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1 Intermolecular Interaction and the Extended Wormlike Chain 2 Conformation of Chitin in NaOH/Urea Aqueous Solution

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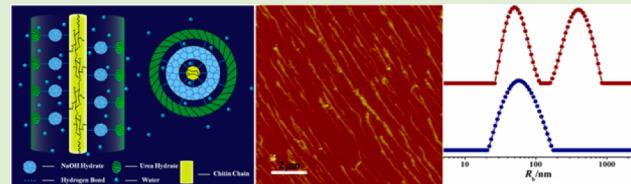
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7 Supporting Information

8 **ABSTRACT:** The intra- and intermolecular interactions of
9 chitin in NaOH/urea aqueous system were studied by a
10 combination of NMR measurements (including ¹³C NMR, ²³Na
11 NMR, and ¹⁵N NMR) and differential scanning calorimetry.
12 The results revealed that the NaOH and chitin formed a
13 hydrogen-bonded complex that was surrounded by the urea
14 hydrates to form a sheath-like structure, leading to the good
15 dissolution. The optimal concentration range, in which chitin was molecularly dispersed in NaOH/urea aqueous, was found to
16 investigate the chain conformation in the dilute solution with a combination of static and dynamic light scattering. The weight-
17 average molecular weight (M_w), radii of gyration ($\langle R_g \rangle_z$), and hydrodynamic radii ($\langle R_h \rangle_z$) values of chitin were determined, and
18 the structure-sensitive parameter (ρ) and persistent length (L_p) were calculated to be >2.0 and ~30 nm, respectively, suggesting
19 an extended wormlike chain conformation. On the basis of these results, chitin nanofibers were fabricated from the parallel
20 aggregation of chitin chain in NaOH/urea system. This work would provide a theoretical guidance for constructing novel chitin-
21 based nanomaterials via “bottom-up” method at the molecular level.



22 ■ INTRODUCTION

23 Chitin, an enticing biopolymer with important functionalities, is
24 the second most abundant organic material in nature after
25 cellulose, existing mainly in the exoskeletons of crabs and
26 shrimps.¹ It has attracted considerable attention due to its
27 notable biofunctionality, biodegradability, and biocompatibility.^{2–5} However, the numerous intermolecular and intra-
28 molecular hydrogen bonds among chitin chains result in its
29 difficult dissolution and further processing. Only a few solvents
30 including *N,N*-dimethylacetamide (DMAc)/lithium chloride
31 (LiCl),⁶ CaCl₂–MeOH,⁷ ionic liquids,⁸ and alkaline–ice
32 mixture,⁹ some strong acids and fluorinated solvents¹⁰ could
33 dissolve chitin. However, these solvents have not been finally
34 used in industry, as they have some limitations such as cost,
35 difficulty for solvent recovery, volatility, and toxicity. Recently,
36 in our laboratory, a novel green solvent of NaOH/urea aqueous
37 solution with low cost has been found to successfully dissolve
38 chitin via freezing/thawing. Moreover, a series of functional
39 chitin-based materials such as hydrogels, aerogels, films, fibers,
40 and microspheres with homogeneous structure and excellent
41 properties have been constructed,^{11–17} but the structure–
42 property correlation of these materials has not been fully
43 understood.

44 It is noted that the chain conformation and the
45 intermolecular interactions between polymer and solvent are
46 important during the material regeneration and processing, as it
47 would help in formulating the polymer processing parameters
48 to fabricate the materials with specific structure and functions.
49 Surprisingly, varying results of chain conformations of chitin in

50 different solvent have been reported.^{6,9,10,18–20} For instance,
51 the chain conformation of chitin was a stiff chain in DMAc/
52 LiCl system,⁶ opposite to random coil in alkaline–ice mixture⁹
53 and NaOH/urea.¹⁸ Furthermore, to date, no systemic studies
54 have been done to explore the intermolecular interactions
55 between chitin and solvent in NaOH/urea system. Therefore, it
56 is necessary to clarify the chain conformation and the
57 interaction between chitin and solvent in NaOH/urea aqueous
58 solution so as to construct chitin-based materials with the
59 unique structure and functions.⁶⁰

61 In the following work, the intermolecular interactions in
62 chitin–NaOH–urea aqueous system were explored by NMR
63 experiments (including ¹³C NMR, ²³Na NMR, and ¹⁵N NMR)
64 and DSC measurements. Moreover, the chain conformation of
65 chitin in NaOH/urea aqueous system was carefully studied by
66 dynamic light scattering (DLS) and static light scattering (SLS)
67 under different conditions including varying temperature and
68 chitin concentration. As a result, (1) the interactions between
69 chitin and solvent of the NaOH and urea molecules were
70 revealed for the first time, on the basis of which the chitin
71 complex model was proposed and (2) the extended wormlike
72 chain conformation of the chitin chain in NaOH/urea aqueous
73 solution was determined. Furthermore, inspired by the fact that
74 extended wormlike chain are excellent candidates for creating
75 nanofibers via self-assembly process,^{21–24} nanofibers were

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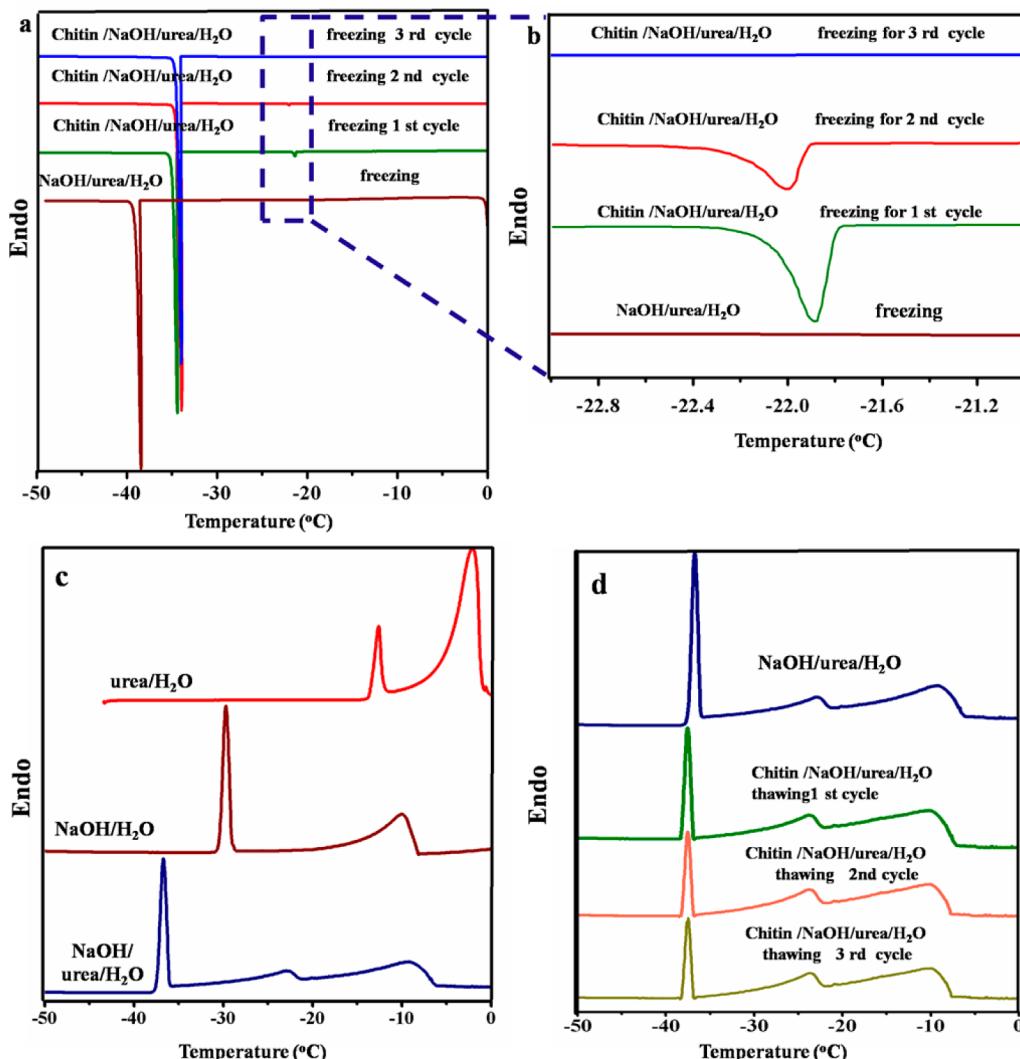


Figure 1. DSC freezing thermograms of NaOH/urea solution and the suspension of chitin in NaOH/urea solution during three times freezing-thawing process (a). Magnified freezing thermograms for NaOH/urea solution and the suspension of chitin in NaOH/urea solution during three times freezing-thawing process (b). DSC thawing thermograms of urea, NaOH, and NaOH/urea solution (c). DSC thawing thermograms of NaOH/urea solution and the suspension of chitin in NaOH/urea solution during three times freezing-thawing process (d).

successfully obtained from the aggregation of chitin chains in a parallel manner from NaOH/urea aqueous system. Taken together, this work would provide a theoretical guidance for constructing nanomaterials via “bottom up” method at the molecular level in the future.

EXPERIMENTAL SECTION

Materials. The commercially available chitin product was purchased from Sigma-Aldrich, coded as chitin-1 without further treatment before use. To demonstrate the universality of the dissolution, chitin powder from Golden-Shell Biochemical in Zhejiang of China was also purchased. To remove the impurity such as protein and mineral substance, the raw chitin powder was purified before use by treating with 5 wt % NaOH solution (at room temperature, overnight), then 7% (V/V) HCl solution (at room temperature, 1 day), and washed with water after each step. Finally, the purified chitin powder was freeze-dried, coded as chitin-2, and stored in desiccators before use, and the yield of the purified chitin powder was 85%. To get lower molecular weight of chitin, chitin-2 was treated with 5 wt % H₂O₂ for 4 h (pH 9, 80 °C) and washed with water. Finally, the purified chitin powder was freeze-dried, coded as chitin-3. The degree of acetylation of chitin-1, chitin-2, and chitin-3 was determined to be

94, 93, and 90%, respectively, from FT-IR spectra according to the following equation.

$$\frac{A_{1560}}{A_{2875}} = 0.0125 \times DA + 0.2 \quad (1)$$

where A_{1560}/A_{2875} is the ratio of the absorption bands at 1560 and 2875 cm⁻¹.²⁵ This result indicated that the purification procedures hardly decreased the degree of acetylation of chitin. NaOH and urea were purchased from Shanghai Chemical Regent in China and used without further purification.

Preparation of the Chitin Solution. The weighted chitin powder was dispersed in a mixture of NaOH, urea, and distilled water in the weight ratio of 11:4:85. Subsequently, the suspension was frozen at -30 °C in a cooling trap for 4 h and then thawed at 5 °C. The freezing/thawing cycle was repeated three times to obtain a transparent chitin solution. The original concentrations of the chitin solution in the range from 0.02 to 1.0 mg/mL were used in the following experiments.

Preparation of Chitin Nanofiber in NaOH/Urea System. Five μL of chitin solution with the original concentration ranging from 0.001 mg/mL to 0.1 mg/mL was placed on mica plate at ambient temperature. After ~10 min, the substrate was washed with amounts of

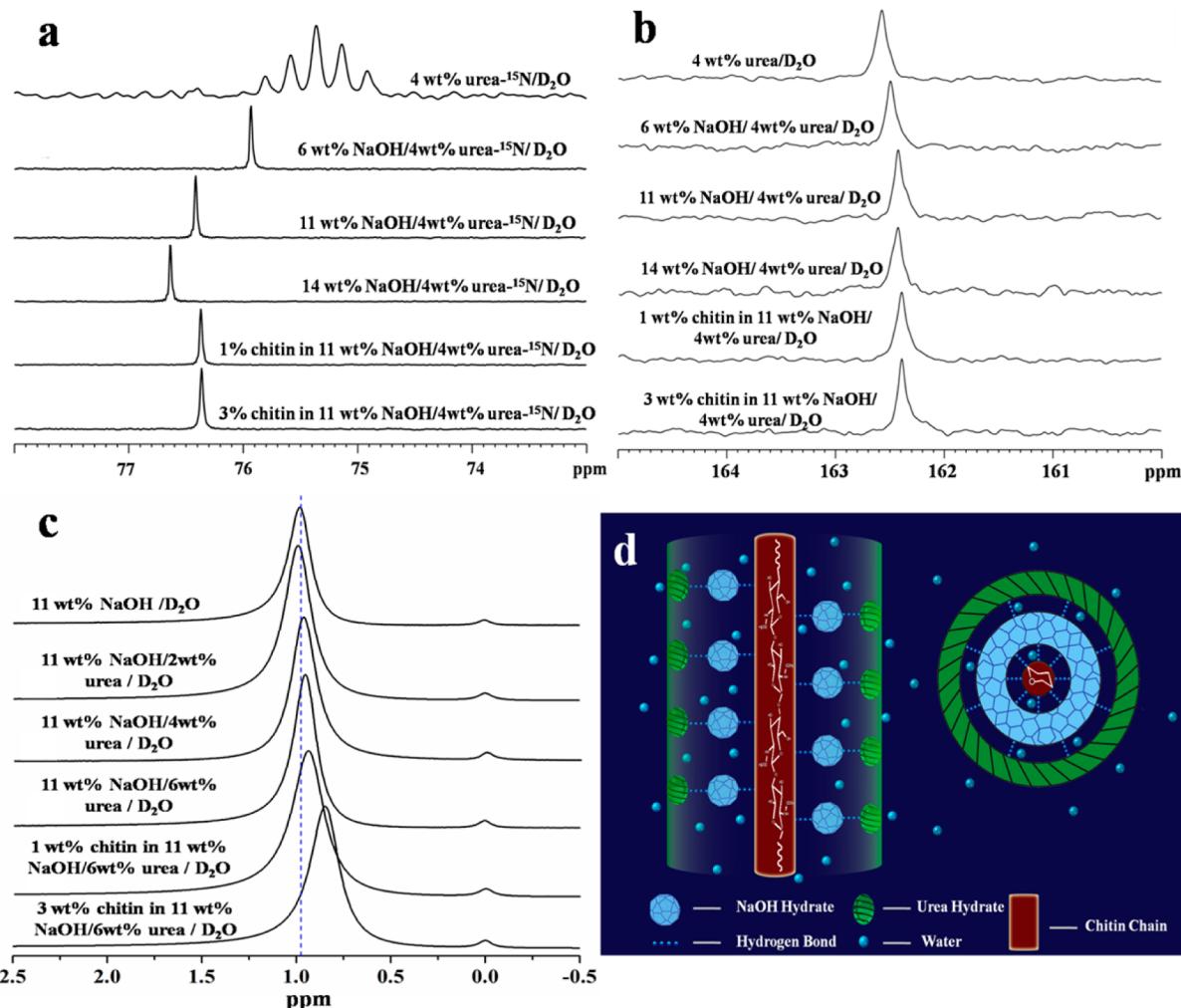


Figure 2. ¹⁵N NMR spectra of urea-¹⁵N/D₂O, NaOH/urea-¹⁵N/D₂O with different contents of NaOH, and chitin in NaOH/urea-¹⁵N/D₂O solutions at 5 °C (a). ¹³C NMR spectra of urea/D₂O, NaOH/urea/D₂O with different contents of NaOH, and chitin in NaOH/urea/D₂O solutions at 5 °C (b). ²³Na-NMR spectra of NaOH/D₂O, NaOH/urea/D₂O, and chitin in NaOH/urea/D₂O solutions with different contents of urea, NaOH, and chitin at 5 °C (c). A model description of chitin complex chain in NaOH/urea aqueous solution (d).

ice water to remove salts such as NaOH and urea for the formation of nanofiber. The substrates were then dried under constant N₂ gas flow. **Characterization.** Differential scanning calorimetry (DSC) was carried out on a PERKIN ELMER PYRISL. Twenty mg of suspension was sealed in a stainless pan. The temperature program consisted of freezing from 5 °C to -50 °C and heating from -50 to 5 °C, both at 1 °C/min, and the cycle was repeated three times. ¹³C NMR measurements of the chitin in 11 wt % NaOH/4 wt % urea/D₂O and 11 wt % NaOH/4 wt % urea/D₂O aqueous solutions were carried out on a Mercury 600 MHz NMR spectrometer (Varian) at 0 °C. For ¹³C NMR measurements, the chitin concentration was 7 wt %. The sodium salt of 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS) was used as an internal reference to determine the chemical shifts. ²³Na and ¹⁵N NMR spectra were recorded on a Bruker AVANCE 600 NMR spectrometer. For the ¹⁵N NMR experiments, nitromethane (CH₃NO₂) was used as an external chemical-shift reference at 382 ppm. ²³Na NMR chemical shifts were externally referenced to aqueous solution of 0.7 M NaBPh₄ at -0.041 ppm. AFM observations were carried out on a Picoscan AFM (Molecular Imaging, Tempe, AZ) in a MAC mode with commercial MAC lever II tips (Molecular Imaging, U.S.A), with a spring constant of 0.95 N/m. TEM images were obtained by using a JEM-2010 (HT) transmission electron microscope (JEOL TEM, Japan). For the TEM observation, the samples were prepared by casting the diluted chitin solution with the concentration of 1 mg/mL onto a holey carbon film, which was supported on a

copper grid. For cryo-TEM, the samples were prepared in a controlled environment vitrification system (CEVS) at 277 K. The chitin/NaOH/urea aqueous solution with the chitin mass fraction of 5 mg/mL was dropped onto a carbon-coated copper grid, which was blotted with two pieces of filter paper, resulting in the formation of thin films suspended on the mesh holes. The samples were quickly plunged into a reservoir of liquid ethane (cooled by nitrogen) at 108 K. The vitrified samples were observed with a FEI Tecnai 20 TEM (200 kV) at ~99 K.

Laser Light Scattering and Viscometry. SLS and DLS were utilized to characterize the molecular weight and chain conformation of chitin in the dilute solution (11 wt % NaOH/4 wt % urea aqueous system) at different temperature (5, 10, 15, and 20 °C). A commercial light-scattering spectrometer (ALV/SP-125, ALV, Germany) equipped with an ALV-5000/E multidigital time correlator and a He-Ne laser (at 632.8 nm) was used at scattering angles θ of 20–150°, respectively. All of the diluted chitin solutions with concentration from 0.02 to 1.0 mg/mL were made optically clean by filtration through 0.45 μm Millipore filters (NYL, 13 mm syringe filter, Whatman, U.S.A). The Rayleigh ratio R_g can lead to the weight-average molar mass (M_w), the z-mean radius of gyration ($\langle R_g \rangle_z$), and the second viral coefficient (A_2) by using

$$\frac{Kc}{R_g} = \frac{1}{M_w} \left(1 + \frac{1}{3} \langle R_g^2 \rangle_z q^2 \right) + 2A_2 c \quad (2)$$

where $K = 4\pi^2 n^2 (dn/dc)^2 / (N_A \lambda_0^4)$ and $q = (4\pi n/\lambda_0) \sin(\theta/2)$, with c , dn/dc , N_A , and λ_0 being the concentration of polymer solution, the specific refractive index increment, Avogadro's number, and the wavelength of light in vacuum, respectively. The dn/dc value for chitin in NaOH/urea aqueous solution was measured using a double-beam differential refractometer (DMR-1020, Otsuka Electronics, Japan) at 632.8 nm to be 0.160 mL/g. A Zimm plot was used to calculate the values of M_w and $\langle R_g \rangle_z$.

In the DLS measurements, the CONTIN program was used for the analysis of the dynamic light-scattering data.²⁶ The hydrodynamic radius ($\langle R_h \rangle_z$) of chitin in dilute solution was calculated by using the following Stokes-Einstein relation as

$$\langle R_h \rangle_z = \frac{k_B T}{6\pi\eta_0 D_z} \quad (3)$$

where k_B is the Boltzmann constant, T is the temperature in units of K, η_0 is the solvent viscosity, and D_z represents the translational diffusion coefficient.

In the viscosity measurement, the reduced viscosity (η_{sp}/c) of the chitin dilute solutions in 11 wt % NaOH/4 wt % urea aqueous system was measured by using an Ubbelohde capillary viscometer at 5 °C.

RESULTS AND DISCUSSION

Interaction in Chitin–NaOH–Urea Solution. As stated in the experiments, chitin was dissolved in NaOH/urea aqueous solution via freezing/thawing cycles, and DSC was thus performed to monitor this dissolution process. Figure 1 shows the DSC profiles for freezing and thawing of the solvent and the suspension of chitin-1 in NaOH/urea aqueous solution. The big exothermic peak was ascribed to the crystalline peak of water in the mixed solution.²⁷ Compared with the freezing profile of solvent (Figure 1a), a small exothermic peak was detected in the temperature range from −23 to −20 °C for the chitin in NaOH/urea solution, whose intensity decreased with an increase in freezing–thawing cycle times and completely disappeared in the third cycle (Figure 1b). This results indicated that the dissolution at the low temperature was a typical enthalpy-driven process, and the dissolution was a physical process, which was supported by results of the ¹³C NMR spectra of chitin in NaOH/urea/D₂O solution (Figure S1 and Table S1 in the Supporting Information). In the thawing profiles of urea/H₂O and NaOH/H₂O (Figure 1c), −12.8 and −31.9 °C of the two sharp endothermic peaks were, respectively, ascribed to the melting of urea hydrates and NaOH hydrates, and the broad peak at ~10 °C corresponded to the free water.²⁸ Clearly, in the thawing profiles of NaOH/urea aqueous solution (Figure 1c), the peaks standing for the melting of NaOH hydrates (−37.5 °) and urea hydrates (−22.6°) moved to the lower temperature, suggesting the interaction between NaOH and urea occurred. Interestingly, in the presence of chitin in NaOH/urea aqueous solution (Figure 1d), the peak corresponding to the melting of NaOH hydrates also moved to the lower temperature, and the enthalpy of the melting of NaOH hydrates decreased significantly with increasing freezing-thawing cycle. Whereas those standing for urea hydrates and free water hardly changed. Furthermore, the enthalpy assigned to urea hydrates and free water almost remained the same (Figure 1d). All of the results indicated that the NaOH hydrates directly interacted with chitin during the dissolution process, which played a key role in the chitin dissolution. It was noted that the chitin solution in NaOH aqueous system without urea tended to gel at ambient temperature (not shown). Therefore, urea may be contributed to stabilize the chitin solution.

To further elucidate the role of urea and NaOH in the dissolution process, we observed a series of ¹⁵N NMR spectra of urea in urea/D₂O solutions with different contents of NaOH and chitin and ²³Na-NMR spectra of NaOH solutions with different contents of urea and chitin at 5 °C (Figure 2). The ¹⁵N signal was multiple due to the ¹⁵N-D spin–spin coupling, which became a singlet and moved to downfield at the presence of NaOH. This suggested that OH[−] ions of NaOH interacted with the amino groups of urea, leading to fast exchange between −ND₂ and D₂O and thus disappearance of the ¹⁵N-D coupling and that the interaction of NaOH with urea reduced the electron cloud density of urea-¹⁵N, leading to higher double-bond character for the C–N bonds.^{29,30} It was noticed that further addition of chitin in the solution hardly changed the chemical shift and the line shape of ¹⁵N signal. Moreover, the ¹³C NMR spectra of urea/D₂O with different contents of NaOH as well as chitin at 5 °C (Figure 2b) showed that the presence of NaOH caused a clear upfield chemical shift drift of ¹³C=O of urea, whereas the ¹³C=O chemical shift was insensitive to the changes in the contents of chitin. Those above results suggested that urea hydrates have no direct interaction with chitin but attached to NaOH hydrates to promote the dissolution and prevent hydrophobic association of chitin, which was consistent with the results from ²³Na-NMR spectra of a series of NaOH solutions with different contents of urea and chitin at 5 °C (Figure 2c), where the chemical shift of ²³Na moved to the upfield in the presence of urea or chitin or both.

On the basis of the previously described analysis, we proposed a schematic model to qualitatively describe the chitin complexes hosted by urea and NaOH hydrates, as shown in Figure 2d. When the mixture of chitin and solvent was cooled to −30 °C, the hydrogen-bonded complex associated with chitin macromolecules and solvent molecules including NaOH, urea, and water clusters in the aqueous solution was created, leading to the disruption of intermolecular hydrogen bonds between chitin chains.

Molecular Weight and Chain Conformation of Chitin in the Extremely Dilute Solution. To investigate the stability of chitin in NaOH/urea aqueous solution, the reduced viscosity of the 0.8 mg/mL chitin-2 solution was measured at 5 °C for 25 h. The results (blue curve in Figure 3) indicated that ~5% loss of the viscosity was observed during the whole test,

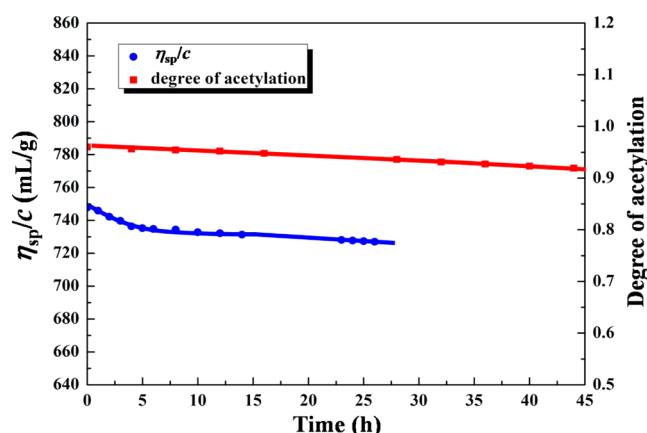


Figure 3. Reduced viscosity (η_{sp}/c) and the degree of acetylation as a function of time ($c = 0.3$ mg/mL, 5 °C).

suggesting that the molecular weight of chitin hardly changed. Moreover, the deacetylation of chitin-2 in this system at 5 °C was measured over 45 h, and only ~5% loss on the degree of acetylation was observed (red curve in Figure 3). These results indicated the chitin structure was stable in the NaOH/urea system, and the chitin solution can be used to study the dilute solution properties of chitin.

Because of the abundance of interchain hydrogen bonds, chitin has a strong tendency to aggregate in aqueous solution. As shown in Figure S2 in the Supporting Information, there were two peaks in the R_h distribution of chitin-1 in NaOH/urea aqueous solution, and with an increase in temperature, the peak corresponding to the aggregates increased. It can be explained as the chitin complexes structure was destroyed at elevated temperature as a result of the peeling of water-soluble shell consisting of NaOH and urea to induce the aggregation, leading to an increase in the apparent weight-average molecular weight. To avoid aggregation, extremely dilute solutions of chitin were prepared to be used for DLS and SLS measurements at relatively low temperature and small scattering angle. Figure 4

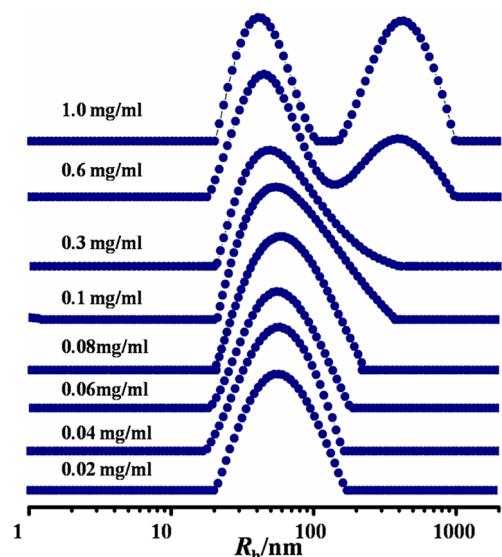


Figure 4. R_h distribution of chitin-1 in NaOH/urea aqueous solution at different concentration (scattering angle θ of 20°).

shows the apparent hydrodynamic radius distributions $f(R_h)$ of chitin-1 in NaOH/urea aqueous solution with concentrations from 0.02 to 1 mg/mL at 5 °C (scattering angle $\theta = 20^\circ$). Clearly, only one peak appeared in the extremely dilute solution with concentration lower than 0.1 mg/mL. In particular, the $f(R_h)$ curves at concentrations equal to and lower than 0.06 mg/mL were symmetrical and almost completely overlapped with each other, demonstrating that only isolated chitin chains (individuals) existed in the solution. With an increase in the chitin concentration to higher than 0.08 mg/mL, peak tailing and broadening were observed, and even two peaks corresponding to individuals and aggregates (fast and slow modes) occurred at a concentration of 0.6 mg/mL. These results indicated that with a slight increase in the chitin concentration, the transition from only individual chains to the coexistence of individuals and aggregates occurred gradually. The previous results further supported that the NaOH/urea aqueous solution could completely dissolve chitin into a true solution, in which chitin was molecularly dispersed in the

aqueous solution. However, the aggregates occurred with increasing the polymer concentration as a result of increasing the intermolecular colliding probability. Therefore, the complex's structure was very sensitive to the polymer concentration and temperature and could be destroyed as either of them increased, resulting in the self-association of chitin. Thus, the chitin individuals coexisted with their aggregates in the dilute solution, and the existence of the aggregate caused the various determination results of the molecular weight and chain conformation. In refs 9 and 18, the concentrations used ranged from 0.05 to 2.25 mg/mL, and the $f(R_h)$ peak tailing and asymmetry were clearly observed, suggesting that the isolated chains and aggregates coexisted, which would lead to higher molecular weight. In view of the observations previously discussed, the optimal measuring conditions under which chitin was molecularly dispersed in the NaOH/urea aqueous solution were very important for the correct characterization of the M_w and R_g values.

As shown in Figure 4, chitin-1 was completely dispersed at the molecular level at concentrations of 0.02 to 0.06 mg/mL, showing one peak in the R_h distribution patterns obtained with DLS. Moreover, to demonstrate the cleanliness of the extremely dilute solution, we tested the time-dependent R_h distribution of chitin-1 at a small angle of 20° for 30 min. The results showed that no difference was observed (Figure S3 in the Supporting Information), indicating the good cleanliness of the chitin solution. Subsequently, the extremely dilute solutions with concentration lower than or equal to 0.06 mg/mL were prepared to determine the exact molecular weight of the chitin samples with SLS. Figure 5 shows the Zimm plots of the chitin-1 and chitin-2 samples in the extremely dilute solution of 0.02 to 0.06 mg/mL. Clearly, the Zimm plots exhibited a good linear relationship, and the values of M_w and $\langle R_g \rangle_z$ were easily calculated from the intercept and the slope of the extrapolation.

The hydrodynamic radius ($\langle R_h \rangle_z$) values were determined according to the Stokes–Einstein relation (eq 3) from DLS and listed in Table 1. It is well known that $\langle R_g \rangle_z$ is related to the actual space occupied by the polymer chain, whereas $\langle R_h \rangle_z$ is the radius of an equivalent hard sphere, which has an identical diffusion coefficient D as the polymer chain in this solution.³¹ The molecular shape of polymers can be described from the value of a structure-sensitive dimensionless parameter ρ (= $\langle R_g \rangle_z / \langle R_h \rangle_z$), which directly reflects the chain conformation.³² By using the $\langle R_g \rangle_z$ and $\langle R_h \rangle_z$ data in Table 1, the ρ values for the individuals of chitin samples in the aqueous solution were estimated to be higher than 2.0, indicating an extended chain conformation of chitin in this solution.³³

Additionally, we used the light-scattering data of three samples to estimate the conformational parameters including the persistence length (L_p) and molar mass per contour length (M_L) according to Benoit and Doty's expression for wormlike chains of polymers by³⁴

$$\langle R_g \rangle_z^2 = \frac{L_p L}{3} - L_p^2 + \frac{2L_p^3}{L} - \frac{2L_p^4}{L^2} [1 - \exp(-L/L_p)] \quad (4)$$

where L is the contour length and $L = M_w/M_L$. Usually, the value of L_p is in the range from 11 to 66 nm, and that of M_L lies in the range from 350 to 810 nm⁻¹ for typical stiff polymers.³⁵ In these findings, the values of L_p and M_L were estimated to be ~30 and ~470 nm⁻¹ (Figure S4 in the Supporting

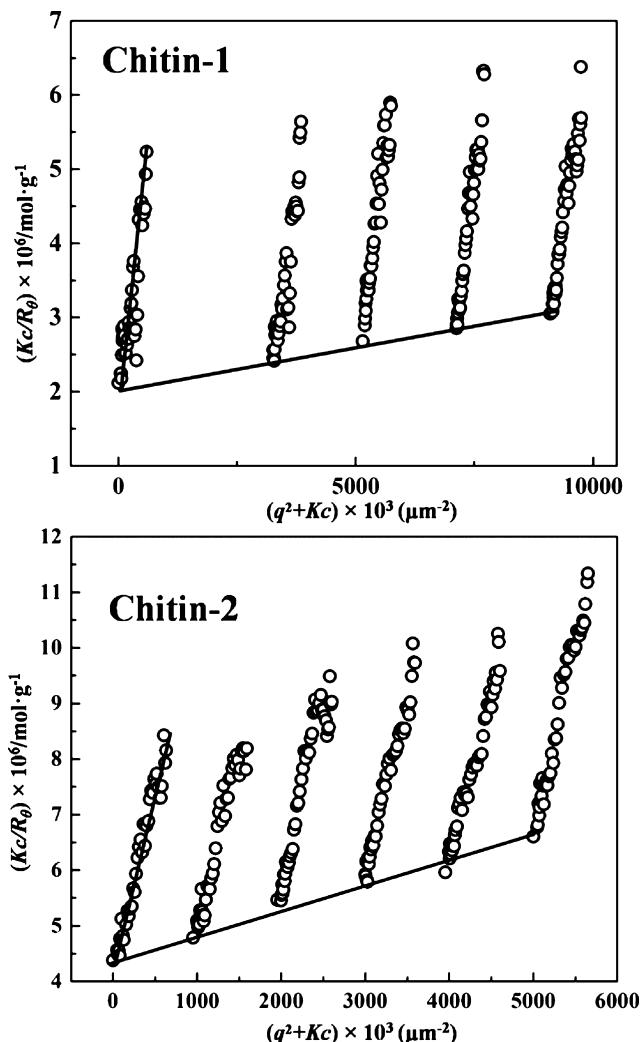


Figure 5. Zimm plots of the chitin-1 and chitin-2 solution in aqueous NaOH/urea at 5 °C.

Table 1. Experiment Results of M_w , $\langle R_g \rangle_z$, $\langle R_h \rangle_z$, and ρ of the Chitin-1, Chitin-2, and Chitin-3 in NaOH/Urea Aqueous System at 5 °C

systems	samples	$M_w \times 10^{-4}$	$\langle R_g \rangle_z$ (nm)	$\langle R_h \rangle_z$ (nm)	ρ
NaOH/urea	chitin-1	53.3	113.7	45.3	2.5
	chitin-2	23.0	61.9	30.1	2.1
	chitin-3	15.0	46.8	22.7	2.1

Information), further suggesting the wormlike chain structure of chitin.

Nanofibers Self-Assembled from Extended Chitin Chains in NaOH/Urea System. In general, the stiff polymer chains tend to be fabricated into nanofibers due to the easy arrangement in a parallel manner.^{21–24} On the basis of the previous analysis, it triggered us to observe whether nanofibers occurred. As a result, nanofibers with an average diameter of 3.6 nm were successfully produced even at the lowest concentration (0.001 mg/mL) (Figure 6a), which was likely to be promoted by destruction of the sheath-like structure of chitin complex, leading to the parallel aggregation of the extended wormlike chitin chains. Interestingly, a slight increase in the chitin concentration led to self-aggregation of nanofibers to

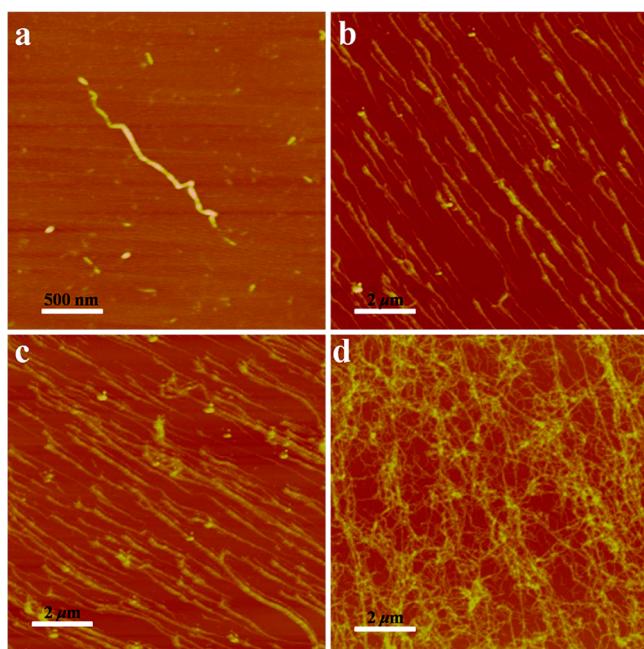


Figure 6. AFM images of the dilute chitin-1 solution in NaOH/urea: (a) 0.001, (b) 0.01, (c) 0.05, and (d) 0.1 mg/mL.

form a longer chain bundle ($h = 8.3$ nm) (Figure 6b,c); then, a film-like structure composed of randomly aggregated nanofiber networks was further constructed with slightly elevated concentration (Figure 6d), which would serve as a chitin nanofiber-based coating for biomaterials in the future. The results from AFM furthered suggested the stiff chain conformation of chitin in the NaOH/urea system, which was consistent with those from TEM. TEM images of the chitin-1 dilute solution and solvent dried at room temperature (Figure 7) were carried out. Ordered architecture of the NaOH crystals in solvent was observed in Figure 7a, whereas chitin nanofibers were formed via aggregation of extended wormlike chitin chain in a parallel manner during the drying process (Figure 7b,c,f). Because of the rapid deep-freezing procedure in liquid nitrogen during sample preparation for cryo-TEM observation process, the chitin–NaOH–urea–water complex and its nanofibers can maintain the original morphology in solution. In the elevated concentration, nanofibers consisting of extended wormlike chitin chain was observed in cryo-TEM (as shown in Figure S5 in the Supporting Information), showing the stiffness. In our findings, the results from TEM, cryo-TEM, and AFM were consistent with those of SLS and DLS to support strongly that the chitin chains existed as the extended wormlike chains, which easily aggregated in a parallel manner to form nanofibers. It would open a complete new pathway for the construction of the chitin-based nanomaterials.

CONCLUSIONS

Chitin was dissolved successfully in NaOH/urea aqueous solution at −30 °C to obtain true solution. The chitin chains were surrounded directly by NaOH hydrates, and the urea hydrate clusters were attached outside of NaOH hydrogen-bonded chitin complex to form sheath-like structure, leading to the good dissolution. The dilute chitin solution in NaOH/urea system was sensitive with temperature and concentration. The measuring conditions under which chitin was molecularly dispersed in the aqueous solution were obtained, and the

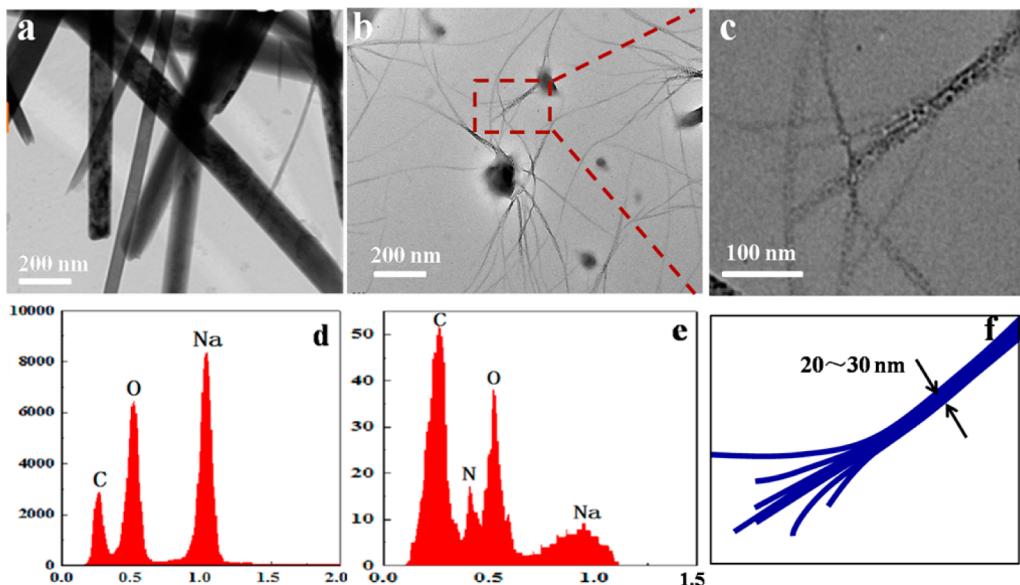


Figure 7. TEM images of the 11% NaOH solution (a), chitin-1 solution with concentration of 1 mg/mL in NaOH/urea (b), magnified image of TEM image b (c), EDS (d,e) corresponding to panels a and b, and a scheme to describe the formation of the nanofibers constructed from the chitin chain complex (f).

416 molecular weight, structure-sensitive parameter, and persistent
417 length of the chitin in the extremely dilute solutions were
418 determined with DLS and SLS, revealing that chitin existed as
419 an extended wormlike chain. The results from TEM, cryo-
420 TEM, and AFM were consistent with those of SLS and DLS to
421 support strongly that the chitin in the NaOH/urea aqueous
422 solution existed as the extended wormlike chains, which easily
423 aggregated in a parallel manner to form nanofibers. This work
424 provided a theoretical guidance for constructing nanomaterials
425 via “bottom-up” method.

426 ■ ASSOCIATED CONTENT

427 ■ Supporting Information

428 ^{13}C NMR chemical shifts of chitin-1 powder and different
429 chitin-1 solutions, the time-dependent R_h distribution of chitin-
430 1 at a small angle of 20° for 30 min, and R_h distribution of
431 chitin-2 in NaOH/urea aqueous solution at different temper-
432 atures (scattering angle $\theta = 90^\circ$). Plot of $\langle R_g \rangle_z$ versus M_w for
433 three chitin fractions in NaOH/urea aqueous solution
434 compared with the theoretical curves for the unperturbed
435 wormlike chain (the solid line) with $q = 30 \text{ nm}$ and $M_L = 470$
436 nm^{-1} , and cryo-TEM image of the chitin solution and the
437 model of the chitin nanofibers. This material is available free of
438 charge via the Internet at <http://pubs.acs.org>.

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443 Notes

444 The authors declare no competing financial interest.

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