Liquid-Crystalline Behavior of Chitosan in Malic Acid

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ABSTRACT: This report describes how the degree of deacetylation and molecular weight of chitosan and the concentrations of sodium chloride and malic acid affect the formation of lyotropic chitosan liquid crystals. Chitosan samples of various degrees of deacetylation were prepared from β-chitin that was isolated from squid pens. They were degraded by ultrasonic irradiation to various molecular weights. The critical concentrations forming chitosan liquid crystals were determined with a polarized microscope. A chitosan sample with a degree of deacetylation of 67.2–83.6% formed cholesteric lyotropic liquid crystals when it was dissolved in 0.37–2.59*M* malic acid. The critical concentrations increased with increasing degrees of deacetylation of chitosan. They decreased with increasing molecular weights or increasing concentrations of sodium chloride and malic acid. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 2670–2675, 2007

Key words: liquid-crystalline polymers (LCP); phase behavior; rheology

INTRODUCTION

Chitin is the second most abundant natural polymer. It is a linear polysaccharide composed of glucosamine and *N*-acetyl glucosamine linked by β-1,4-glycosidic bonds.[1,2] Both the shells of shrimps, crabs, and mollusks and the cell walls of fungi and algae contain chitin as one of their major structural components.[2–4] The deacetylation of chitin with a hot alkali solution or an enzyme gives rise to chitosan, whose physicochemical properties depend closely on its degree of deacetylation (DD) and molecular weight (MW). Chitosan is a nontoxic, biocompatible, and biodegradable polymer. Because of its specific properties, chitosan has a number of applications in biological medicine, food, agriculture, cosmetics, textiles, nanoparticles, water engineering, and so forth.[1–6]

Ogura et al.[7] reported that chitosan formed cholesteric liquid crystals when 30–90% chitosan (90% DD) was dissolved in a 10% acetic acid solution. Their results also indicated that hydroxypropyl chitosan and acetopropyl chitosan demonstrated both lyotropic and thermotropic liquid-crystalline behaviors. Rout and coworkers[8–10] reported that *N*-phthalolyl chitosan, *N*-phthalolyl-3,6-di-*O*-acetyl chitosan, and *N*-phthalolyl-3,6-*O*-(butyl carbamate) chitosan had a lyotropic liquid-crystal property in dimethyl sulfoxide. The critical concentrations (*C**'s; i.e., the lowest concentrations to form lyotropic liquid crystals) of these polymers were 0.25–0.27, 0.1, and 0.5%, respectively. However, *C** of *N*-phthalolyl-3,6-di-*O*-acetyl chitosan in dioxane was as low as 0.07%. It is apparent that both the substitution group and solvent may affect the liquid-crystalline behaviors of chitosan and its derivatives. Kim and Lee[11] reported that chitin derivatives, including propyl chitin, hydroxypropyl chitin (HPC), and dihydroxypropyl chitin, had cholesteric lyotropic liquid-crystal behavior when their concentration was increased to greater than 30% in 99% formic acid. They also reported that the side chain in HPC contributed to the flexibility and decrease in the crystallinity. Murray and Neville[12] reported that the effects of the pH, DD, and temperature on the liquid-crystalline behavior of chitosan. Hu et al.[13] reported the mesophase transition and its kinetics for chitosan in solutions of chitosan and dichloroacetic acid (DCA). The mesophase formation of chitosan in DCA involves nucleation and growth. The isothermal kinetics of mesophase formation from an isotropic phase were described by an Avrami equation with exponent *n* close to 1, which suggested that the formation of the chitosan mesophase was a process of instantaneous nucleation and

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one-dimensional rodlike growth in the temperature range investigated. Dong and coworkers[14–20] reported that chitin, chitosan, and its derivatives form lyotropic liquid crystals. C^* of chitosanous materials decreased with increasing polymeric MW. The influence of MW on C^* was smaller for more flexible chitosan molecules. C^* of chitosan derivatives increased with the increasing chain length of the substitute group. The influence of the degree of molar etherification (DME) of HPC on C^* was insignificant when DME was smaller, but the effect became distinct when DME was greater than or equal to 3. Furthermore, they reported that chitooligosaccharides with MWs of 2.3 and 4.3 kDa could form cholesteric liquid-crystal phases in the proper solutions.

Malic acid is a natural dicarboxylic acid. Bodnár et al.[21] reported that malic acid served as a crosslinking agent and led to an intermolecular covalent condensation reaction during the preparation of hydrophilic chitosan nanoparticles. Park et al.[22] reported that κ-carrageenan/chitosan films could be prepared by the codissolution of κ-carrageenan and chitosan in different organic acids, including malic acid, acetic acid, citric acid, and lactic acid. Malic acid caused the largest increase in both the tensile strength and the elongation of composite films prepared with 2% ascorbic acid. The addition of high concentrations of malic acid appeared to reinforce the crosslinking interactions provided by ascorbic acid to carrageenan and chitosan.

The objectives of this study were to examine the effects of the DD and weight-average molecular weight (M_w) of chitosan and the concentrations of sodium chloride (NaCl) and malic acid on the formation of chitosan liquid crystals. In addition, the relationship between the conformation of chitosan and liquid-crystal formation was explored.

EXPERIMENTAL

Materials

Squid pens were donated as a gift from Shin Dar Bio-Tech Co., Ltd. (Taoyuan, Taiwan). Chemicals were reagent- or analytical-grade and were obtained from Merck (Darmstadt, Germany).

Preparation of chitosans with different MWs

The preparation of β-chitin was modified slightly from the procedure reported by Kurita et al.[23] One hundred grams of squid pen powder (40–60 mesh) was immersed in 500 mL of a 1*M* hydrochloric acid solution overnight. It was washed to neutrality and drained. Then, the sample was soaked in 500 mL of 2*M* sodium hydroxide and kept at the ambient temperature overnight. Subsequently, it was reacted in 500 mL of a 2*M* sodium hydroxide solution at 100°C for 4 h, washed, and dried to produce β-chitin. Then, chitosans was obtained by the deacetylation of β-chitin in hot alkali, as reported by Tsaih and Chen.[24,25] The DDs of these chitosan samples were 67.2, 73.3, 76.2, and 83.6%, respectively. Chitosan samples with different MWs were prepared by ultrasonic degradation.[26,27] The properties of these samples are listed in Table I.

Determination of the DD of chitosan

IR spectroscopy was used to determine the DD of chitosan.[28] Chitosan powder was mixed with potassium bromide, desiccated, and tableted. A Bio-Rad (Hercules, CA) FTS-155 IR spectrometer measured the absorption spectrum. The DD was calculated with the following equation:

$$\mathrm{DD} = 100 - (A_{1655}/A_{3450}) \times 115$$

where A_{1655} and A_{3450} are the absorbances at 1655 and 3450 cm^{-1}, respectively.

Determination of the M_w values of chitosan

Size exclusion chromatography was used to determine the M_w values of the chitosan samples.[24,29] Two columns packed with TSKgel were used ($G4000PW_{XL}$ and $G6000PW_{XL}$, Tosoh Co., Ltd., Tokyo, Japan). The mobile phase consisted of 0.2*M* acetic acid/0.1*M* sodium acetate and 0.008*M* sodium azide. A sample concentration of 0.1% (w/v) was loaded and eluted at a flow rate of 0.5 mL/min with an LDC Analytical Constametric 3500 pump (Riviera Beach, FL). The elution peak was detected with a Gilson (Middleton, WI) M132 RI detector. The data were analyzed with Chem-Lab software (Scientific Information Service Co., Taipei, Taiwan). Pullulan standards (Shodex, Kawasaki, Japan) with different M_w values were used as markers. The M_w values of the samples were calculated from the pullulan calibration curve with the Chem-Lab software.

Determination of the intrinsic viscosity [η] and calculation of the persistencelength (q) for chitosan

Chitosan solutions of different concentrations (0.01–0.1%) in 0.055*M* acetic acid/0.01*M* sodium acetate

TABLE I
M_w Values (kDa) of Chitosans with Four Different DDs After Ultrasonic Degradation

	Ultrasonic degradation time (h)			
DD (%)	0	2	12	24
67.2	461	217	103	48.2
73.3	510	259	116	55.7
76.2	553	269	130	58.3
83.6	498	234	119	52.3

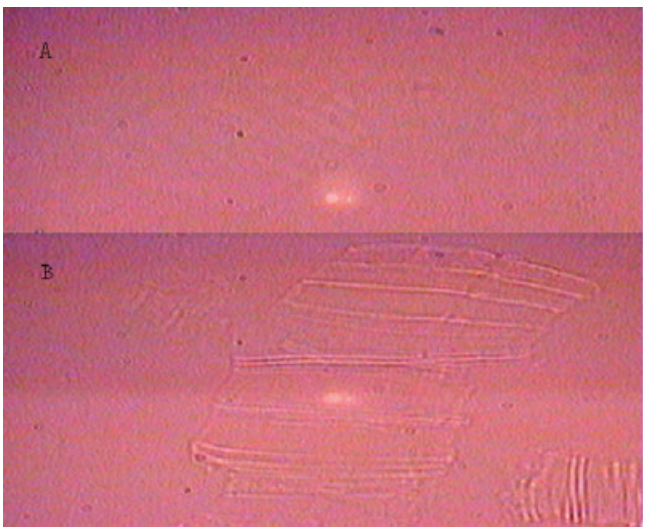

Figure 1 Polarized light microscopy pictures of chitosan solutions (DD = 67.2%, M_w = 461 kDa) in 1.85*M* malic acid with different chitosan concentrations: (A) 0.1 and (B) 1.0 wt % (10 × 10). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(pH = 4) containing 0–0.20*M* NaCl were prepared. Each solution was passed through a 0.45-μm filter (Lida, St. Louis, MO) to remove insoluble materials. A capillary viscometer (no. 75, Cannon-Fenske, State College, PA) was filled with 5 mL of a sample and equilibrated in a water bath (TMV 40, Tamson, Zoetermeer, Holland) with an extra thermostat (B403, Firstek, Taipei, Taiwan) to maintain the temperature at 25 ± 0.1°C. 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. The reduced viscosity was plotted against the concentration, with the intercept being [η].[30]

q was calculated with the model proposed by Yamakawa and Fujii[31] and subsequently modified by Kienzle-Sterzer et al.:[32]

$$[\eta](\mathrm{mL/g}) = 2.6 \times 10^{23}\,(2q/M_L)^{3/2}M^{1/2}$$

$$M_L = M/L$$

$$L = (M/m)l$$

$$m = 161 + 42(1 - \mathrm{DD})$$

where M_L is the molecular weight per unit contour length, M is the molecular weight, m is the molecular weight of the repeat unit, L is the contour length, and l is the unit contour length (the length of glucosamine, i.e., 0.533 nm).

Determination of *C** of chitosan liquid crystals

Chitosan solutions of different concentrations in an interval of 0.1% (w/w) were prepared separately in small glass vials. The solvent was malic acid, with concentrations ranging from 0.37 to 2.96*M* used to examine the effect of malic acid on *C**. In other parts of this study, the concentration was kept constant at 1.85*M*. The vials were tightly sealed and aged at the ambient temperature for 24 h. Aliquots of the solution were dripped and sandwiched between two glass slides. The slides were mounted onto a Nikon (Tokyo, Japan) MDA502AA E400 polarized microscope for visual observation at 25°C. The minimal concentration of chitosan at which the birefringence appeared was recorded as *C**.[7,8,14–20]

RESULTS AND DISCUSSION

Effect of the concentrations of malic acid

For the chitosan sample with DD = 67.2% and M_w = 461 kDa, a typical fingerprint texture indicative of the formation of cholesteric liquid crystals was observed at 1% chitosan [Fig. 1(B)]. Such a texture could not occur at lower concentrations [Fig. 1(A)], suggesting that chitosan liquid crystals in malic acid were lyotropic.

*C** for chitosan liquid-crystal formation decreased with increasing concentrations of malic acid (Table II). *C** reached the minimum (0.2%) when the concentration of malic acid was raised to 1.85–2.59*M*. When the concentration of malic acid increased, more amino groups of chitosan molecules could be protonated by the proton groups from the acid molecules. Positively charged amino groups may repel one another and force the chitosan molecules to assume an extended conformation. On the other hand, the neutralization of positively charged amino groups by the acidic groups will favor a more compact conformation and a smaller contour. According to Flory's theory, a macromolecule that has a smaller contour and a smaller axis ratio tends to have higher

TABLE II
Effect of the Malic Acid Concentration on *C of Chitosan Liquid Crystals[a]**

Malic acid (*M*)	0.37	0.74	1.11	1.48	1.85	2.22	2.59	2.96
pH	3.10	2.93	2.81	2.76	2.71	2.66	2.60	2.55
*C** (w/w %)	1.0	1.0	0.5	0.5	0.2	0.2	0.2	—[b]

[a] DD = 67.2%; M_w = 461 kDa.
[b] No liquid-crystalline pattern was observed.

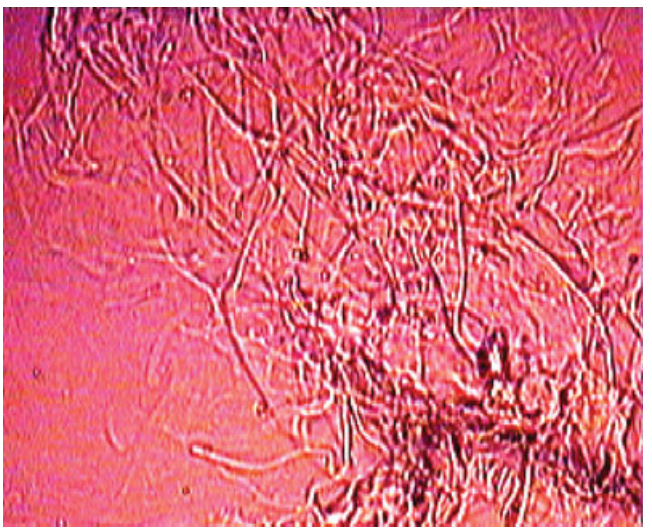

Figure 2 Polarized light microscopy picture of a chitosan solution (DD = 67.2%, M_w = 461 kDa) in 2.96*M* malic acid (10 × 10). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

*C**. However, our results reveal that liquid crystals formed more readily with an increasing concentration of malic acid, even though more positively charged amino groups might have been neutralized by the acidic groups. This might be due to the fact that the first electroviscous effect reduced the intermolecular distance and resulted in the formation of more hydrogen bonds and liquid crystals among chitosan molecules. It is also probable that malic acid molecules formed crosslinks with chitosan molecules[21] and facilitated the formation of liquid crystals. These results indicate that the formation of chitosan liquid crystals in malic acid behaves in a similar fashion to the instantaneous nucleation and growth process of lyotropic chitosan in DCA.[13]

No liquid-crystal phase was observable at a malic acid concentration of 2.96*M*. At this extremely high concentration of malic acid, the intermolecular interactions became so excessive that chitosan molecules turned into fiberlike structures and precipitated from the solution (Fig. 2).

Effect of M_w of chitosan

*C** decreased with increasing M_w of chitosan (Table III). These results are in agreement with data of Wang et al.[14] and Dong et al.[15,17] In other words, a longer polymer chain (higher M_w) resulted in lower *C**. For the samples with the lowest M_w value at each DD, that is, 48.2 (DD = 67.2%), 55.7 (DD = 73.3%), 58.3 (DD = 76.2%), and 52.3 kDa (DD = 83.6%), no fingerprint texture could be observed even at high concentrations of chitosan. In addition, for the samples with a DD of 83.6%, an M_w greater than or equal to 234 kDa was required to form liquid crystals at NaCl concentrations less than or equal to 0.10%. This suggests that M_w of chitosan must be high enough so that intermolecular forces become sufficient for the formation of a liquid-crystal phase.

We have observed that in the absence of NaCl, chitosan was unable to form liquid crystals in malic acid when M_w was below 48.2 (when DD was kept constant at 67.2%), 55.7 (DD = 73.3%), 58.3 (DD = 76.2%), or 119 kDa (DD = 83.6%). Consequently, no

TABLE III
Effects of the NaCl Concentration and Chitosan M_w on *C (v/v %) and *q* of Chitosan in 1.85*M* Malic Acid Liquid Crystals**

		NaCl (%)				
DD (%)	M_w (kDa)	0	0.01	0.05	0.10	0.20
67.2	461	0.14	0.14 (57.8)	0.14 (49.8)	0.072 (45.8)	0.057(45.1)
	217	1.43	1.43 (54.4)	1.43 (46.3)	0.72 (42.1)	0.36 (41.1)
	103	2.87	2.87 (52.3)	2.87 (43.1)	2.15 (37.9)	1.43 (36.6)
	48.2	—[a]	—[a] (51.6)	—[a] (39.2)	—[a] (34.4)	—[a] (32.9)
73.3	510	0.72	0.72 (55.3)	0.57 (47.0)	0.36 (45.2)	0.22 (43.6)
	259	2.15	2.15 (52.1)	1.43 (45.0)	1.43 (40.1)	0.72 (37.7)
	116	4.3	4.3 (49.9)	4.3 (38.9)	2.87 (35.8)	2.15 (34.2)
	55.7	—[a]	—[a] (45.7)	—[a] (37.4)	—[a] (33.0)	—[a] (32.5)
76.2	553	1.43	1.43 (52.8)	0.72 (45.9)	0.5 (42.5)	0.36 (41.4)
	269	2.87	2.87 (50.4)	2.15 (44.8)	1.43 (38.5)	0.72 (37.2)
	130	5.02	5.02 (47.2)	4.3 (38.7)	3.58 (35.7)	2.15 (33.5)
	58.3	—[a]	—[a] (44.9)	—[a] (37.0)	—[a] (32.9)	—[a] (30.7)
83.6	498	3.58	3.58 (43.9)	2.87 (38.7)	2.15 (35.5)	1.43 (33.1)
	234	5.02	5.02 (43.3)	4.3 (36.4)	3.58 (33.7)	2.87 (32.2)
	119	—[a]	—[a] (42.1)	—[a] (35.5)	—[a] (32.5)	7.89 (31.5)
	52.3	—[a]	—[a] (41.6)	—[a] (31.8)	—[a] (27.8)	—[a] (27.6)

The data in parentheses represent *q* (nm), which was determined with the same amounts of chitosan in the 0.055*M* acetic acid/0.01*M* sodium acetate solvent system with a pH value of 4.

[a] No liquid-crystalline pattern was observed.

C^* value could be determined. In contrast, chitosan with an M_w value of 13.5 kDa was found to have a C^* value of 44% in formic acid.[17] Recently, Dong et al.[20] reported that the C^* values in formic acid were 36 and 32% for chitosan samples with M_w values as low as 2.3 and 4.3 kDa, respectively. The interactions among chitosans with various lengths, organic acids, and solvent molecules differed greatly with respect to the charge density, hydrophobic interaction, and hydrogen-bonding propensity.[33] Consequently, the M_w values of chitosan had a significant influence on C^*.

Effect of q of chitosan

Table III also shows that C^* decreased with increasing q of chitosan. At the same DD, as q of chitosan increased, the molecules became more extended and resulted in lower C^*.

According to Flory's rodlike chain model, the theoretical critical concentration fraction required to form an ordered phase [C_1 (v/v %)] is a function of q, the chain length (L), and the diameter (d) of the polymer chain:[19,34]

$$C_1 = (8/X_k)(1 - 2/X_k)$$

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

where X_k is an axis ratio equal to $2q/d$, ρ is the density of the polymer, M_0 is the molar mass of the repeat unit, N_A is Avogadro's constant, and L_0 is the length of the repeating unit along the polymeric chain. For chitosan, ρ is 1.5 g/mL, and L_0 is 0.515 nm.

The theoretical critical concentration of a semirigid polymer chain [C_2 (v/v %)] also can be deduced with the Khokhlov–Semenov–Onsager–Ann formula:[17,18,20]

$$C_2 = (d/2q)[3.34 + 11.94(L/2q) + 6.34(L/2q)^2]/\{(L/2q)[1 + 0.586(l/2q)]\}$$

C_1 and C_2, calculated with these equations, are shown in Table IV. Both C_1 and C_2 decreased with increasing q of chitosan. This is consistent with the data of q and C^* in Table III. However, both C_1 and C_2 in acetic acid were much higher than the experimental values (C^*) in malic acid. These results suggest that the intermolecular interactions among chitosan molecules in malic acid are much higher than those existing in the solvent system containing acetic acid. It is possible that the higher tendency to form hydrogen bonds among chitosan molecules in malic acid might lead to higher intermolecular attractive forces.

Effect of NaCl

Table III shows that C^* decreased with an increasing concentration of NaCl. The negatively charged chloride ions could have been attracted to the positive amino groups ($—NH_3^+$) in chitosan. These ions could neutralize the positive charges, form a double layer, and allow more hydrogen bonds to form between adjacent chitosan molecules. It has been demonstrated that chitosan molecules become more coiled at higher salt concentrations.[30] Thus, higher salt concentrations would lead to lower q values for chitosan molecules. According to Flory's model, one would expect a higher C^* value because X_k becomes smaller under these conditions. To the contrary, the data in Table IV suggest that the neutralization of the positive charges of the ammonium ions within the chitosan structure might have provided an environment favorable to the formation of hydrophobic interactions, hydrogen bonding, and liquid-crystalline structure. It appears that not only q but also the intermolecular interactions influence the liquid-crystalline behavior of chitosan.

TABLE IV
[η], q, C_1, and C_2 Values of Chitosan in 0.055*M* Acetic Acid/0.01*M* Sodium Acetate (pH = 4) Containing 0.01*M* NaCl

DD (%)	M_w (kDa)	[η] (mL/g)	q (nm)	C_1 (v/v %)	C_2 (v/v %)
67.2	461	1142	57.8	13.3	18.0
	217	711	54.4	14.1	19.1
	103	531	52.3	14.7	19.9
	48.2	319	51.6	14.9	20.2
73.3	510	1321	55.3	13.9	18.8
	259	1174	52.1	14.8	20.0
	116	919	49.9	15.6	21.1
	55.7	539	45.7	17.0	23.0
76.3	553	2014	52.8	14.6	19.7
	269	1423	50.4	15.4	20.8
	130	1034	47.2	16.4	22.2
	58.3	711	44.9	17.2	23.2
83.6	498	2091	43.9	17.3	23.4
	234	1461	43.3	17.6	23.8
	119	1109	42.1	18.1	24.5
	52.3	720	41.6	18.3	24.8

Effect of DD of chitosan

Both M_w and DD of chitosan influence C^* (Fig. 3). C^* increased when the DD of chitosan was raised from 67.2 to 83.6%. At lower DDs, more acetyl groups remained attached to the amino groups on the C2 position of the glucosamine residue. Their carbonyl groups could contribute to the formation of intermolecular and intramolecular hydrogen bonds. Not only did they make the chitosan become more rigid and extended, but they also facilitated the formation of a liquid-crystalline mesophase because C^* was inversely related to the polymer chain length.[34] Rout et al.[8] observed that *N*-phthalolyl-3,6-di-*O*-acetyl chitosan formed a liquid-crystalline mesophase at a lower concentration with respect to *N*-phthalolyl

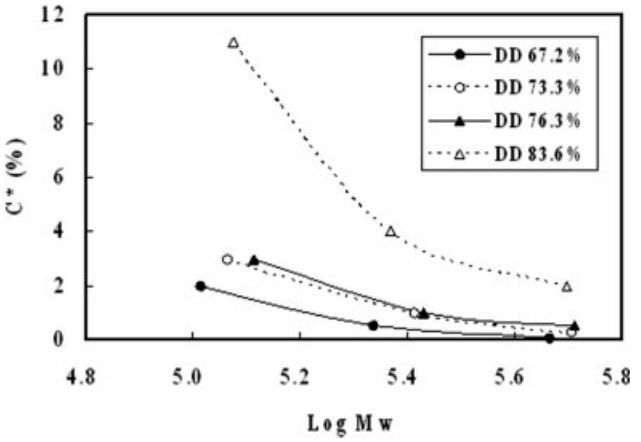

Figure 3 Logarithmic plot of M_w versus C^* (% w/w) for chitosans with different DDs in 1.85*M* malic acid/0.2% NaCl.

chitosan in the organic solvents dimethyl sulfoxide and dioxane. The presence of acetyl groups seemed to induce the same effect on C^* in their solvent system and ours. These results, however, do not completely correspond with the data recently published for chitosan/DCA liquid crystals.[19] Dong et al.[19] noticed that C^* decreased with increasing DD from $\sim$ 20 to 100%, but it increased with increasing DD from 0 to $\sim$ 20%. The reason that heterogeneously deacetylated chitosan with a relatively high DD in malic acid (this study) showed liquid-crystalline behavior similar to that of reacetylated chitosan with a low DD in DCA[19] is waiting to be explored.

CONCLUSIONS

Liquid crystals were prepared by the dissolution of a relatively low concentration of chitosan in malic acid. The polarized micrograph and concentration dependence of the fingerprint texture formation suggested that they were cholesteric lyotropic liquid crystals. C^* was closely related to M_w and DD of chitosan as well as the concentrations of malic acid and NaCl. Malic acid is less pungent than acetic acid. We expect that this novel liquid-crystal system of chitosan could be applied to the controlled release of pharmaceutical or cosmetic ingredients. The application research related to this study is currently underway in our laboratory.

References

1. Knorr, D. Food Technol 1984, 38, 85.
2. Muzzarelli, R. A. A. Carbohydr Polym 1996, 29, 309.
3. Austin, P. R.; Brine, C. J.; Castle, J. E.; Zikakis, J. P. Science 1981, 212, 749.
4. Muzzarelli, R. A. A. Carbohydr Polym 1983, 3, 53.
5. Li, Q.; Dunn, E. T.; Grandmaison, E. W.; Goosen, M. F. A. In Applications of Chitin and Chitosan; Goosen, M. F. A., Ed.; Technomic: Lancaster, PA, 1997; p 3.
6. Ravi Kumar, M. N. V. React Funct Polym 2000, 46, 1.
7. Ogura, K.; Kanamoto, T.; Sannan, T.; Tanaka, K.; Iwakura, Y. In Chitin and Chitosan; Proceedings of the Second International Conference on Chitin and Chitosan; Hirano, S.; Tokura, S., Eds.; Japanese Society of Chitin and Chitosan: Sapporo, Japan, 1982; p 39.
8. Rout, D. K.; Li, K.; Pulapura, S. K.; Gross, R. A. Macromolecules 1993, 26, 5999.
9. Rout, D. K.; Pulapura, S. K.; Gross, R. A. Macromolecules 1993, 26, 6007.
10. Rout, D. K.; Barman, S. P.; Pulapura, S. K.; Gross, R. A. Macromolecules 1994, 27, 2945.
11. Kim, S. J.; Lee, Y. M. In Chitin and Chitosan: The Versatile Environmentally Friendly Modern Materials; Zakaria, M. B.; Muda, W. M. W.; Abdullah, M. P., Eds.; Universiti Kebangsaan: Bangi, Malaysia, 1955; p 155.
12. Murray, S. B.; Neville, A. C. Int J Biol Macromol 1998, 22, 137.
13. Hu, Z.; Wu, L.; Wu, D.; Chen, S. J Appl Polym Sci 2001, 80, 1770.
14. Wang, J.; Dong, Y.; Liu, H.; Yuan, Q.; Mei, X. Chem J Chin Univ 1999, 20, 474.
15. Dong, Y.; Wang, J.; Liu, H.; Yuan, Q.; Li, Z. Acta Polym Sin 1999, 4, 431.
16. Dong, Y.; Yuan, Q.; Wu, Y.; Wang, J.; Wang, M. Polym J 2000, 32, 326.
17. Dong, Y.; Qiu, W.; Ruan, Y.; Wu, Y.; Wang, M.; Xu, C. Polym J 2001, 33, 387.
18. Dong, Y.; Wu, Y.; Wang, J.; Wang, M. Eur Polym J 2001, 37, 1713.
19. Dong, Y.; Xu, C.; Wang, J.; Wu, Y.; Wang, M.; Ruan, Y. J Appl Polym Sci 2002, 83, 1204.
20. Dong, Y.; Wang, H.; Zheng, W.; Zhao, Y.; Bi, D.; Zhao, L.; Li, X. Carbohydr Polym 2004, 57, 235.
21. Bodnár, M.; Hartmann, J. F.; Borbély, J. Macromol Symp 2005, 227, 321.
22. Park, S. Y.; Lee, B. I.; Jung, S. T.; Park, H. J. Mater Res Bull 2001, 36, 511.
23. Kurita, K.; Tomita, K.; Ishii, S.; Nishimura, S.-I; Shimoda, K. J Polym Sci Part A: Polym Chem 1993, 31, 2393.
24. Tsaih, M. L.; Chen, R. H. J Appl Polym Sci 1999, 71, 1905.
25. Tsaih, M. L.; Chen, R. H. J Appl Polym Sci 2003, 88, 2917.
26. Tsaih, M. L.; Chen, R. H. J Appl Polym Sci 2003, 90, 3526.
27. Tsaih, M. L.; Tseng, L. Z.; Chen, R. H. Polym Degrad Stab 2004, 86, 25.
28. Baxter, A.; Dillon, M.; Taylor, K. D. A.; Roberts, G. A. F. Int J Biol Macromol 1992, 14, 166.
29. Chang, K. L. B.; Tai, M.-C.; Cheng, F.-H. J Agric Food Chem 2001, 49, 4845.
30. Tsaih, M. L.; Chen, R. H. Int J Biol Macromol 1997, 20, 233.
31. Yamakawa, H.; Fujii, M. Macromolecules 1974, 7, 128.
32. Kienzle-Sterzer, C. A.; Rodriquez-Sanchez, D.; Rha, C. K. In Chitin, Chitosan, and Related Enzymes; Zikakis, J. P., Ed.; Academic: London, 1984; p 383.
33. Domard, A. In Advances in Chitin Science; Domard, A.; Roberts, G. A. F.; Vårum, K. M., Eds.; Jacques Andres: Lyon, France, 1997; Vol. II, p 410.
34. Flory, P. J. Proc R Soc London Ser A 1956, 234, 73.