

**Full Paper:** A series of derivatives of chitosan – *N*-alkyl (methyl, ethyl, propyl and butyl) chitosans – were synthesized from completely deacetylated chitosan. The degree of substitution (from 0.15 to 0.81) of the *N*-ethyl chitosan were obtained by controlling the molar ratio of the reactants. All the products showed lyotropic liquid-crystalline properties regardless of the length of the side chains and the degree of substitution. The critical concentration ( $C^*$ ) of the samples were measured by both microscopy and refractometry.  $C^*$  seemed not to vary with the degree of substitution ( $ds$ ) in the case of a given substituent chain, but rose dramatically depending on the length of the substituent group as this was varied from methyl to butyl. The results were explained according to Flory's classical theory as well as experimental of X-ray diffraction measurements.

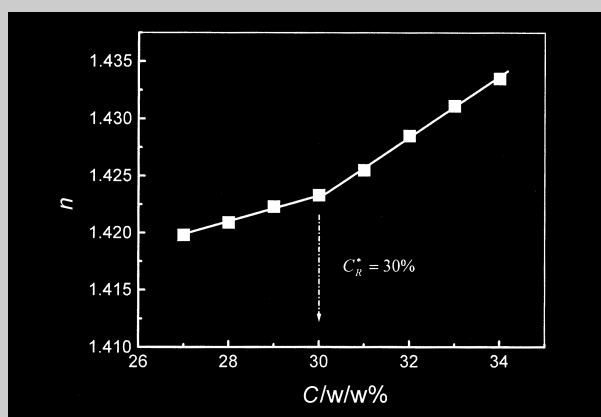


Illustration of measurement of  $C_R^*$  by refractometry in NECS3/formic acid solution.

## Studies on Lyotropic Liquid-Crystalline *N*-Alkyl Chitosans in Formic Acid

Yusong Wu, Yanming Dong,\* Ling Chen, Jianying Huang, Jun Li

Department of Materials Science and Engineering & State Key Laboratory for Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen, 361005, China

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### Introduction

Chitin, a very important biosynthesized functional polymer with the repeating unit  $\beta$ -(1,4)-2-deoxy-2-acetamido-D-glucopyranose, is widely found in the bodies of several living organisms. Chitosan, commercially manufactured from chitin, can be considered as a partially or completely deacetylated production of chitin.<sup>[1]</sup> The amino group at the ring C<sub>2</sub>-position and the hydroxyl groups at the ring C<sub>3</sub>- and C<sub>6</sub>-position respectively, have at least two important influences on the physical and chemical properties of chitosan itself. On one hand, strong intermolecular and intramolecular hydrogen bonds might be formed due to these groups; this results in chitosan having a very poor solubility (only soluble in some proton solvents). On the other hand, many chemical modifications can be readily carried out by introducing other new functionalities into either the amino group or the hydroxyl groups, or into both.<sup>[2–8]</sup>

Several rigid or semi-rigid natural polymers have been found to form a lyotropic mesophase. Examples include

polypeptides,<sup>[9]</sup> proteins,<sup>[10]</sup> poly(nucleic acids)<sup>[11–14]</sup> and cellulotics.<sup>[15–17]</sup> Chitosan and its derivatives belong to another family of liquid-crystalline biopolymers, which was first reported in 1982 by Ogura et al.<sup>[18]</sup> Since then, quite a number of lyotropic solutions of chitosan derivatives have been found.<sup>[19–25]</sup> However, only a few authors report regarding the effects of structural factors on the critical concentration for forming the lyotropic mesophase.<sup>[24,25]</sup>

*N*-Alkyl chitosan is one of the most important derivatives of chitosan, with favorable properties for casting into films and chelating transitional metal ions. It is broadly used in cosmetics, environment protection, textiles, pharmaceuticals etc.<sup>[26,27]</sup> However, the liquid-crystalline properties of *N*-alkyl chitosan have not previously been reported. In this paper, we focus our attention on a new series of lyotropic species (*N*-methyl chitosan, *N*-ethyl chitosan, *N*-propyl chitosan and *N*-butyl chitosan, noted as N-MCS, N-ECS, N-PCS and N-BCS, respectively). The influences of chemical structures (the length

of the side chain and the degree of substitution) on the critical concentration of these derivatives are investigated.

## Experimental Part

### Preparation of *N*-Alkyl Chitosan

Chitosan from crab shell with a degree of deacetylation of 84% and a viscosity-average molecular weight of  $7.4 \times 10^5$  was used for this study. This commercially purchased raw material (from Xiamen Second Pharmaceutical Factory, Xiamen) was treated with 50% aqueous NaOH solution under  $N_2$  at  $80^\circ C$  for 8 h, three times to produce acetyl-free chitosan according to the process previously described.<sup>[28]</sup> It was purified by dissolution in 1% aqueous acetic acid and precipitation with 10% aqueous NaOH solution.

*N*-Alkyl chitosan was then prepared according to the methods described in literature.<sup>[26,27]</sup> 2.0 g of acetyl-free chitosan was dissolved in 200 mL of 15% acetic acid aqueous solution at room temperature. A large excess of formaldehyde (or acetaldehyde, propionaldehyde, *n*-butylaldehyde) was added to the solution. The mixture was stirred for 2 h at room temperature. Then the Schiff's base produced was reduced by adding 1.7 g of  $NaBH_4$  (in 15% aqueous solution) and stirring for 1 h at room temperature. After being washed several times in succession with ethanol, ethyl ether and distilled water, the product was finally dried at  $40^\circ C$ . The reaction pathway and chemical structure of *N*-alkyl chitosan are illustrated in Figure 1.

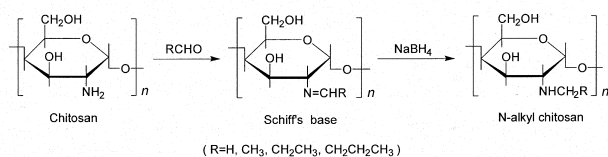


Figure 1. The preparation and chemical structure of *N*-alkyl chitosan.

### Measurements

IR spectra were recorded with a Nicolet Avator 360 FT-IR (US) by the KBr pellet method.

The elemental analyses were measured with a CE 1110 CHNS—O elemental analyzer (Italy).

$^1H$  NMR spectra were obtained with a Varian Unity NMR spectrometer at 500 MHz (US).  $CD_3COOH/D_2O$  was used as solvent.

X-ray diffraction (XRD) spectra were carried out using a Rigaku Rotaflex D/max-C XRD (Japan).

The critical concentration  $C^*$  required to form the ordered phase was determined in two ways. (i) A series of *N*-alkyl chitosan/formic acid solutions of concentrations differing by intervals of 1 wt.-% were prepared separately in small glass vials. The vials were tightly sealed so that the solvent could not evaporate. The solutions were aged for 3 d at  $25^\circ C$  before use, and then sandwiched between two glass slides to form liquid-crystal boxes. An Olympus BH2 polarized microscope (Japan) was used for observation. The concentration at

which the birefringence appeared at  $25^\circ C$  was defined as  $C_p^*$ . (ii) The refractive indexes  $n$  of each of the prepared solutions was measured at a constant temperature of  $25^\circ C$ . Then, the curve of  $n$  vs.  $C$  was plotted. The curve showed an inflection because of different refractive indexes of the solutions of the isotropic and anisotropic phase. This point was defined as  $C_R^*$ .

## Results and Discussion

### Characterization of *N*-Alkyl Chitosan

In the IR spectra of the *N*-alkyl chitosans (not shown), the absorption bands at 2875, 2920 ( $-CH_2-$  and  $-CH$  stretch) and at  $1455\text{ cm}^{-1}$  ( $C-H$  bend) all showed increased intensity compared with those of chitosan itself, as a result of the introduction of the alkyl side chains. Moreover, the strength of these bands increased when the introduced carbon chains increased in length from methyl to butyl. New absorption bands at 2966 ( $-CH_3$  stretch) and  $1370\text{ cm}^{-1}$  ( $-CH_3$  bend) appeared in the IR spectra of *N*-alkyl chitosan. Simultaneously, the bands at  $1603\text{ cm}^{-1}$  (representing the  $-NH_2$  of chitosan) decreased as the  $-NH_2$  group was substituted. A new band at  $1650-1670\text{ cm}^{-1}$  ( $-NHR$ ) appeared in the IR spectra of *N*-alkyl chitosan.

$^1H$  NMR spectroscopy was employed for further confirmation of structure of *N*-alkyl chitosan. The most typical signals of *N*-alkyl chitosans appeared at 2.6 ppm (for NMCS) or  $<2.0$  ppm (for NECS, NPCS and NBCS), which are assigned to the protons of methyl groups in the introduced alkyl chains.

The results of both FT-IR and  $^1H$  NMR spectroscopies indicated the successful introduction into chitosan of alkyl chains with different carbon numbers. The  $ds$  of the products was determined by means of both elemental analysis and  $^1H$  NMR spectroscopy. The results of the characterization and the preparation conditions of *N*-alkyl chitosan are listed in Table 1.

Table 1. Preparation conditions and degree of substitution ( $ds$ ) of *N*-alkyl chitosan.

| Polymer                   | Code   | $\frac{n_{glu}}{mol} : \frac{n_{ald}}{mol}$ | $ds$                                    |
|---------------------------|--------|---|---|
| <i>N</i> -methyl chitosan | N-MCS  | 1 : 10                                      | 0.75 <sup>b)</sup>                      |
| <i>N</i> -ethyl chitosan  | N-ECS1 | 1 : 2 <sup>a)</sup>                         | 0.15 <sup>c)</sup>                      |
| <i>N</i> -ethyl chitosan  | N-ECS2 | 1 : 2                                       | 0.26 <sup>c)</sup>                      |
| <i>N</i> -ethyl chitosan  | N-ECS3 | 1 : 8                                       | 0.47 <sup>c)</sup>                      |
| <i>N</i> -ethyl chitosan  | N-ECS4 | 1 : 10                                      | 0.72 <sup>c)</sup> (0.75) <sup>b)</sup> |
| <i>N</i> -ethyl chitosan  | N-ECS5 | 1 : 15                                      | 0.81 <sup>c)</sup>                      |
| <i>N</i> -propyl chitosan | N-PCS  | 1 : 10                                      | 0.77 <sup>b)</sup>                      |
| <i>N</i> -butyl chitosan  | N-BCS  | 1 : 10                                      | 0.74 <sup>b)</sup>                      |

a) Reaction time was 0.5 h.

b) Determined by  $^1H$  NMR spectroscopy.

c) Determined by elemental analysis.

## Lyotropic Behavior of *N*-Alkyl Chitosans

All the products could be dissolved in formic acid, dichloroacetic acid and acrylic acid and to form lyotropic liquid crystals. To study the influence of the chemical structures on the critical behavior of the lyotropic liquid-crystalline solutions, the critical concentration  $C^*$  of *N*-alkyl chitosans with side chains of different length and with different degree of substitutions was determined in formic acid solutions.  $C^*$ , at which the formation of the lyotropic mesophase begins, is an important physical parameter for evaluating the rigidity of the chains of some polymers. Several methods have been introduced to determine  $C^*$  of liquid-crystalline polymer cellulose and its derivatives.<sup>[29–34]</sup> In this paper, two methods (polarized microscopy and refractometry) are used. A typical curve for the refractometry measurement is illustrated in Figure 2. The results of  $C^*$  determination are shown in Table 2 and 3.

It can be seen from Table 2 and 3 that the results  $C_p^*$  and  $C_R^*$  measured by two different methods are almost consistent with each other, supporting the reliability of the results. The results also indicated that the  $C^*$  of NECS was clearly not influenced by the degree of substitution ( $ds$ , from 0.15 to 0.81), though the results seemed

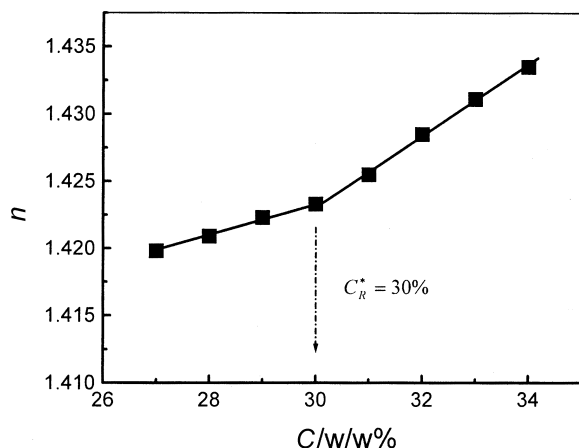


Figure 2. Illustration of measurement of  $C_R^*$  by refractometry in NECS3/formic acid solution.

Table 2. Critical concentration for *N*-ethyl chitosan with different degrees of substitution.

| Sample ( $ds$ ) | N-ECS1 (0.15) | N-ECS2 (0.26) | N-ECS3 (0.47) | N-ECS4 (0.72) | N-ECS5 (0.81) |
|-----------------|---------------|---------------|---------------|---------------|---------------|
| $C_p^*$         | 31            | 30            | 31            | 31            | 30            |
| $C_R^*$         | 32            | 32            | 30            | 30            | 30            |

Table 3. Critical concentration for chitosan and *N*-alkyl chitosan with side chains of different length.

| Sample ( $ds$ ) | CS (0) | N-MCS (0.75) | N-ECS4 (0.75) | NPCS (0.77) | N-BCS (0.74) |
|-----------------|--------|--------------|---------------|-------------|--------------|
| $C_p^*$         | 20     | 26           | 31            | 40          | 49           |
| $C_R^*$         | 20     | 26           | 30            | 38          | 46           |

to be higher than for chitosan itself. However, the  $C^*$  rises constantly as the substituent groups are varied from methyl to butyl in the case of the same  $ds$  ( $0.75 \pm 0.02$ ).

### Explanation of the Results

The liquid-crystal phase of a rod-like chain is predicted to occur at  $C^*(v/v)$  according to Flory's well-known equation:<sup>[35]</sup>

$$C^*(v/v) \cong (8/x)(1-2/x) \quad (1)$$

where  $x$  is the axis ratio  $q/r$  or  $2q/d$  (where  $q$  represents the persistence length,  $d$  and  $r$  represent the diameter and radius of chain, respectively). As  $q \gg d$ , the above formula can be rewritten approximately as:

$$C^*(v/v) \propto d/q \quad (2)$$

So generally speaking,  $C^*$  is affected by both  $d$  and  $q$  for a given liquid-crystalline polymer. Because  $C^*(v/v)$  is close to  $C^*(w/w)$ , which we have measured and represented as  $C^*$  previously,  $C^*(w/w)$  is also affected by both  $d$  and  $q$ .

Firstly, the rigidity of the molecular chain decreases as a result of the breaking down of the inter- and intramolecular hydrogen bonds in chitosan, which results in a decrease in  $q$ . This explains why the introduction of the alkyl chains results in the  $C^*$  increasing even through the introduced chains are short (e. g., methyl) or  $ds$  is very small.

Secondly, the diameter of the macromolecule may be altered by introduction of the alkyl chain.  $d$  increases continuously as the size of the substituent group varies from methyl to butyl; the longer the introduced side chains; the bigger the value of  $d$ . Nevertheless,  $d$  did not change much for a given side chain when  $ds$  was increased. This could explain the dramatic increase in  $C^*$  with the increase in the size of the substituent groups when the  $ds$  was kept constant. It could also explained the observation that  $C^*$  did not change despite an increase of the  $ds$ , if the substituent group was kept constant.

The above explanations can be further confirmed by means of XRD experiments. XRD results for *N*-alkyl chitosan were shown in Figure 3 and 4. It can be seen that the *N*-alkyl chitosans studied gave two peaks at about  $2\theta = 20^\circ$  and  $2\theta = 5^\circ-10^\circ$ , respectively. The positions of these two dispersion peaks were not affected by an increase in  $ds$ , if the substituent group was the same (Figure 3). This indicates that the  $ds$  was not the primary factor influencing the size of crystal cells of *N*-alkyl chitosan. However, the peaks of smaller angle, which may correspond to the space between molecules in an ordered structure, always shifted to a smaller angle as the substituent groups varied from methyl to butyl; the longer the introduced side-chain, the greater the angle shift (Figure 4). This indicates that a longer side chain leads to a "fat-

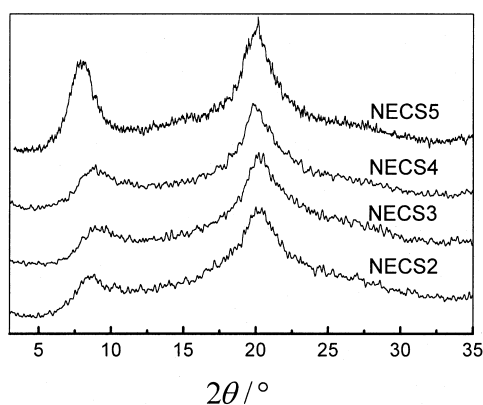


Figure 3. XRD patterns of *N*-ethyl chitosan with different degree of substitution.

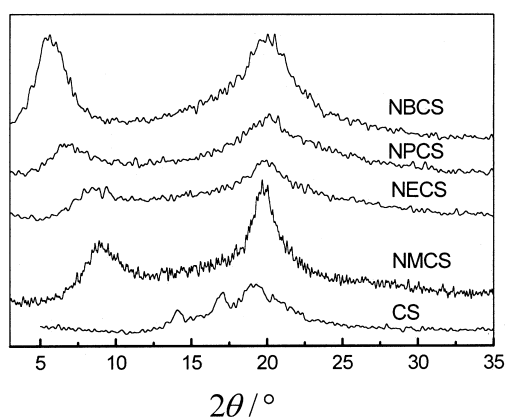


Figure 4. XRD patterns of chitosan and *N*-alkyl chitosan with substituted side chains of different length.

ter" molecular chain, i.e., a larger  $d$  value, and a larger  $C^*$  value could be achieved.

## Conclusion

We successfully prepared a series of *N*-alkyl chitosans with controlled  $ds$ . All the products showed lyotropic liquid-crystalline properties regardless of the length of the side chains and the degree of substitution. The critical concentration of the samples were determined by means of both microscopy and refractometry, which gave identical data.  $C^*$  was found not to vary with the  $ds$  in the case of a given carbon side chain, but rose consistently as the length of substituent group varied from methyl to butyl. The results agree with the previous work on other chitosan derivatives.<sup>[24, 25]</sup>

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