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Multiple crystalline morphologies of *N*-Alkyl chitosan solution cast films

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Summary

In addition to spherulites, two novel crystalline morphologies, i. e. need-like crystal and sheet-like crystals were found in formic acid solution cast films of *N*-methyl chitosan and *N*-ethyl chitosan during casting at 25°C and relative humidity 65%. Their size was larger in 1~2 order of magnitude than chitin needle-like single crystals and chitosan lamellar single crystals reported previously due to the high molecular weight. The formation mechanism of these crystalline morphologies was discussed.

Introduction

Chitin represents an important polymer, which is the second most abundant in nature, and serves as a renewable resource in many applications. In living systems chitin occurs in the form of micro-fibrils. The micro-fibrils have been clearly brought to evidence by electron microscopists who have called them crystallites as well [1]. The structure of the crystallites has been researched mainly with X-ray diffraction by many authors [2~8]. But so far most works had concentrated on the crystallographic studies, few works concerned about the crystalline morphology of chitin/chitosan and its derivatives. Murray et al.[9] and Sakamoto et al.[10] demonstrated spherulites from α-chitin. Dong et al.[11] reported spherulites of cyanoethyl chitosan developing from cholesteric mesophase. Chanzy's group investigated needle-like single crystals of chitin [12] and lamellar single crystals of chitosan [13,14] using electron microscopy and electron diffraction. Helbert et al. observed α-chitin single crystals with a shape of flat ribbon [15]. All single crystals studied previously were prepared from very dilute solutions of low molecular species, e.g. chitosan with DP=35 [13].

In this paper, in addition to spherulites, large needle-like crystals and sheet-like crystals with the similar shapes to typical needle-like single crystals [12] and lamellar single crystals [13] were prepared from cast films of high molecular weight *N*-alkyl chitosan. The preparation method and size of these novel crystals were completely different with the previous studies. The morphology of these crystals and the formation mechanism were discussed in detail.

Experimental

Chitosan from crab shell with degree of deacetylation 84% and viscosity average molecular weight of 7.4×10^5 was used. Chitosan raw material was purified by dissolved in 1% aqueous acetic acid solution and precipitated with 10% aqueous NaOH solution. The purified chitosan was then treated with 50% NaOH solution under N_2 at 100°C for 4 hr three times to produce acetyl-free chitosan according to the process previously described [16]. Two kinds of *N*-alkyl chitosan i. e. *N*-methyl chitosan (denoted as MCS) and *N*-ethyl chitosan (denoted as ECS) were then prepared referring to the method of literature [17~18]. The degree of substitution of MCS and ECS is 0.75 and 0.84 respectively, as measured by a VARIAN UNITY ^1H NMR spectrometer at 500 MHz.

The concentrated MCS or ECS / formic acid solutions were prepared readily by mixing MCS or ECS powder with formic acid (98~100%, guaranteed reagent produced by NACALAI TESQUE INC.) in sealed glass vials. The concentration (in weight) of solutions was 15% for MCS and 20% for ECS, which was the lowest concentration to observe multiple crystalline morphologies in cast films for MCS and ECS respectively. The solutions aged for one week at room temperature before use. Each solution was sandwiched between a glass slide and a Teflon film. The Teflon film was removed to expose the solution. There were two methods to prepare sample films. Preparation method 1: the solutions on glass slide were used directly for in situ crystallization observation at 25°C and relative humidity 65% with an OLYMPUS polarized optical microscope (POM). The solutions became films after evaporation of formic acid in few days. Preparation method 2: the solutions on glass slide were transferred further into a vacuum system (about 10^{-3} Torr) to dry at 25°C for 1 day. After withdrawn from the vacuum system, the solutions were put immediately onto the POM sample stage for in situ crystallization observation at 25°C and relative humidity 65%. Finally both kinds of sample films were also detected after Pt sputtering with a JEOL JSM-6320F scanning electron microscope (SEM) using a low accelerating voltage of 5KV to avoid electron beam damage. Table 1 is the lists of the sample films.

Table 1 Lists of the sample films studied in this paper

Sample film	Initial concentration of solution in formic acid	Polymer	Preparation method of sample film[a]
15% MCS-1	15%	MCS	method 1
20% ECS-1	20%	ECS	method 1
15% MCS-2	15%	MCS	method 2
20% ECS-2	20%	ECS	method 2

[a] See the text.

Results and discussion

Observation of spherulites

For 15%MCS-1 and 20%ECS-1, there was no any spherulite at the beginning of solution casting. But after 1~2 day typical spherulites occurred in all sample films. Figure 1a shows the POM micrographs of the typical spherulites in 20%ECS-1. Unlike the small crystallites usually found in natural polymers such as cellulose, chitin and chitosan, the spherulites of these chitosan derivatives are multi-crystallite aggregation with a radial fibrillar morphology which are generally observed in ordinary synthetic polymers. The size of these spherulites can reach several millimeters so that they can be seen even by the naked eyes. The growth rate of the spherulites was slow. Normally it is 1-4 μm/min.

On the other hand, for 15%MCS-2 and 20%ECS-2 the spherulites started to grow in few minutes after the beginning of observation. Figure1b shows a spherulite growing in 15%MCS-2. In the samples prepared using method 2, the growth rate was faster. For instance, it was 23 μm/min in the case of 15%MCS-2 .

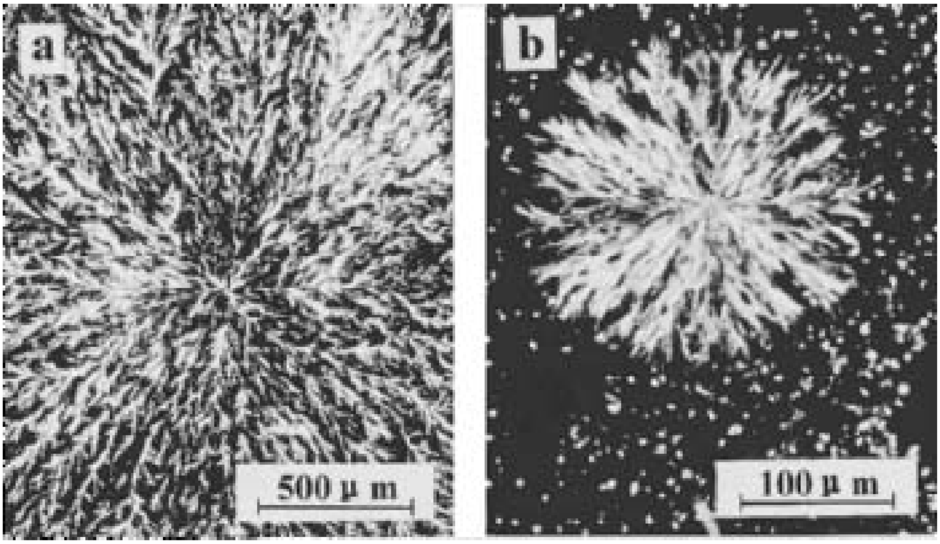

Figure 1 POM micrograph of the spherulites in (a) 20%ECS-1 and (b) 15%MCS-2

Observation of needle-like crystals and sheet-like crystals

It is interesting to note that the spherulites were not the final morphology of crystallization for MCS and ECS. After storing few weeks at 25°C and relative humidity 65%, the crystalline morphology changed dramatically. There were two kinds of new crystalline morphology occurred. On the spherulites of all samples, plenty of needle-like crystals appeared. Figure 2 illustrates a typical example of these needle-like crystals. They decorated the spherulite so that the morphology of spherulite seemed unchanged, but actually the needle-like crystals were wholly on the surface of the film. Although there was a distribution of size for these needle-like crystals, an average length was about 50μm and the width was about 2μm. Taking account of the positive birefringence nature, the molecular chains may align at the long axis of the needle. The shape of these needle-like crystals was similar to needle-like single crystals prepared from low molecular weight chitin in very dilute solutions

[12]. Nevertheless the size was very different. The average length and width of the latter were only few μm and 10~20nm respectively. Thus the former was larger in 1~2 order of magnitude. The variation of molecular weight can be regarded as the major reason of different size for these needles.

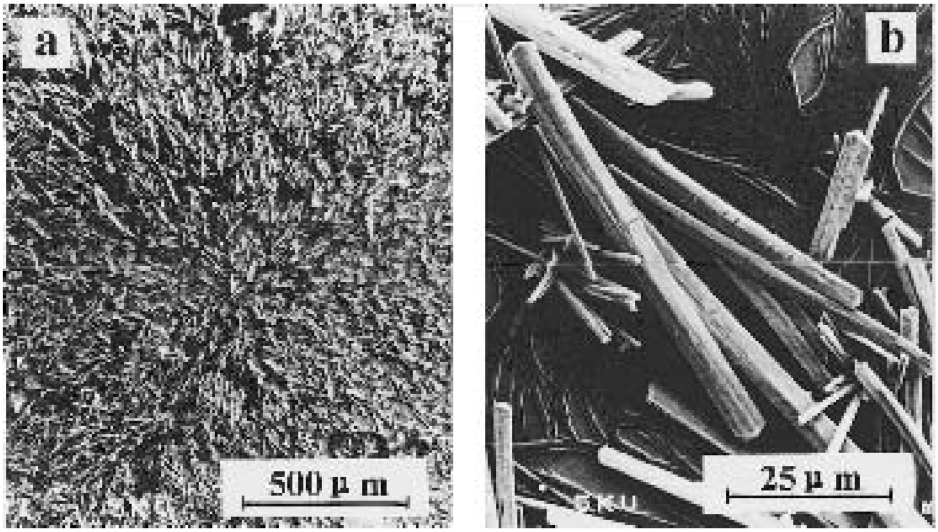

Figure 2 SEM micrograph of the needle-like crystals in 20%ECS-1 film. (a) The needles decorate the original spherulite and orient at the same direction as the original fibrils of spherulite; (b) Details of the needles.

The second new crystalline morphology was sheet-like crystals. Normally they appeared in the area that had not spherulites or at the edge of spherulite. Each thin single crystal had a square-like shape with typical sides of ~20μm and a thickness of around 2μm. From Figure 3 it can be noticed that the thin sheet-like crystals were often overlapped. The orientation of molecular chains in sheets is not clear. Wide angle X-ray diffraction experiments showed no obvious orientation as a whole of the sample. An electron diffraction is necessary to detect the chain arrangement, but the determination is impossible because of the huge thickness of the sheets. Taking account of the average length of chain is about 2μm according to the molecular weight, which agreed to the thickness of the sheet, the chains may be vertical to the surface of the sheets. Table 2 compares sheet-like crystals with lamellar single crystals reported previously. They had similar shape, but the size was largely different.

Table 2 Comparison between sheet-like crystals studied in this paper and lamellar single crystals reported previously

Morphology	Polymer	DP	concentration of solution	Length and width	Thick-ness	Re-ference
lamellae single crystals	Chitosan	35	0.15%	0.2~0.5μm	12nm	[13]
Sheet-like crystals	MCS and ECS	~4600	15 and 20%	~20μm	~2μm	This work

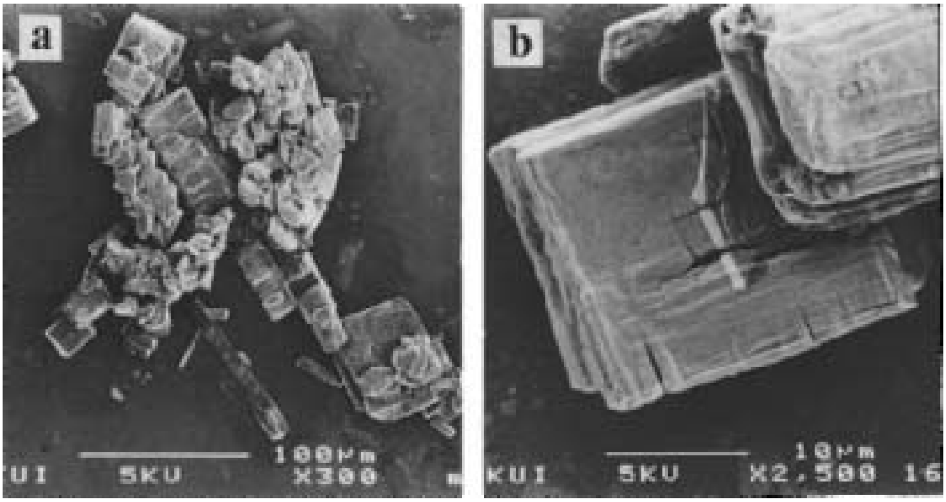

Figure 3 SEM micrograph of the sheet-like crystals in 15%MCS-2 film. (a) The sheet-like crystals overlap each other; (b) Details of the sheet-like crystals.

Discussion of formation mechanism

Chitosan shows hydrophilic properties because of the hydroxyl group and amino group. It is well known that twist glucosamine units of commercial chitosan or its derivatives always contain combined water molecule. Several works reported that water molecules involve the unit cell of chitosan crystals [2~8]. The influences of acidic solvents on crystal structure of chitosan were also investigated [7]. In this paper, we found moisture and remained solvent played an important role on the formation of crystalline morphologies.

In order to know the formation mechanism of spherulites in films, we measured the weight of the sample during the observation of crystallization. It was found that the weight of the sample decreased in all the samples prepared using method 1, but increased in all the samples prepared using method 2. For the former, the solvent in the solutions evaporate continuously (see Figure 4a) and formic acid content remained in films was about 30-35% in 6 hr and reached a constant value of about 18% after 10 days. The start time of spherulite formation was from 6 hours to few days depending on the nucleating condition. It means that there were 18-35% formic acid remained in films during crystallization, so the crystallization happened in concentrated solutions, the solvent acts an important role in the crystallization. But for the later, the weight of sample indicated that there was almost no solvent in the films, which were as received from the vacuum system. It is interesting to know how the spherulites can be formed without solvent. Figure 4b shows that the weight of film rose because it absorbed moisture. The water content reached a constant value of about 20% after 6 hours. At the same time the growth of the spherulites can be observed almost from the beginning (the water content was ~5%) after the samples were taken off the vacuum. It means that the crystallization can occurred at a reasonable speed only in the water content of ~5%- 20%. A small amount of water is necessary for the crystallization, because water was used as a plasticizer, which increases the mobility of polymer chains. The phenomenon can be regarded as solvent induced crystallization, which is well known in some commercial polymers such as Nylon 6, whose crystallization can also be induced by water [19].

The actual solvent (including water) content in the film during the formation of both needle-like crystals and sheet-like crystals was measured to be ~20%, which was the constant value after solvent evaporation or moisture absorption. So the small molecules such as remained solvent or absorbed water have been proved to play a very important part also in the formation of these two crystalline morphologies. The two crystalline morphologies formed after secondary crystallization from spherulites. So it suggests that water and solvent were promoter for the perfection process of crystals.

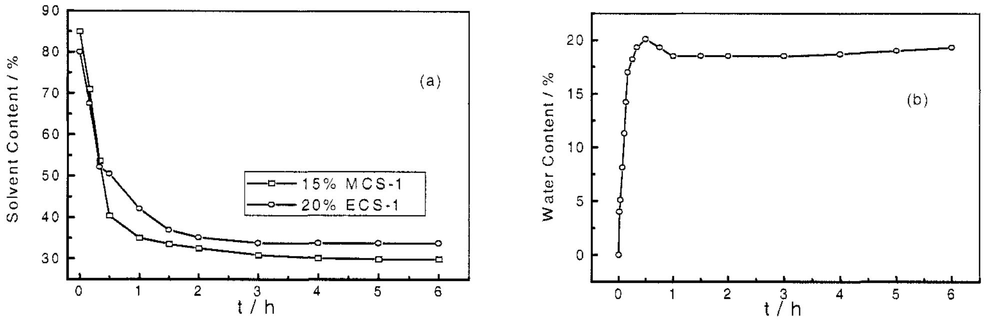

Figure 4 The plot of solvent or water content in cast films vs. observation time. (a) 15%MCS-1 and 20%ECS-1; (b) 20%ECS-2.

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