Influence of Degree of Deacetylation on Critical Concentration of Chitosan/Dichloroacetic Acid Liquid-Crystalline Solution

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ABSTRACT: We prepared chitosans with various degrees of deacetylation (DDAs) by mixing completely deacetylated chitosan and acetic anhydride at room temperature without serious degradation and O-substitution. We obtained a standard curve to measure DDA by plotting the IR absorbance ratio of A_{1560}/A_{2880} against the known DDAs (from 1–100%) of 10 specimens. The effect of DDA on the critical concentration (C^*) of chitosan/dichloroacetic acid solutions required to form mesophase was investigated by optical methods. A maximum C^* value of 23 wt % appeared at a relative medium DDA (~20%). The effect was explained by the disordering of chains with medium composition ratios of the copolymer of glucosamine and *N*-acetyl glucosamine. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 1204–1208, 2002

Key words: chitin; chitosan; liquid-crystalline polymers; degree of deacetylation; critical concentration

INTRODUCTION

Chitin, poly(1,4-2-acetamido-2-deoxyβ-D-glucose), is the second most abundant natural polymer. Chitosan is the partially or completely deacetylated product of chitin. Chitosan can be regarded as a copolymer of $\beta1\rightarrow4$-D-glucosamine and $\beta1\rightarrow4$-*N*-acetyl-D-glucosamine with varying composition. The properties of chitosan depend on the average degree of deacetylation (DDA).[1] One of the properties is liquid-crystalline behavior.[2]

Since 1982, the development of lyotropic mesophases for semirigid chitosan and its derivatives has been studied by some groups.[2–13] Ogura et al. reported that chitosan, (hydroxypropyl) chitosan, and (acetoxypropyl) chitosan form a cholesteric phase at concentrated solutions.[2] Sakurai and colleagues studied liquid-crystal structure in chitosan films and fibers prepared form liquid-crystalline solutions.[3–5] Terbojevich et al. determined the persistence length of chitosan to be 22 nm in 0.1 mol/L CH_3COOH + 0.2 mol/L NaCl.[6] Rout and colleagues found cholesteric solutions in *N*-phthalolyl chitosan and *N*-phthaloyl-3,6-di-O-acetyl chitosan dissolved in organic solvents.[7–9] Ratto et al. investigated the mesophase of water/chitosan systems with differential scanning calorimetry.[10] Dong and colleagues demonstrated the lyotropic liquid crystallinity of some chitosan derivatives and studied structure factors such as molar mass and degree of substitution on critical concentration (C^*) forming the liquid-crystal phase.[11–13]

Table I   Preparation Conditions of Chitosan Specimens with Various DDAs

Specimen Number	Molar Ratio (Acetic Anhydride : Chitosan)	Concentration of Acetic Acid Solution (%)	State of Product in Reaction System	DDA[a] (%)
1[b]	—	—	—	99.8
2	0.02 : 1	1	Dissolve	94.2
3	0.04 : 1	1	Dissolve	92.8
4	0.10 : 1	1	Dissolve	81.4
5	0.20 : 1	1	Dissolve	64.2
6	0.60 : 1	1	Gelation	47.4
7	0.80 : 1	1	Gelation	37.5
8	1.00 : 1	1	Gelation	19.1
9	3.50 : 1	0.3	Precipitate	7.6
10	10.00 : 1	0.3	Precipitate	1.2

[a] Determined with element analysis.
[b] Completely deacetylated chitosan prepared in this lab, which was the raw material of other specimens.

The aim of this work was to examine the effect of DDA on C^*. Terbojevich et al.[6] compared the persistence length of chitosan A (DDA = 58%) and chitosan B (DDA = 85%), and found they are only slightly different, which implies that these two chitosans may have similar C^*'s. Recently, Hu et al.[14] studied chitosan samples with DDAs from 71 to 89% and reported they had the same C^*'s (10–11 wt %). Nevertheless, the DDA scope of these studies are limited. In this study, the research scope of DDA was developed to encompass the entire range.

EXPERIMENTAL

Materials

Chitosan (from crab shell) with a DDA of 84% and a viscosity average molecular weight of 7.4×10^5 g/mol was purchased from Xiamen Second Pharmaceutical Factory (Xiamen, China). All commercially available solvents and reagents (acetic acid, dichloroacetic acid, acetic anhydride, acetone, ethanol, and sodium hydroxide) were used without further purification.

Preparation of Chitosan with Different DDAs

We purified chitosan raw material by dissolving it in a 1% aqueous acetic acid solution and precipitating with a 10% aqueous NaOH solution. The purified chitosan was then treated with 50% NaOH solution under N_2 at 100°C for 4 h; this procedure was repeated two times to produce completely deacetylated chitosan.

The completely deacetylated chitosan was homogeneously N-acetylated according to literature method.[15] This chitosan (1 g) was dissolved in 50 mL of 1 or 0.3% aqueous acetic acid. Methanol (250 mL) was mixed with the solution. In different batches, different molar ratios of acetic anhydride (see Table I) were added to the solution by strong stirring. After reacting for 2 h at room temperature, the mixture was poured into a 10% NaOH solution. The precipitated polymer was filtered off, washed well with ethanol and then with acetone and air-dried. For the gel-like mixture, the product was immersed in fresh ethanol several times and then dried *in vacuo*.

Measurements

The elemental analyses were carried out by means of a CE 1110 CHNS—O elemental analyzer (Italy). DDA was calculated from the N/C ratio according to the following formula:

$$\frac{\text{N(Found)}}{\text{C(Found)}} = \frac{14}{96 - 24\,\text{DDA}}$$

The IR absorption spectra were measured with a Nicolet Avator 360 Fourier transform infrared (FTIR) spectrometer (USA) by the KBr pellet method.

The C^* required to form the ordered phase was determined with an Olympus polarized microscope (Japan). Chitosan/dichloroacetic acid solutions with different concentrations with gradations of 1 wt % were prepared separately in small glass vials. The vials were tightly sealed so that
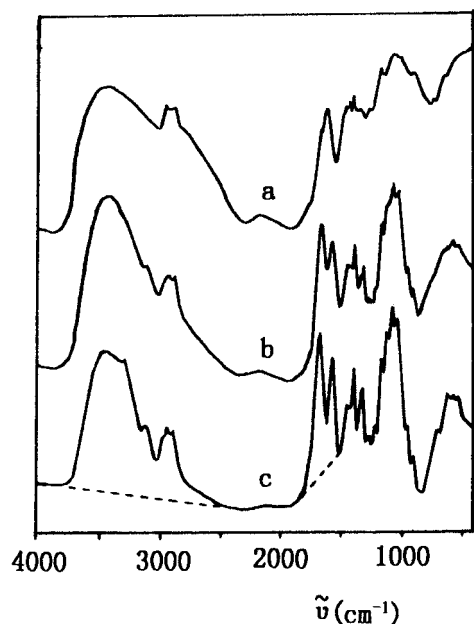
Figure 1 FTIR absorption spectra of chitosans with different DDAs: (a) DDA = 0.998, (b) DDA = 0.474, and (c) DDA = 0.012. The dashed line shows the drawing method of baseline.

Figure 2 A_{1560}/A_{2880} versus DDA.

the solvent could not evaporate. The solutions were aged for 2 weeks at 20°C before use and then sandwiched between two glass slides to form the liquid-crystal box. The concentration at which the birefringence was just noticed at 20°C was defined as C^*.

Intrinsic viscosity $[\eta]$ was determined in 0.1 mol/L CH_3COONa + 0.2 mol/L CH_3COOH buffer solution at 30°C.

Thermogravimetry curves were determined with a Netzsch STA 409 thermal analysis instrument (Germany) under N_2 at 10°C/min.

RESULTS AND DISCUSSION

Characteristics of Chitosan Specimens

Commonly, slightly N-deacetylated chitins have also been regarded as chitin, and partially or completely deacetylated chitins have been called chitosan. In this article, we use the united name chitosan for deacetylated chitins with various DDAs.

Table I lists the preparation conditions, phenomena, and DDA values of 10 chitosan specimens. The DDAs of these specimens covered almost the whole DDA range.
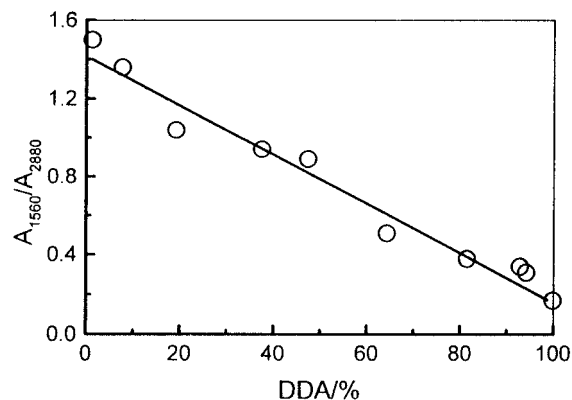
The FTIR spectra of three typical specimens are shown in Figure 1. There was no C=0 stretching band of ester at ~1730 cm^{-1}, which indicates that no substitution occurred on hydroxyl groups during acetylation. The structure difference of these 10 chitosan specimens was only the degree of N-acetylation.

The DDA values of these specimens could be proven by FTIR. The amide band at 1560 cm^{-1} could be used as probe band, and the C—H band at 2880 cm^{-1} could be used as reference band. We then obtained a good linear curve (with correlation coefficients 0.99) by plotting the absorbance ratio of A_{1560}/A_{2880} against the known DDAs of these 10 specimens (see Fig. 2).

The FTIR results support the element analysis determination of DDA value. Therefore, Figure 2 can also be used as a standard curve for the measurement of the DDA of unknown chitosan. This standard curve has extended the DDA measure-

Table II $[\eta]$ of Chitosan Specimens with Different DDAs

	Specimen		
	1	3	5
DDA (%)	99.8	92.8	64.2
$[\eta]$ (mL/g)	450	429	361
K × 10^3 (mL/g)	16.800[a]	6.598[b]	0.014[c]
a[d]	0.81[a]	0.88[b]	1.12[c]
$\overline{M}_V$[e] × 10^{-5}	2.93	2.95	2.82

[a] For DDA = 100%.
[b] For DDA = 91%.
[c] For DDA = 69%.
[d] From ref. 17.
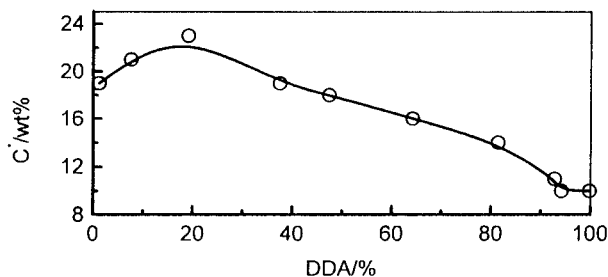[e] Viscosity average molecular weight.

Figure 3 Relation between DDA and C^* in the chitosan/dichloroacetic acid system.

ment scope of literature method[16] to almost the whole scope (1–100%).

The 10 chitosan specimens were prepared at very mild N-deacetylation condition so that no serious degradation of the molecular chain occurred. Table II lists $[\eta]$'s of some specimens, showing only slight decrease in $[\eta]$. Because K and a in the Mark–Houwink equation $[\eta] = kM_v^a$ change with DDA,[17] these specimens were prepared without evident variation of molar mass.

Relationship Between DDA and C^*

Figure 3 illustrates the relationship between the DDA and C^* of chitosan. As shown in Figure 3, C^* did not vary monotonously with DDA. A maximum C^* value appeared at medium DDA. Although C^* changed slightly in the DDA scopes of

58–85% and 71–89%, as shown by refs. 6 and 14, respectively, a very strong variation of C^* from 10% (for DDA = 99.8%) to 23% (for DDA = 19.1%) was obtained in this study.

The critical volume fraction (V_2') of semirigid chains could be predicted by Flory's well-known equation:[18]

$$V_2' \cong (8/x)(1 - 2/x)$$

where x is the axis ratio of rods. $x(= 2q/d)$ depends on the persistence length (q) and the diameter of the chain (d):

$$d = (M_0/\rho \cdot N_A \cdot L_0)^{1/2}$$

where M_0 is molar mass of repeat unit, ρ is the density of the polymer, N_A is Avogadro's constant, and L_0 is the shadow length of repeat unit along chain.

Given that $q \approx 22$ nm for chitosan with DDA = 58–85%,[6] $\rho = 1.5$ g/cm³,[3] $M_0 = 161$, and $L_0 = 0.515$ nm (half of crystalline parameter b in the unit cell of a chitosan crystal[3]), V_2' was calculated to be 10.4% (v/v); therefore, C^* was 10.0% (w/w) calculated with the density value of 1.5 g/cm³ (for chitosan) and 1.573 g/cm³ (for dichloroacetic acid).

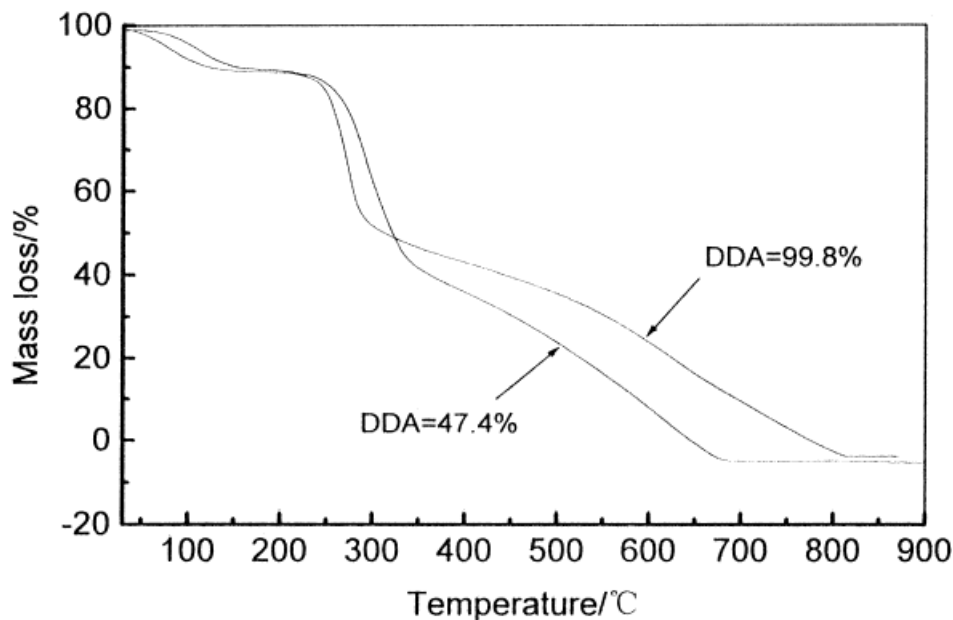As shown in Figure 3, only chitosan samples with a high DDA agreed with the anticipated

Figure 4 Thermogravimetry curves of chitosans with DDA = 0.998 and 0.474.

Table III Thermogravimetry Results of Some Chitosan Specimens

DDA of Chitosan (%)	Mass Loss at 400°C Including Combined Water (%)	Mass Loss at 400°C Excluding Combined Water (%)
99.8	53.0	48.3
92.8	52.1	47.2
64.2	53.4	48.9
47.4	61.8	58.3
37.5	60.5	57.2
19.1	56.0	50.0
1.2	52.7	50.4

value. The high C^* value at medium DDAs (about 20%) may be explained by the disordering of the chain in medium composition ratios of copolymer. The disordering weakens the intermolecular and intramolecular hydrogen bonding and decreases the rigidity of the chain. As a result, C^* rises obviously with the decrease of chain stiffness.

The thermogravimetry experiments were carried out to illustrate the weakening of the interactions of molecules. As shown in Figure 4 and Table III, higher mass losses at 400°C in chitosan with DDAs = 37.5 or 47.4 could be observed than in chitosans of both ends (i.e., DDA = 99.8 and 1.2%), whether or not combined water was included. This implies that the intermolecular hydrogen bonds of relative medium DDA were fewer and more easily broken. Although the degradations in thermogravimetry experiments may include the scission of main chains or side groups, this effect is similar for the segment of $\beta1{\rightarrow}4$-D-glucosamine and $\beta1{\rightarrow}4$-N-acetyl-D-glucosamine, which can be shown by the similar mass loss of chitosans with DDAs = 99.8 and 1.2% (see Table III). The only difference between chitosans with medium DDAs and chitin or completely deacetylated chitosan is the intermolecular hydrogen bonding, which can be weakened by the disordering of the sequence structure.

CONCLUSIONS

Ten chitosan samples with different DDAs (from 1 to 100%) were prepared by the mixture of completely deacetylated chitosan and acetic anhy-

dride without serious degradation and O-substitution, instead of by the alkali method from chitin with serious degradation. The influence of DDA on the boundary concentration C^* from isotropic to liquid-crystalline phase for chitosan/dichloroacetic acid solutions was investigated by polarized optical microscopy. A maximum C^* value of 23% was observed at DDA = ~20%. The effect was explained by the weakening of hydrogen bonding. The thermogravimetry results illustrated the explanation. In addition, we introduced a standard curve to measure the DDA of chitosan by plotting the IR absorbance ratio of A_{1560}/A_{2880} against the known DDAs (from 1 to 100%).

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