

LYOTROPIC LIQUID CRYSTALLINE BEHAVIOR OF FIVE CHITOSAN DERIVATIVES*

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Abstract Five chitosan derivatives, i.e. O-butyryl chitosan, O-benzoyl chitosan, N-phthaloyl chitosan, N-maleoyl chitosan and O-cyanoethyl chitosan, were prepared from chitosan. All of them had better solubility than chitosan, and demonstrated lyotropic liquid crystalline behavior in various solvents. The critical liquid crystalline behavior of three O-substituted chitosan derivatives was evidently different from two N-substituted analogues. Typical fingerprint textures of cholesteric phase were only observed in three O-substituted derivatives. The critical concentration (v/v%) of three O-substituted derivatives does not depend on the acidity of acidic solvents.

Keywords Chitosan, Derivatives, Lyotropic liquid crystal

INTRODUCTION

Chitosan is a N-deacetylation product of chitin, the principle component of several living organisms such as fungi and arthropods. This polysaccharide has a repeating structural unit of β -1-4-linked-2-amino-2-deoxy-D-glucopyranose. The primary amino group at the ring C₂ position and the primary and secondary hydroxyl functionalities at the ring C₆ and C₃ position, respectively, are amenable to selective chemical modification to improve its solubility and other properties for medical, pharmaceutical, agricultural and technological applications.

Since 1976, lyotropic and thermotropic liquid crystals of cellulose derivatives have been widely studied^[1-3]. The cholesteric phase was found in most cellulose derivatives. Although chitosan is structurally similar to cellulose and its chain may be sufficiently rigid to facilitate the formation of mesophases, researches on liquid crystalline behavior of chitosan and its derivatives are limited. Chitosan^[4], hydroxypropyl chitosan^[4], acetoxypropyl chitosan^[4], phthaloyl chitosan^[5] and acetyl phthaloyl chitosan^[5] have been shown to form lyotropic cholesteric phases at suitable concentrations. Acetoxypropyl chitosan has been shown to form thermotropic cholesteric phase^[4].

The present study aims to prepare five chitosan derivatives. Four of them were found to be novel liquid crystalline polymers. The liquid crystalline behavior of these five chitosan derivatives was investigated and compared.

EXPERIMENTAL

Materials

Chitosan was purchased from Yuhuan chemical factory(China) and was further deacetylated before

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use. Its degree of deacetylation was determined to be 78% by FTIR spectroscopy^[6]. All commercially available solvents and reagents were used without further purification.

Synthesis

Preparation of O-butyryl chitosan^[7]

Chitosan powder(2.1 g) was added to methanesulphonic acid (11 mL) and stirred at 0°C. Butyric anhydride (20.5 mL) was added dropwise and the total mixture was stirred at 0 ~ 5°C for 2 h. The resulting gel was stored at -20°C overnight. Then the thawed product was precipitated by pouring into acetone (200 mL), filtered and extracted for 18 h with acetone at 0°C, after which the acylated chitosan was dried in vacuo. The yield was 3.3 g.

Preparation of O-benzoyl chitosan^[8]

Chitosan powder (1.0 g) was added to methanesulphonic acid (5.2 mL) and stirred at 0°C. Benzoyl chloride (1.1 mL) was added dropwise and the mixture was stirred at 0~5°C for 2 h and kept at -20 °C overnight. The product was precipitated by pouring into acetone(150 mL), filtered and extracted for 18 h with acetone at 0°C. The product was then dried in vacuo. The yield was 1.4g.

Preparation of N-phthaloyl chitosan^[5]

Chitosan powder(1.0 g) was dissolved in 1% aqueous acetic acid(100 mL). The polymer was made insoluble by the addition of methanol (100 mL) followed by 4% NaHCO₃ aqueous solution (100 mL). After the mixture was stirred at room temperature for 2 h, the gel-like precipitate was collected by filtration, washed with distilled water and then partially dried using the Buchner filtration apparatus. This wet precipitate was suspended in DMF and stirred overnight, the DMF was removed by filtration, and fresh DMF was added. After 1 h of stirring, the solvent was replaced using 30 mL fresh DMF. Then phthalic anhydride (2.75 g) was added to the preswollen gel. The reaction was carried out at 110°C for 5 h. The product was precipitated by pouring into acetone (200 mL), filtered, extracted for 5 h with acetone and then dried in vacuo. The yield was 1.3 g.

Preparation of N-maleoyl chitosan

Maleic anhydride (2.0 g) was added to the preswollen gel as described above. The reaction was carried out at 110 °C for 6 h. The product was precipitated by pouring into ethanol (200 mL), filtered, extracted for 5 h with ethanol and then dried in vacuo. The yield was 1.3 g.

Preparation of O-cyanoethyl chitosan

Chitosan powder (0.5 g) was added to acrylonitrile (30 mL) and stirred. 5% NaOH aqueous solution (1 mL) was added dropwise and the total mixture was stirred at room temperature overnight. The product was filtered, extracted with acetone and dried in vacuo. The yield was 0.64 g.

Sample Preparation

Different concentrations of polymer solutions with an interval of 2% (in w/w%) were prepared separately in small glass vials. The vials were tightly capped with Teflon caps so that the solvent cannot evaporate. The solutions were aged for 3 days at room temperature before use, and were then sandwiched between two glass slides to form the sample cells. The cells were sealed from all sides using epoxide resin to prevent solvent evaporation and aged for 4 days before observation under a polarized microscope. The concentration at which the birefringence was just observed was defined as the critical concentration. The liquid crystal textures were also observed using these sample cells.

Instruments

An OLYMPUS polarized microscope was used for texture observation and critical concentration measurement and a NICOLET 740 FTIR spectrometer was used for chemical structure analysis.

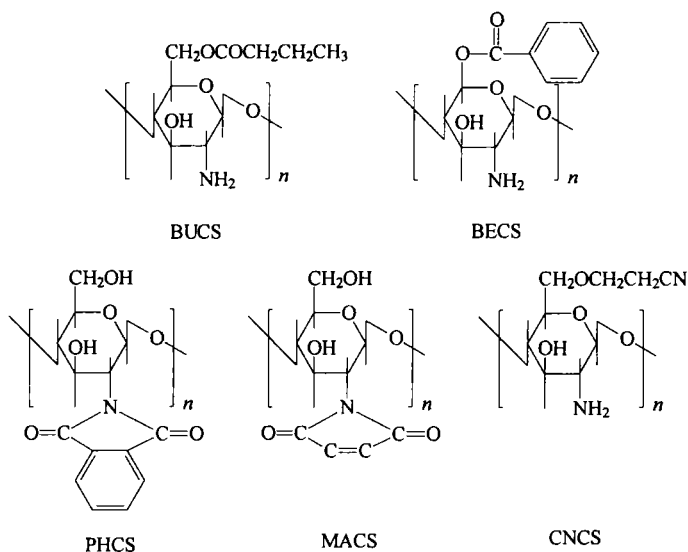
RESULTS AND DISCUSSION

Chemical Structure

The characteristic IR absorptions of the five chitosan derivatives are summarized below.

O-butyryl chitosan (hereafter BUCS)(cm^{-1}): 1741(*s*, sha), 1207(*vs*, sha) and 779(*s*, sha); O-benzoyl chitosan (hereafter BECS)(cm^{-1}): 1722(*s*, sha), 1202(*vs*, sha) and 781,714(*s*, sha); N-phthaloyl chitosan (hereafter PHCS)(cm^{-1}): 1778(*s*, sha), 1715(*vs*, sha), 1606(*w*, sha) and 720(*s*, sha); N-maleoyl chitosan (hereafter MACS)(cm^{-1}): 3095(*w*, b), 1720(*vs*, sha), 1631(*s*, sha), 1483(*w*, sha) and 695(*s*, sha); O-cyanoethyl chitosan (hereafter CNCS)(cm^{-1}): 2251(*s*, sha).

Although the position and the degree of substitution were rather complicated, for instance, O-phthaloylation and O-maleoylation may take place to some degree, schematic chemical structures of these five chitosan derivatives are drawn in Scheme 1. The degree of substitutions of BUCS, BECS, PHCS, MACS and CNCS were roughly calculated from the yield to be 1.42, 0.67, 0.92, 0.40 and 0.65, respectively.



Scheme 1 Chemical structure of BUCS, BECS, PHCS, MACS and CNCS

The Solubility Properties

The solubility properties of the five chitosan derivatives were estimated at first, because they are the key requirements for lyotropic liquid crystalline behavior.

It is known that chitosan is insoluble in ordinary organic solvents, and only soluble in acidic solvents such as formic acid, dichloroacetic acid or dilute organic acids, because of its strong micelle structure due to inter- or intra- molecular hydrogen bonds among hydroxyl and amino groups. But the five chemically modified chitosan derivatives showed much better solubility in organic solvents than chitosan. The introduction of hydrophobic groups into the chitosan molecule evidently enhanced its solubility in common organic solvents.

From Table 1, it can be seen that all five chitosan derivatives are soluble or slightly soluble in common organic solvents such as formamide, N, N-dimethyl formamide, N, N-dimethyl acetamide or pyridine in addition to acidic solvents. What's more, BUCS and BECS are water-soluble. The best solvents are those, whose solubility parameters are between 20.5 and 24.8.

Although the solubility properties of chitosan derivatives are affected by the degree of substitution, all products prepared in our conditions had a medium degree of substitution, and some comparison of solubility properties among five derivatives can still be made. From Table 1, it can

also be noticed that O-substituted species are able to dissolve in more solvents than N-substituted analogues. For example, PHCS and MACS were not soluble in weak acids such as acetic acid and acrylic acid due to the absence of free amino groups.

Table 1. Solubility^a of chitosan and its derivatives in various solvents

Solvents	$\delta^b/(\text{MPa})^{1/2}$	CS	BUCS	BECS	PHCS	MACS	CNCS
Trifluoroacetic acid	17.6	S	S	S	S	S	S
Ethyl acetate	18.6	I	I	I	I	I	I
Tetrahydrofuran	18.3	I	I	I	I	I	SS
Benzene	18.8	I	I	I	I	I	SS
Chloroform	19.0	I	I	I	I	I	SS
Acetone	20.3	I	I	I	I	SS	SS
1,4-Dioxane	20.5	I	SS	SS	S	I	I
Acetic acid	20.7	S	S	S	I	I	S
M-Cresol	20.9	S	S	S	S	I	S
Pyridine	21.9	I	S	S	S	SS	SS
N, N-Dimethyl acetamide	22.1	I	S	S	S	S	S
Dichloroacetic acid	22.5	S	S	S	S	S	S
Acrylic acid	24.6	S	S	SS	I	I	S
Dimethyl sulfoxide	24.6	I	S	S	S	S	SS
N, N-Dimethyl formamide	24.8	I	S	S	S	S	S
Formic acid	24.8	S	S	S	I	S	S
Ethanol	26.0	I	S	I	I	I	SS
Methanol	29.7	I	S	I	I	I	SS
Formamide	39.3	I	S	S	SS	S	S
Water	47.9	I	S	S	I	I	I

a. S, soluble; I, insoluble; SS, slightly soluble

b. Solubility parameters, from Brandrup J. and Immergrant E.H., "Polymer Handbook, Third Edition," John Wiley and Sons, New York, 1989, VII: 519, Table 5~8

The Liquid Crystalline Texture

All of the five chitosan derivatives demonstrated lyotropic liquid crystalline ordering. Some examples of liquid crystalline textures observed in polarized microscope were shown in Fig.1. Three kinds of textures can be observed, they are the fingerprint texture, alternative polarized field and planar texture. Observation results are summarized in Table 2.

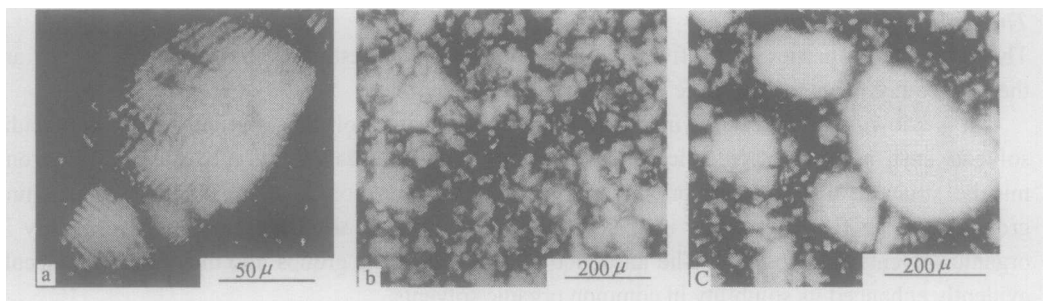


Fig. 1 Various liquid crystalline textures of chitosan derivatives
 (a) Fingerprint texture of BUCS in 50% DMSO; (b) Alternative polarized field of BECS in 40% DMAC; (c) Planar texture of PHCS in 35% 1, 4-dioxane

It is known that when a cholesteric liquid crystalline solution is held between glass plates, polydomains in which regions of parallel equidistant lines are seen sporadically can be observed. This is the so-called fingerprint texture, a typical texture of cholesteric phase. Table 2 shows that

fingerprint textures only appear in three O-substituted derivatives, and never in two N-substituted derivatives no matter what kind of solvent was used. The reason for this remains to be investigated. In some polymer/solvent systems such as BUCS/trifluoroacetic acid, BUCS/DMSO, CNCS/trifluoroacetic acid, CNCS/dichloroacetic acid, CNCS/formamide, CNCS/DMAc, CNCS/DMF and CNCS/*m*-cresol, the fingerprint-like domains were abundant, with each piece of domain normally kept isolated. The pitch of the helix, which can be calculated from the "fingerprint", varied from one domain to another.

Table 2. Liquid crystalline textures that have been observed in solutions of chitosan and its derivatives

Texture	CS	BUCS	BECS	PHCS	MACS	CNCS
Fingerprint texture	Yes	Yes	Yes	No	No	Yes
Alternative polarized field	No	Yes	Yes	Yes	Yes	Yes
Plannar texture	No	Yes	Yes	Yes	Yes	Yes

On the other hand, alternative polarized field and plannar texture occurred in all five derivatives.

In so-called alternative polarized field texture, the bright and dark regions alternatively change when the polarizers rotate. In so-called plannar texture, some isolate bright domains can be observed. The boundaries of domains are often clear. All molecules in a domain arrange itself in one direction. The two textures described above can be observed in both nematic and cholesteric mesophases. The birefringence in texture of these derivatives is much stronger than in that of the chitosan itself. It implied that the liquid crystalline behavior of these derivatives is better than the chitosan.

No thermotropic liquid crystalline behavior can be observed in all of these chitosan derivatives by DSC determination.

The Critical Concentration

The weight fraction C_w^* and volume fraction C_v^* at incipient phase separation of the anisotropic phase were experimentally measured for different chitosan derivative/solvent systems. The results of critical concentration determination are listed in Table 3. DMSO and four acidic solvents were used to prepare the solutions. The acidity of the four acidic solvents are quite different. The pK_a values of trifluoroacetic acid, dichloroacetic acid, acrylic acid and acetic acid are 0.5, 1.26, 4.26 and 4.76, respectively^[9].

From Table 3, it is of interest to note that the critical liquid crystalline behavior of three O-

Table 3. Critical concentration values of chitosan and its derivatives in various solvents

Polymer	Critical concentration	Trifluoro acetic acid	Dichloro acetic acid	Acrylic acid	Acetic acid	Dimethyl sulfoxide
CS	C_w^*	0.04	0.04	0.05	0.06	
	C_v^*	0.08	0.08	0.08	0.08	
BUCS	C_w^*	0.12	0.12	0.16	0.16	0.08
	C_v^*	0.17	0.17	0.17	0.16	0.09
BECS	C_w^*	0.18	0.18	0.22	0.24	0.22
	C_v^*	0.23	0.24	0.23	0.24	0.25
PHCS	C_w^*	0.14	0.24			0.24
	C_v^*	0.17	0.30			0.23
MACS	C_w^*	0.18	0.12			0.22
	C_v^*	0.22	0.16			0.21
CNCS	C_w^*	0.14	0.14	0.18	0.20	
	C_v^*	0.19	0.20	0.20	0.20	

substituted chitosan derivatives is obviously different from two N-substituted derivatives. All critical concentration values (v/v%) of the O-substituted chitosan derivatives in acidic solvents are independent of the acidity of solvent. It indicated that the lyotropic liquid crystalline behavior of O-substituted chitosan is related to the free amino groups. In solution, the rigidity of chain depends not only on the chemical structure of molecules, but also on the solvation of macromolecules. The solvation in these systems was achieved basically by the protonation of amine group. Since the protonation of amine with acid is rapid and easy even though the acid is weak, the rigidity of chain (therefore the critical concentration C_v^*) did not depend on the acidity of solvents.

From Table 3, it can also be noticed that the critical concentration values of all derivatives are higher than those of chitosan itself. The decrease of chain rigidity of chitosan after substitution can be explained by the disruption of the inter- or intra- molecular hydrogen bonds in chitosan.

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