Chem. Pharm. Bull. **44**(7) 1372—1375 (1996) Vol. 44, No. 7

Physicochemical Properties of Chitosan Film Prepared on a Metal Plate Loaded with Electric Charge

Hiraku IKEDA,*[a] Shigeo YAMAMURA,[b] Kozo TAKAYAMA,[a] and Tsuneji NAGAI[a]

Department of Pharmaceutics, Hoshi University,[a] Ebara 2–4–41, Shinagawa-ku, Tokyo 142, Japan and Department of Clinical Pharmacy, Toho University,[b] Miyama 2–2–1, Funabashi-shi, Chiba 274, Japan.

Received February 16, 1996; accepted April 22, 1996

A novel method to prepare a chitosan film using a newly developed apparatus in which the cast plate was loaded an electric charge was previously reported. In this study, the film prepared with or without the electric charge was characterized by X-ray diffractometry, differential scanning calorimetry (DSC), a microwave molecular orientation analyzer and an optical birefringence analyzer.

X-Ray diffraction patterns indicated that the film prepared with an electric charge (VF) had higher crystallinity than the film prepared without an electric charge (NVF). These findings suggested that well-crystallized chitosan fibers were formed during the preparation of VF. Furthermore, chitosan fibers in VF had more preferred orientation. The DSC findings also indicated that the crystallinity of VF was higher than that of NVF. An electric charge during preparation of chitosan film might help making a particular fiber arrangement of chitosan macromolecules. The mechanical properties of VF and NVF reported previously were discussed from physicochemical characteristics of the films.

Key words electric charge; chitosan film characterization; crystallite; X-ray diffraction; differential scanning calorimetry

Previously, we reported a novel method to prepare a chitosan film on the metal plate loaded with an electric charge.[1)] Mechanical strength and cross sectional morphology of the film prepared with an electric charge (VF) were greatly different from those of the film prepared without an electric charge (NVF).

Here, to characterize the natures of VF and NVF physicochemically, we employed some sophisticated analytical techniques such as wide-angle X-ray diffractometry, differential scanning calorimetry (DSC), microwave molecular orientation analyzer (MOA)[2—4)] and a three dimensional optical birefringence analyzer.[5—11)] Furthermore, the difference in the mechanical property of VF and NVF was discussed from their physicochemical properties.

Experimental

Materials Chitosan, derived from crab shell chitin with about 95% deacetylation, was generously supplied from Dainichi Seika Color & Chemicals Mfg. Co., Ltd., Tokyo, Japan. The relative viscosity of a 0.2% chitosan aqueous solution (at pH 2.0) was 4.11 at 37 °C as determined with an Ubbelohde viscometer. The average molecular weight of chitosan was determined to be 4.4×10^5, by gel-permeation chromatography. Other chemicals used were of reagent grade. A stainless steel flat plate was purchased from Queen Rose Co., Ltd. Teflon sheet (thickness of 0.3 mm) was purchased from Nichiasu Co., Ltd. A direct currency power supplier was purchased from Sunhayato Co., Ltd.

Preparation of Chitosan Film Chitosan films were prepared by using the apparatus shown in Fig. 1 in the same way as reported previously.[1)] Briefly, 0.8% chitosan solution was prepared by using 1% (v/v) acetic acid solution as a solvent. This solution (160 ml) was poured on the metal plate in the apparatus. The upper plate was connected to the (−) electrode and the lower one was connected to the (+) electrode of the battery (15.2 V) which supplied direct currency. The solvent was naturally dried up in the draft chamber at room temperature. The direct currency was supplied during the drying. Acetic acid and water remaining in the film were removed completely under vacuum for two days. Chitosan films prepared with and without electric charge supplied were abbreviated as VF and NVF, respectively.

X-Ray Diffractometric Method The wide-angle X-ray diffraction patterns of the chitosan film were measured using a RINT-2500 diffractometer (Rigaku, Tokyo, Japan). Both the symmetrical-reflection and symmetrical-transmission geometries were employed to investigate the crystallite orientation in the films.[12)] A diffracted X-ray beam was monochromated by using a bent-type graphite monochrometor, and a scintillation counter was used as a detector. Other conditions were as follows: voltage, 60 kV; current, 200 mA (for symmetrical-reflection method), 250 mA (for symmetrical-transmission method); divergence and scatter slits, 1.0°; receiving slit, 0.30 mm. Scattering intensities were monitored with the fixed-time step-scanning method in the range of 5—40° (2θ) at an interval of 0.01° (2θ).

Differential Scanning Calorimetry (DSC) A differential scanning calorimeter (Model DSC 8240B, Rigaku Denki, Tokyo, Japan) was used. The film was cut into small pieces (about 2 × 2 mm) and used as samples. The sample was heated up from room temperature to 300 °C at a rate of 10 °C/min.

Microwave Molecular Orientation Analyzer (MOA) Macromolecular orientation of chitosan in the film was measured by a microwave

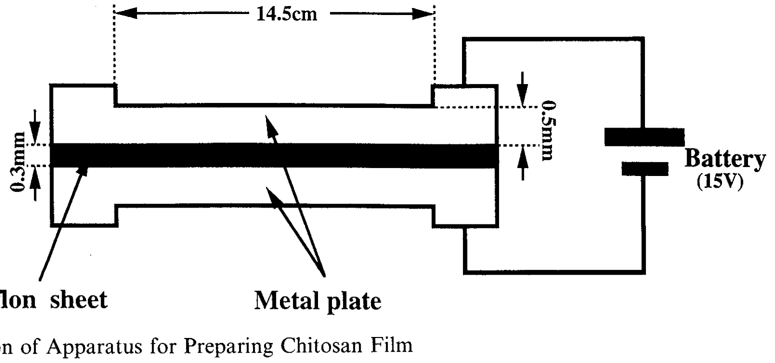

Fig. 1. Schematic Representation of Apparatus for Preparing Chitosan Film

---

* To whom correspondence should be addressed.

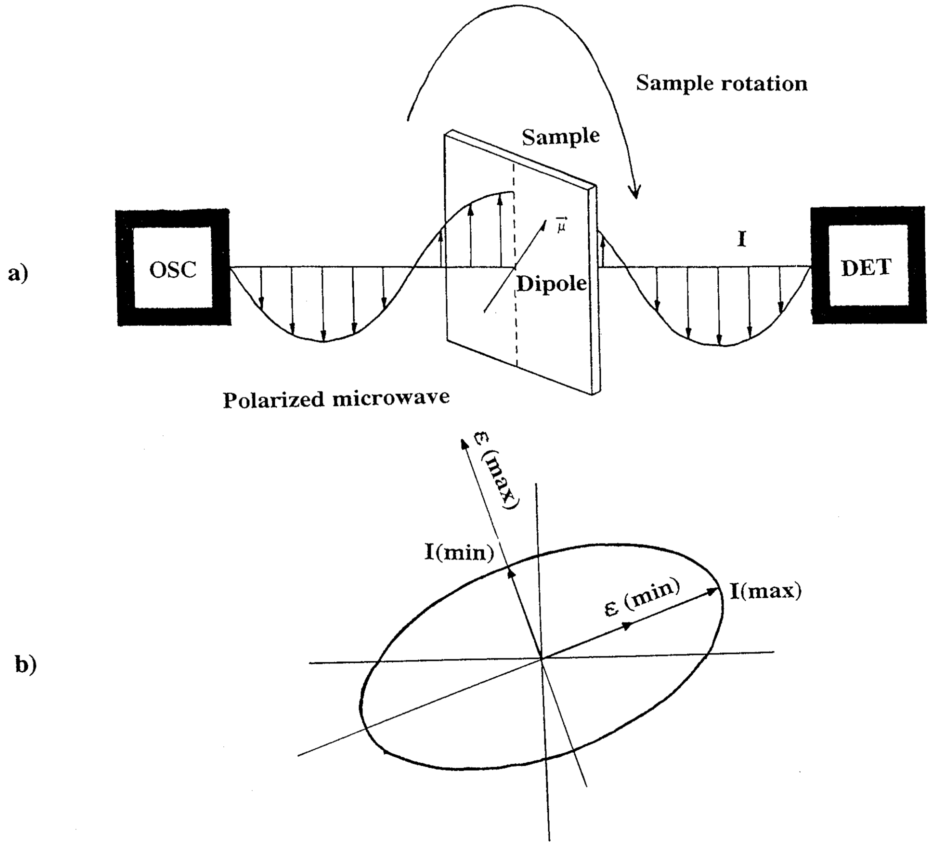

Fig. 2. Principle of Measurement for Molecular Orientation Ratio by Molecular Orientation Analyzer

OSC, oscillator; DET, detector; I, transmitted microwave intensity; ε, dielectric constant; μ, dipole moment.

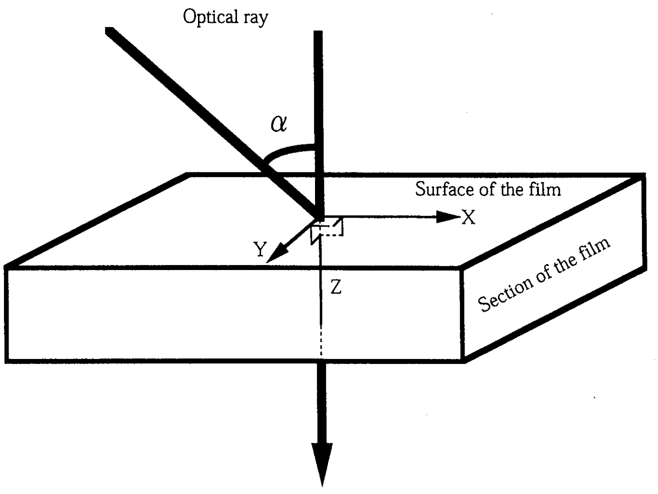

Fig. 3. Principle of Measurement for Three Dimensional Optical Birefringence Analyzer

X, *Y* and *Z* mean the optical directions which are perpendicular each others.

molecular orientation analyzer (MOA-2012A, New Oji Paper Co., Ltd., Tokyo, Japan).[2-4] Figure 2 shows the principle of MOA. The incident microwave intensity should be changed by mutual interactions between polarized microwave and the dipole of the molecules in the sample (Fig. 2a). The frequency of the incident microwave was 12.6 GHz. The transmitted microwave intensities were detected at 360 points in all rotating directions (Fig. 2b), and plotted as a distance from origin on an arbitrary scale.

Optical Birefringence Analysis Refractive indices (*N*) along *X*, *Y* and *Z* axes of the film were measured by an optical birefringence analyzer (Kobra-21ADH, New Oji Paper Co., Ltd., Tokyo, Japan).[5-11] Figure 3 schematically shows the principle of this analytical method. An optical ray (590 nm) was projected to the surface of the film at angles of various α. From phase differences with an angle of α, a reflecture index along *Z* axis was evaluated. The difference in reflective index between two axes was regarded as a birefringence (ΔN). For example, $\Delta N(x-z)$ expresses the difference of reflective index between *X* and *Z* axes. Because there was no restriction for selecting *X* and *Y* axes on the film, $\Delta N(x-y)$ is consistent within 0.00005.

Results

In wide-angle X-ray diffractometry, the symmetrical-transmission and symmetrical-reflection geometries were employed to investigate the crystallite orientation of chitosan macromolecules in the film. Figure 4 shows the geometrical arrangement of the symmetrical-transmission and symmetrical-reflection methods schematically. In the symmetrical-reflection and the symmetrical-transmission geometries, the diffraction of X-ray occurs only from *hkl* planes parallel and normal to the film surface, respectively.[12]

Figure 5 shows X-ray diffraction patterns in VF and NVF. In the diffraction pattern of VF, X-ray diffraction peaks were observed at 8.6° (2θ) and 11.8° (2θ) in both the symmetrical-reflection and symmetrical transmission geometries. On the other hand, very broad diffraction peaks were observed at 9.3° (2θ) and 15.2° (2θ) in the pattern in NVF only by the symmetrical-reflection geometry. Because of poor sensitivity of symmetrical-transmission geometry, diffraction peaks would not be observed in NVF measured by symmetrical-transmission geometry. These findings may suggest that the crystallinity of chitosan in VF was better than that in NVF. Normal

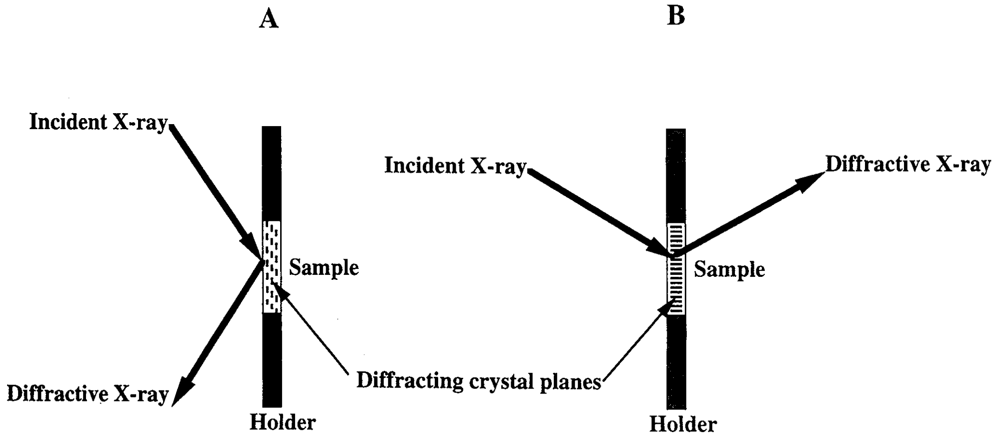

Fig. 4. Schematic Representation of Symmetrical Reflection (A) and Transmission (B) Geometry

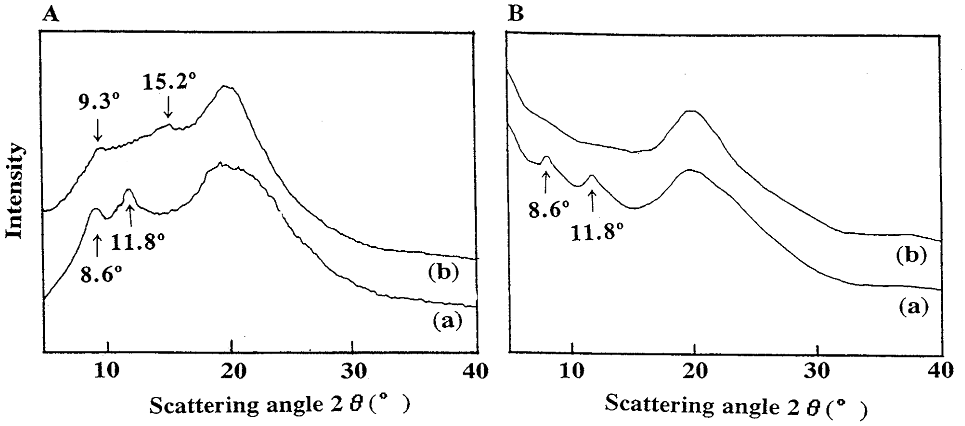

Fig. 5. X-Ray Diffraction Patterns of Chitosan Films

A, Symmetrical-reflection method; B, Symmetrical-transmission method. (a) VF, (b) NVF.

Table 1. Crystal Lattice Constants[13] and Diffracting Plane Indices of Observed Diffraction Lines

Crystal lattice constants		2θ (observed)	Diffracting plane indices	2θ (calculated)
a (Å)	13.8	8.6	040	8.69
b (Å)	40.7	9.3	111	9.17
c (Å)	16.3	11.8	022	11.76
β	96.48	15.2	221	15.23

orientation of chitosan molecule to the film surface was also suggested in NVF.

Several crystal structures of chitosan and its salts have been reported.[13] The crystal structure of chitosan in VF and NVF was estimated by a peak matching. Assuming the monoclinic structure reported by Cairns *et al.*, all diffraction peaks were attributable to the respective diffraction of *hkl* plane as shown in Table1. The diffracting plane indices of the peaks at 9.3° (2θ) and 15.2° (2θ) were not attributable to any *hkl* planes, when other crystal lattice constants were used.

Ogawa *et al.* reported the crystallinity of chitosan membranes which were prepared in the aqueous acetic acid solution.[14] They found that the ratio of amount of anhydrous crystals increased when the lower molecular weight chitosan was used in the membrane preparation from the low concentration of chitosan solution, whereas the amount of hydrated crystals increased with an increase of the molecular weight of chitosan. Reflection peaks at around 10° (2θ) and 15° (2θ) were attributed to the hydrated and anhydrous crystals, respectively. Although the clear and sharp X-ray patterns were not obtained in this study, it is likely that the reflection peak in NVF at 15.2° (2θ) and the peak in VF at 11.8° (2θ) would be attributable to the anhydrous and hydrated crystals, respectively. The difference in the diffracting planes observed between VF and NVF suggested that the orientation of chitosan fibers was modified by an electric charge.

Figure 6 shows the DSC thermograms of VF and NVF. An endothermic peak of melting was observed at 173 °C in VF, while an extremely broad peak was observed at 126 °C in NVF. The DSC findings also indicate that the crystallinity of chitosan in VF was better than that in NVF.

The transmitted intensities of polarized microwave were detected at 360 points in VF and NVF. Macromolecular orientation can be detected by the anisotropy of transmitted intensity of polarized microwave. When macromolecules in the film have any orientation, the MOA chart shows anisotropic figures and the degree of orientation would be defined by the ratio of I(max) and I(min) in Fig. 2. In the present study, the degree of orientation is described as macromolecular orientation ratio (MOR=

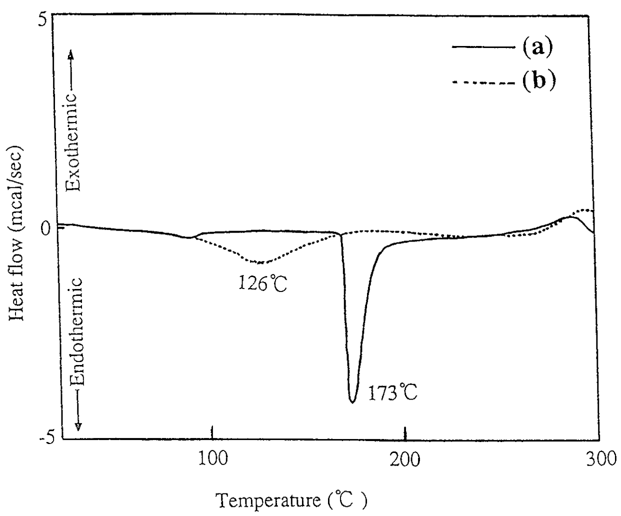

Fig. 6. Differential Scanning Calorimetric Curves of Chitosan Films
(a) VF, (b) NVF.

Table 2. Birefrengence by Optical Birefrengence Analyzer

	Nx	Ny	Nz	$\Delta N(x-y)$	$\Delta N(y-z)$	$\Delta N(x-z)$
VF	1.40174	1.40170	1.39656	0.00003	0.00514	0.00518
NVF	1.40104	1.40102	1.39794	0.00002	0.00307	0.00310

N, refractive index; ΔN, birefrengence index.

$I(\max)/I(\min)$). As the results of MOA, MOR values were estimated as 1.00 in VF and 1.01 in NVF, respectively. This suggests that there is little orientation of macromolecules of chitosan parallel to the surface of the films.

Table 2 shows the birefringence of VF and NVF. The birefringence $\Delta N(y-z)$ and $\Delta N(x-z)$ shows macromolecular orientation in the sample. Namely, larger $\Delta N(y-z)$ and $\Delta N(x-z)$ indicate macromolecular orientation normal to the surface of the film. The birefringence, $N(x-z)$ and $N(y-z)$, in VF was larger than that in NVF, suggesting that chitosan fibers in VF and NVF exhibited molecular orientation, and the degree of orientation in VF was stronger than that in NVF.

Discussion

The results of X-ray diffraction and DSC measurements indicated that chitosan in VF had higher crystallinity rather than that in NVF. These findings suggest that longer and/or well-crystallized chitosan fibers were formed with an electric charge. Molecular orientation of chitosan fibers and anisotropic structure of chitosan film were found in both VF and NVF.

Longer chitosan fibers would make better network structure and macromolecular orientation. In VF, chitosan fibers were considered to be twisted around each other. On the other hand, a poor network structure of chitosan fibers would be formed in NVF. An electric charge might help making a certain fiber arrangement of chitosan macromolecules. As reported previously, the tensile strength test showed that VF was broken after yielding, while NVF was broken without yielding.$^{1)}$ These phenomena may arise from the difference of aggregation nature of chitosan macromolecules in these films. The yielding behavior of VF is caused by the elongation of the network structure made of chitosan fibers. The absence of yielding behavior shown in NVF is due to the poor net work structure of chitosan fibers.

Chitosan is widely investigated as a device for the sustained or controlled release of many kind of drugs.$^{15-19)}$ Drug release phenomena through chitosan films may significantly be affected by the difference of molecular orientation or anisotropic structure in VF and NVF. Permeation studies of several kinds of drugs through VF and NVF are now in progress.

Acknowledgments The authors are grateful to K. Sakai of Measuring Instrument Development DEPT of New Oji Paper Co., Ltd., for his generous support and valuable advice in the molecular orientation analysis and birefringence measurement of chitosan films. They are also grateful to H. Morita for assistance in the experimental work.

References

1) Ikeda H., Uchiyama K., Takayama K., Nagai T., *Chem. Pharm. Bull.*, **43**, 2211—2214 (1995).
2) Osaki S., *Convertech.*, **15**, 15—21 (1987).
3) Osaki S., *Convertech.*, **16**, 23—28 (1988).
4) Osaki S., Japan. Patent 61-083946 (1986).
5) Osaki S., *Convertech.*, **17**, 17—21 (1989).
6) Osaki S., *Convertech.*, **17**, 24—26 (1994).
7) Sakai K., *Plastics Age*, **40**, 152—156 (1994).
8) Sakurai K., Takagi M., Takahashi T., *Sen-i Gakkaishi*, **40**, 246—253 (1984).
9) Sakamoto K., *Kobunshi Ronbunshu*, **48**, 671—678 (1991).
10) Kim J. C., Ruslim C., Watanabe T., Miyata S., *Polymer Preprints Japan*, **44**, 1585—1586 (1995).
11) Sakamoto K., *Hyomen*, **32**, 28—37 (1994).
12) Fukuoka E., Makita M., Yamamura S., *Chem. Pharm. Bull.*, **39**, 3313—3317 (1991).
13) Cairns P., Miles M. J., Morris V. J., Ridout M. J., Brownsey G. J., Winter W. T., *Carbohydr. Res.*, **235**, 23—28 (1992).
14) Ogawa K., Yui T., Miya M., *Biosci. Biotech. Biochem.*, **56**, 858—862 (1992).
15) Kawashima Y., Lin S. Y., Kasai A., Handa T., Takenaka H., *Chem. Pharm. Bull.*, **33**, 2107 (1985).
16) Inoue K., Machida Y., Sannan T., Nagai T., *Drug Design Delivery*, **2**, 165—175 (1988).
17) Kanke M., Katayama H., Tsuzuki S., Kuramoto H., *Chem. Pharm. Bull.*, **37**, 523—525 (1989).
18) Chandy T., Sharma C. P., *Biomaterials*, **14**, 939—944 (1993).
19) Miyazaki S., Nakayama A., Oda M., Takada M., Attwood D., *Biol. Pharm. Bull.*, **17**, 745—747 (1994).