con-

formation was in rod shape, regardless of the molecular weight difference. No molecular weight-induced conformational changes took place. When adding urea in solution, because intramolecular hydrogen bonds are disrupted, electrostatic repulsion becomes the major force that maintains the conformation of chitosan. So, the molecular weight-induced conformational transition of chitosan becomes alleviative. Furthermore, doses of urea promoting or destroying hydrogen bond formation do not agree with the results in the literature.

The effect of different concentrations of urea on the intrinsic viscosities ($[\eta]$) of 10 different weight average molecular weight chitosans (83% DD) in 0.01M HCl solution were determined. Then, a logarithmic $[\eta]$ versus logarithmic M_w plot was used to determine the Mark–Houwink equations. The effects of urea concentration on chitosan conformational changes and on the shift of the break point of the Mark–Houwink equation were elucidated.

MATERIALS AND METHODS

Preparation of the Same Degree of Deacetylation but Different Molecular Weight Chitosan

Chitin was prepared by the method of Chen et al.²⁰ from shrimp (*Solemocera promininitis*) waste. Chitosan was prepared by alkali deacetylation with 50% (w/v) NaOH at 100°C for 3 h with a solid/alkali solution ratio of 1 : 20 to obtain 83 \pm 1% DD chitosan.¹¹ The same DD but different molecular weight (78–914 kDa) chitosans were then obtained by ultrasonic degradation (950E, Crest, Trenton, NJ) in solutions of 1% (w/v) chitosan in 5% (v/v) acetic acid aqueous solution for various times (0–60 h) at 80°C. After ultrasonic degradation, the chitosans were precipitated with a 2N NaOH solution and washed with water, then dried in a freeze drier (UNITOP 800L, Virtis, Gardiner, NY).¹³

Infrared spectroscopy (FTS 155, Bio-Rad, Cambridge, MA) was used to determine the DD of these chitosans samples,²¹ which were all 83 \pm 1%.

Determination of the Weight Average Molecular Weight

The static light-scattering method was used to measure the M_w of the chitosan samples.^{1,13,19,22}

Different concentrations (0.001–0.1 g/L) of chitosans in 0.01N HCl/0.19N NaCl solutions were prepared. The solvent and solutions were filtered through 0.02 μm (Anotop, Whatman, Clifton, NJ) and 0.45 μm (Lida, Kenosh, WI, USA) filters, respectively. The scattered light intensity was measured between 30° and 140° by a Malvern light-scattering photometer (Malvern, 4700, Mal, UK) at 632.8 nm and $30 \pm 0.1^\circ\text{C}$. The M_w was calculated from a Zimm plot processed by Malvern software (version 1.26 for Windows). Every sample measurement was repeated five times. Refractive index increments (dn/dc) of chitosan solutions equaled 0.189 mL/g and were determined by an interferometric refractometer (Optilab 903, Wyatt, Santa Barbara, CA).

Determination of the Intrinsic Viscosity

Different concentration (0.01–0.1%) solutions of chitosan in 0.01M HCl containing 0, 2, 3, 4, and 6M urea were prepared. The solution was passed through a 0.45- μm filter (Lida) to remove insoluble materials. The capillary viscometer (No. 75, Cannon-Fenske, State College, PA) was filled with 5 mL of sample and equilibrated in a water bath (Tamson, TMV 40, Zoetermeer, The Netherlands) with an extra thermostat (Firstek B403, Taipei) to maintain the temperature at $30 \pm 0.1^\circ\text{C}$. The sample was passed through the capillary once before the running time was measured. Each sample was measured three times. The running times of the solution and solvent were used to calculate the relative viscosity, specific viscosity, and reduced viscosity.⁴

Calculation of the Mark–Houwink Exponents 'a', 'a*', and 'a**'

$\log [\eta]$ was plotted against $\log M_w$ with the slope of the plot being the Mark–Houwink exponent.^{2–5,23–24} The Mark–Houwink exponents, 'a', 'a*', and 'a**', represent chitosans whose M_w s are between 78 and 914 kDa, chitosans whose M_w s are smaller than the break point, and chitosans whose M_w s are larger than the break point, respectively.

RESULTS AND DISCUSSION

Effect of Concentration of Urea on Conformational Changes of Chitosan

The Mark–Houwink exponent a of chitosan in 0.01M HCl is 0.715 [Fig. 1(a)]. By adding 2, 3, 4,

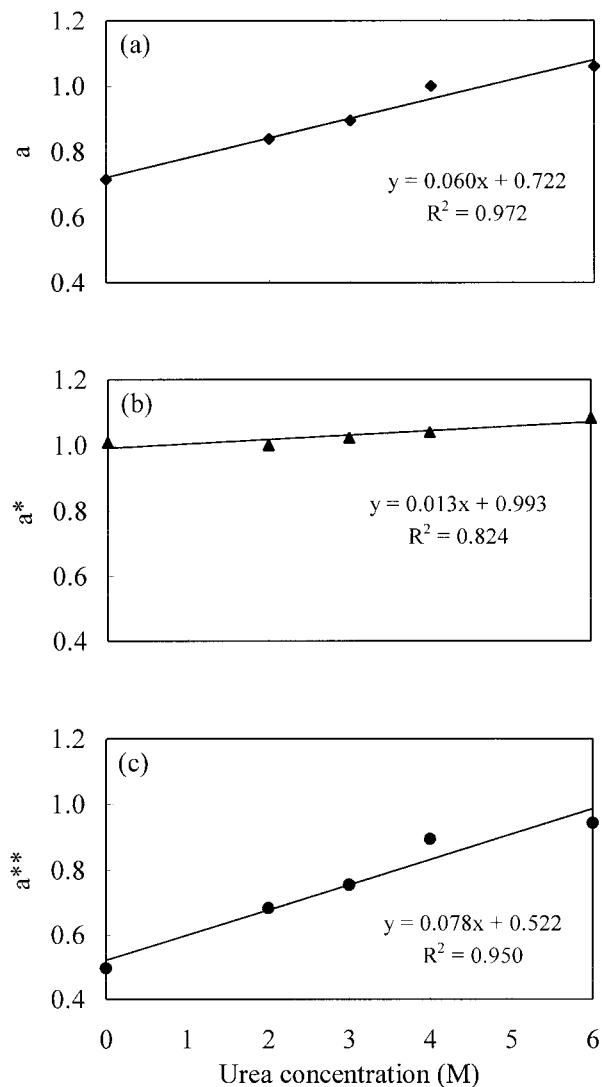


Figure 1 Linear relationship of Mark–Houwink exponents a , a^* , and a^{**} with urea concentration.

and 6M urea to the chitosan solutions, the Mark–Houwink exponent a increased to 0.839, 0.894, 1.00, and 1.060, respectively [Figs. 1(a) 2]. When the solution contained a concentration of 4M urea or greater, results indicate that the conformation of the chitosan samples changed from a random coil to a rod shape. When the concentration of urea is higher than 6M, chitosan solubility rapidly decreases. Effects of urea concentration on chitosan conformation can not be studied at urea concentrations higher than 6M due to solubility limitations.

Effects of urea on the conformation of polysaccharides such as xanthan and chitosan have been reported. Southwick et al.¹⁵ reported that the conformation of xanthan changes from order to dis-

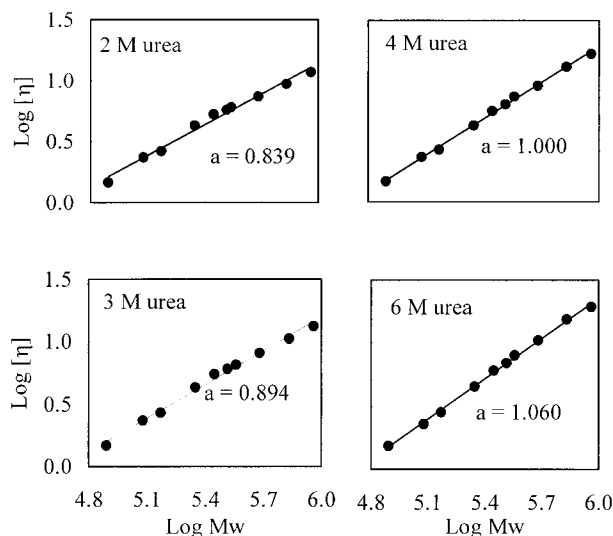


Figure 2 Double logarithmic plots of intrinsic viscosity ($[\eta]$) and weight average molecular weight (M_w) and Mark-Houwink exponents a of chitosans in $0.01M$ HCl solution with different concentrations of urea at $30 \pm 0.1^\circ\text{C}$.

order when urea is added to the solution. Sklyar et al.¹⁷ reported that the relative viscosity increases linearly with increasing urea concentration. Chen et al.¹⁸ and Gamzazade et al.²⁵ reported that increased urea concentration in chitosan solutions results in increasing intrinsic viscosity and further in an increase of persistence length and a decrease in chain flexibility, and so in turn the molecules become more extended.¹⁹ Therefore, conformational changes of xanthan¹⁵ and chitosan¹⁷⁻¹⁹ were attributed to hydrogen bonds of polymers being disturbed by urea. The extent of hydrogen bond changes is proportional to the concentration of urea added. Results in Figure 1(b,c) show a^* and a^{**} values also increasing with increasing urea concentration. This indicates that both conformations of chitosans whose M_w s are larger or smaller than the break point both become more extended by increasing urea concentrations. Since conformations of smaller molecular weight chitosans are rod-shaped, even without urea (at $0M$) [Fig. 1(b)], increasing urea concentrations in solution result in small effects in increasing the Mark-Houwink exponent a^* [as indicated in Fig. 1(b), there was no significant change in the chitosan conformation with increasing concentration of urea, i.e., the slope was 0.013]. However, for those larger molecular weight chitosans, the effects of urea on their conformation are remarkable. Increasing

urea concentration resulted in a remarkable increase in the value of the Mark-Houwink exponent a^{**} . Values of a^{**} increased from 0.497 at $0M$ urea to 0.944 at $6M$ urea [Fig. 1(c)]. The conformation of larger molecular weight chitosans changed from a sphere at $0M$ urea to a random coil at 2 and $3M$ urea and further to a rod shape at concentrations higher than $4M$. This might indicate that conformations of larger molecular weight chitosans change more pronouncedly with increasing urea concentration, e.g., larger molecular weight chitosans are more susceptible to influence by urea. This might also indicate that increasing urea concentrations in larger M_w chitosan solutions result in gradual conformational changes and might indicate urea-induced conformation change occurred in $0.01M$ HCl solutions contained more than $4M$ urea.

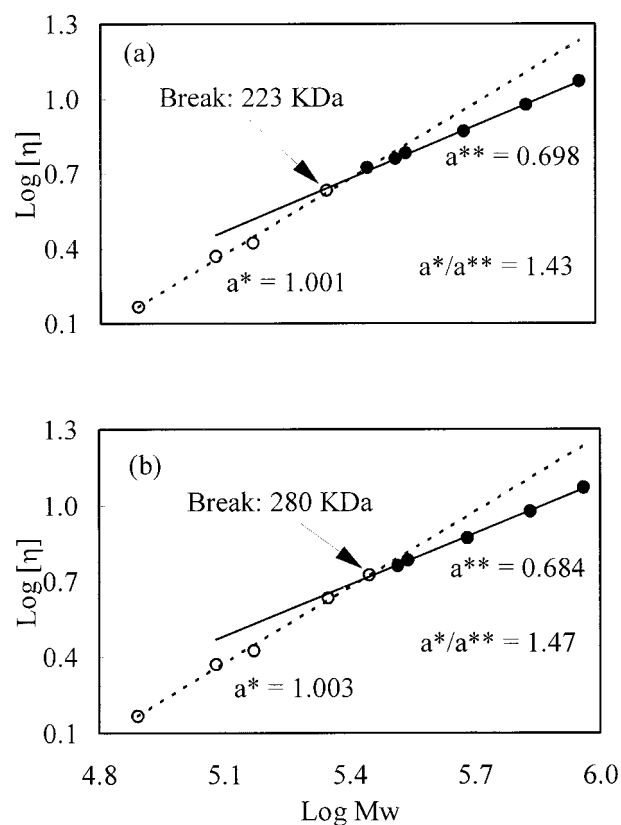


Figure 3 Plots of $\text{Log} [\eta]$ versus $\text{Log} M_w$ for chitosans in $0.01M$ HCl/ $2M$ urea solution at $30 \pm 0.1^\circ\text{C}$ to obtain Mark-Houwink exponents a^* and a^{**} from the slopes of regression lines, including chitosans whose M_w s are smaller and larger than the break point, respectively. (Break point at 223 and at 280 kDa are used in (a) and (b), respectively. (\circ) data associated with a^* ; (\bullet) data associated with a^{**} .)

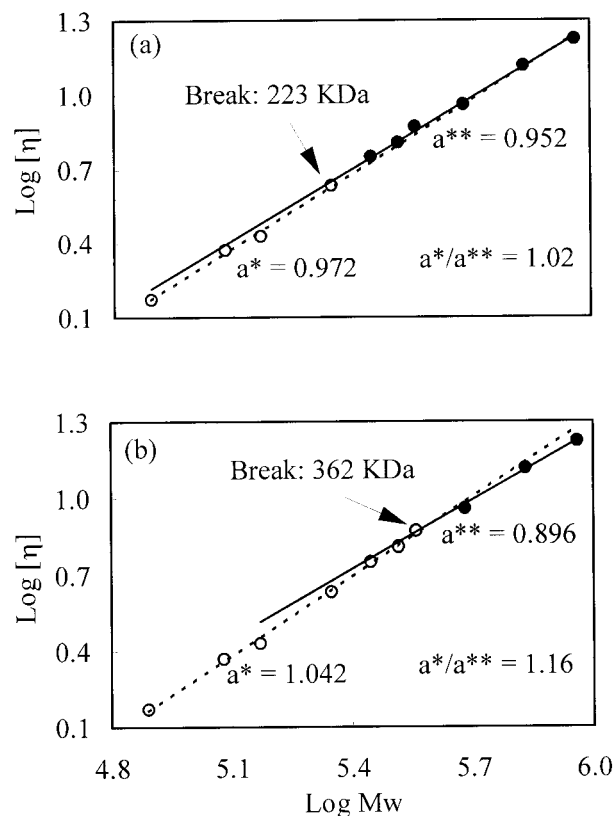


Figure 4 Plots of $\text{Log} [\eta]$ versus $\text{Log} M_w$ for chitosans in $0.01M$ HCl/ $4M$ urea solution at $30 \pm 0.1^\circ\text{C}$ to obtain Mark-Houwink exponents a^* and a^{**} from the slopes of regression lines including chitosans whose M_w s are smaller and larger than the break point, respectively. (Break point at 223 and 362 kDa are used in (a) and (b), respectively. (○) data associated with a^* ; (●) data associated with a^{**}).

Effect of Urea Concentration on the Shift of Break Point of Mark-Houwink Equation

Results in Figure 3(a) shows that using 223 kDa as the break point a^* is 1.001, whereas a^{**} is 0.698. The results indicate that the conformation of chitosans on either side of the break point are different. For example, conformation of chitosans on the lefthand side of the break point are a rod shape, whereas those on the righthand side of the break point are spherical. Values of a^*/a^{**} indicate the tendency for the occurrence of conformational change, e.g., higher a^*/a^{**} values indicate higher tendency of molecular weight-induced conformation changes. However, when using 280 kDa as the break point, a^* , a^{**} , and a^*/a^{**} were calculated to be 1.003, 0.684, and 1.47, respectively [Fig. 3(b)]. Results imply that after adding $2M$ urea to a chitosan solution, the break point of

molecular weight-induced conformational change shifts to 280 kDa. Results in Figure 4(a,b) show the break point of molecular weight-induced conformational change has shifted to 362 kDa, as the concentration of urea in the chitosan solution is increased to $4M$. This may be attributed to urea's hindering the hydrogen bonds and causing the conformation of higher M_w chitosans to become extended, therefore resulting in their shapes becoming similar to those of lower M_w chitosans. Results indicate that the break point shifts to a higher M_w range. Furthermore, the larger the M_w of chitosan, the more the extent of entanglement and also the larger the number of intramolecular hydrogen bonds formed, thus resulting in a more compact conformation.

CONCLUSION

Adding urea to the chitosan solutions, chitosan molecules become extended due to hydrogen bonds being disturbed. The conformations of these resulting higher M_w chitosans become similar to smaller M_w chitosans. The higher the M_w of chitosan, the higher the concentration of urea needed to disrupt the intramolecular hydrogen bonds. Therefore, the break point shifts to a higher M_w range with increasing urea concentrations.

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