Chitosan Film Prepared on a Metal Plate Loaded with Electric Charge

Hiraku IKEDA,*[,a] Kazumi UCHIYAMA,[b] Kozo TAKAYAMA,[a] and Tsuneji NAGAI[a]

Department of Pharmaceutics[a] and Department of Pharmaceutical Analytical Chemistry,[b] Hoshi University, Ebara-2-4-41, Shinagawa-ku, Tokyo 142, Japan. Received May 15, 1995; accepted August 1, 1995

A novel chitosan film was prepared using an electric charge generated on a cast plate and was given characteristic physical properties. Two metal plates were adhered together with Teflon sheet between them. An acetic acid solution of chitosan was poured on the upper plate and a negative charge was supplied galvanostatistically on the metal plate. The solution was dried in order to make the film at room temperature. The cross sectional morphology and the physical properties of tensile strength, breaking elongation and work energy of breaking of the film were compared with those of film prepared without supplying electric potential. The film prepared with an electric charge (VF) was curled gently in water and then gradually flattened. The film prepared without charge (NVF) was curled tightly in water and maintained this shape for a long time. In the cross sectional structure of VF, a line pattern parallel to the film surface was observed microscopically, while a vertical pattern was seen in NVF. The presence of electron on the plate was confirmed by measuring the accumulated volume of negative potential using an oscilloscope connected to the metal plate.

Key words chitosan film; metal plate; electric potential; morphology; tensile strength

Chitin and its deacetylated product, chitosan, are known as biodegradable,[1] low toxic[2] and biocompatible polymers.[3,4] These polymers and their derivatives have certain pharmacological actions: an anti-infective,[5] an immunopotentiating,[6,7] and a wound healing acceleration effect.[8] Application of these polymers in medical fields has been made in surgical sutures[3] and artificial skin membranes for the treatment of full-thickness burn.[4] Chitosan was also applied as a device for the sustained or controlled release of many kind of drugs.[9-13]

In the present study, we investigated a novel method to prepare chitosan film using a newly developed apparatus in which an electric charge was supplied galvanostatistically on cast plates. Physical properties of the film were measured and compared with those of film prepared on a plate without supplying electric potential.

No macromolecular manipulation technique has yet been reported for chitosan film, but new pharmaceutical applications might be found if it were possible to control the film molecular orientation.

Experimental

Materials Chitosan, derived from crab shell chitin with about 95% deacetylation, was generously supplied by Dainichi Seika Color & Chemicals Mfg. Co., Ltd. The relative viscosity of a 0.2% chitosan aqueous solution (at pH 2.0) was 4.11 and 37 °C as determined with an Ubbelohde viscometer. The average molecular weight of chitosan was determined by using gel-permeation chromatography to be 4.4×10^5. Other chemicals used were of reagent grade. Stainless steel flat plate was purchased from Queen Rose Co., Ltd. Teflon sheet (0.5 mm thick) was purchased from Nichiasu Co., Ltd. The direct current power supplier was purchased from Sunhayato Co., Ltd.

Preparation of Chitosan Film An 0.8% chitosan solution was prepared. One (v/v)% acetic acid solution was used as a solvent. This solution was poured on the upper metal plate in the apparatus (Fig. 1a) and the solvent was naturally dried in the draft chamber at room temperature. Acetic acid and water remaining in the film were removed in a vacuum over 2 d: acetic acid remaining in the film after this procedure was less than 1%. Thickness of the film was measured with a micrometer and expressed as the average of three points.

Method of Loading Electric Potential on the Plate Two metal plates were adhered together with a Teflon sheet between them (Fig. 1b). The upper plate was connected to a (−) electrode and the lower one was connected to a (+) electrode of the battery (15.2 V) which supplies direct current. The direct current was supplied during the drying.

Microscopic Observation The chitosan film prepared as above was cut vertically with a razor. The cross section was covered with gold and examined by a scanning electron microscope (JEOL, SEM JSM-T200).

Measurement of Tensile Strength Mechanical strength of the chitosan film (1 × 3 cm) was measured with a tensiometer (Model 4204, Instron Co., Ltd.) The stress changes before breaking the film were monitored. Stretching and chart speeds were adjusted to 200 and 1000 mm/min, respectively. Results were represented as the mean ±S.D. for three determinations.

Confirmation of Electric Potential on the Plate A gadget including the circuit for switching and voltage-amplification was made to measure the volume of the electric potential on the plate as shown in Fig. 2. The cathode of the circuit was connected with anode of an oscilloscope (Digital Storagescope, Type DS-8600, Iwasaki Tsushin Co., Ltd.). Since the metal plates on either side of the Teflon sheet are believed to act as an electric condenser, the plate would be depolarized by turning on the switch in the circuit. Thus the decrease of voltage was monitored on the oscilloscope as a function of time. The volume of electric potential can be given as the following equation[14]:

$$\ln(V) = \ln(V_0) - t \times (R \times C)$$

where V is the voltage at time t, V_0 is the initial voltage, R is the resistance and C is the electric capacity of the metal plates.

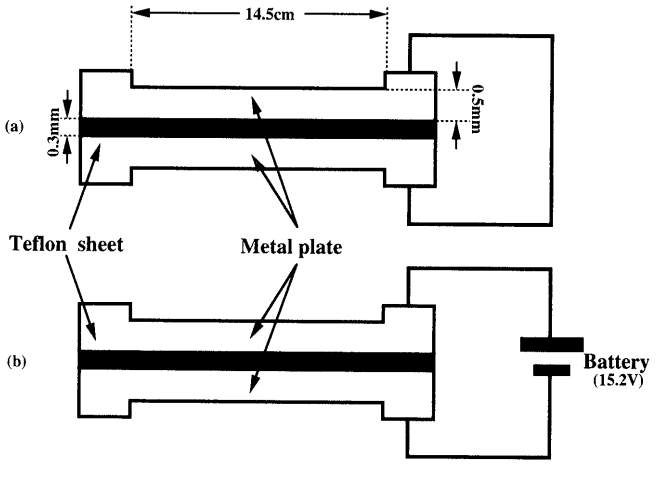

Fig. 1. Schematic Representation of Apparatus for Preparing Chitosan Film

(a) for NVF, (b) for VF.

* To whom correspondence should be addressed.

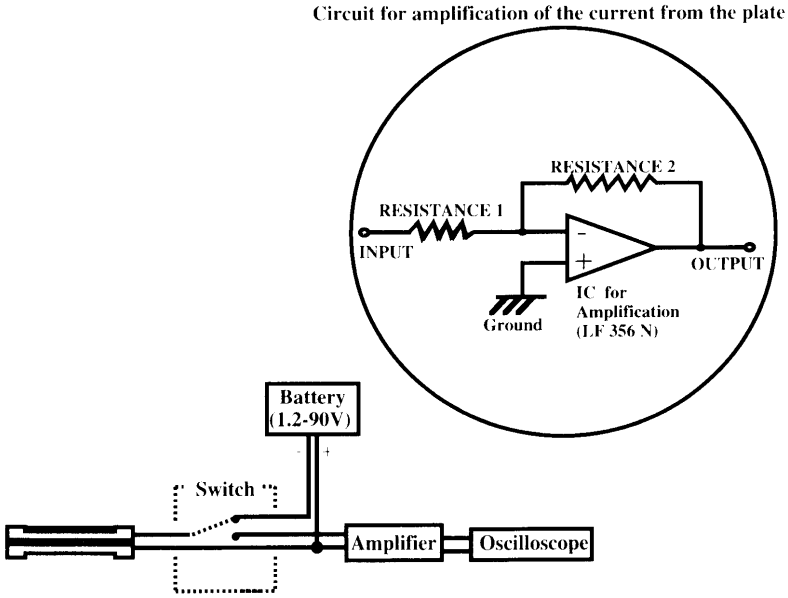

Fig. 2. Schematic Representation of Circuit for Measuring the Voltage from the Metal Plate

Results

Morphological Changes of Chitosan Film in Water Progress of morphological changes of the chitosan films with or without the electric charge were observed in water (Fig. 3).

The film prepared with the electric charge (VF) curled gently from various areas of the edge in water at the initial stage, and then gradually became flat. While the film prepared without charge (NVF) exhibited significantly different changes in water, curling tightly at 60 s and maintaining this shape even after 24 h. Figure 4 shows microphotographs of the cross sectional structure of VF and NVF. In VF, patterns parallel to the film surface were observed, while vertical ones were seen in NVF.

Mechanical Strength of VF and NVF Figure 5 shows the mechanical strength of VF and NVF determined by a tensiometer. The average thickness of VF and NVF was 0.17 and 0.20 mm, respectively with no significant difference between them. VF showed the break point after yielding by stretching, while NVF broke instantly without yielding. Based on the results observed above, the breaking stress, the elongation ratio and work of breaking were calculated. The breaking stress was almost the same between the two, however, the elongation and work of breaking of VF were significantly greater than those of NVF (Fig. 6).

Electric Charge Generated on the Cast Plate To confirm the existence of negative potential on the plate, a circuit for switching and a voltage amplifier were made as shown in Fig. 2. Using this gadget, the decrease of voltage in the plate was monitored after turning on the switch in the circuit. Semi-logarithmic plots of voltage against time showed a fairly good linear line (Fig. 7).

The electric capacity can be estimated from the slope of these plots. Since such results would be affected by the electron in the connecting code between the metal plate and the gadget, the electric capacity of the plate was estimated by subtracting the capacity in the code from that in the metal plate and the code. Results are sum-

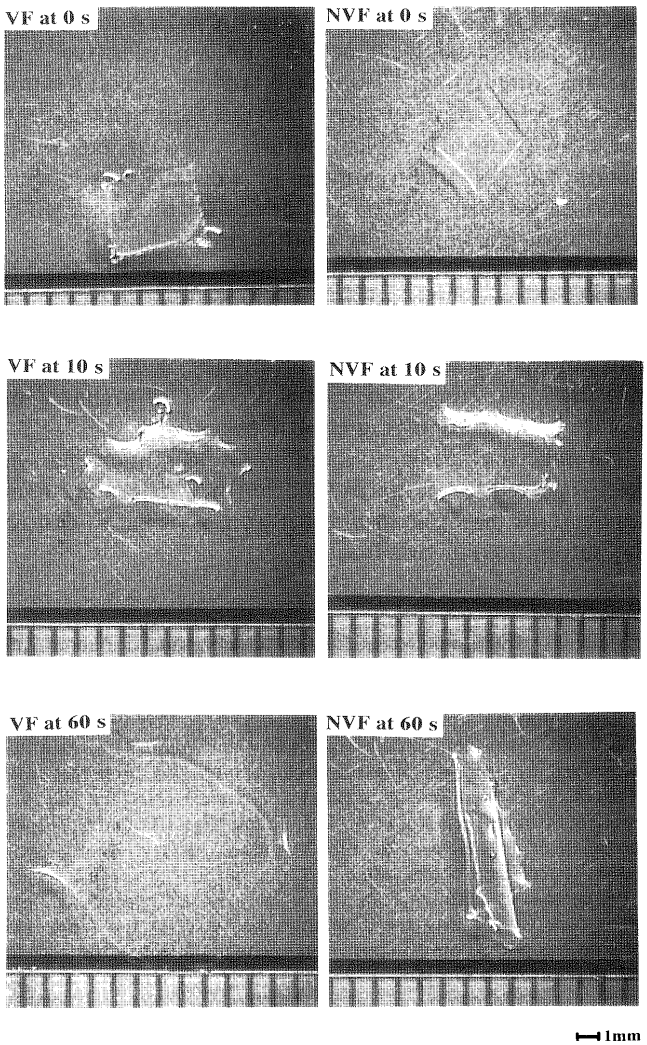

Fig. 3. Progress of Morphological Changes of Chitosan Film in Water

VF, prepared supplying an electric charge; NVF, prepared without supplying an electric charge.

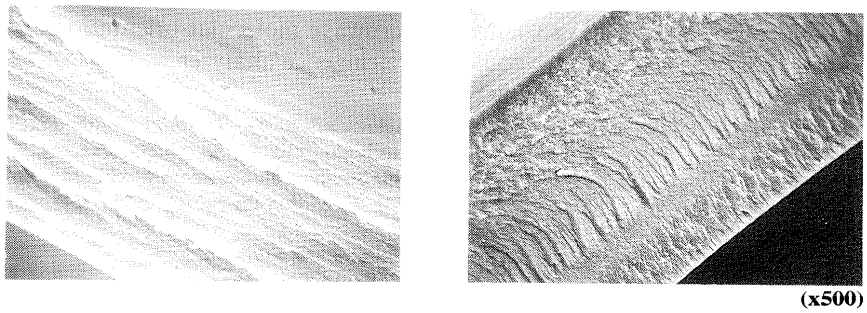

Fig. 4. Scanning Electron Micrographs of Cross Section of Chitosan Films

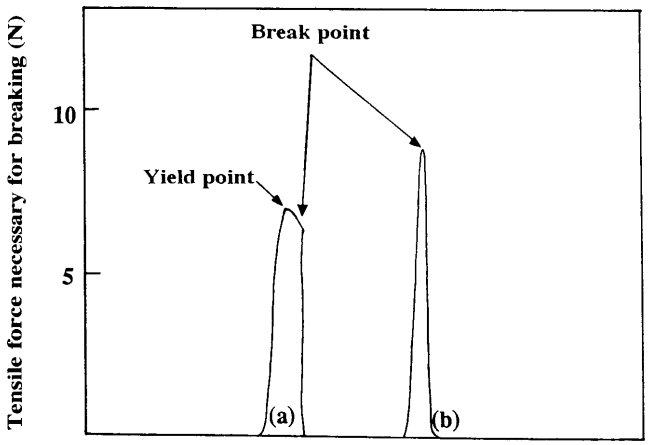

Fig. 5. Tensile Force Necessary for Breaking
Stretch speed: 200 mm/min, chart speed: 1000 mm/min. (a) VF, (b) NVF.

Table 1. Electric Capacity of the Metal Plate

Supplied voltage (V)	Capacity (nF)		
	Plate+code	Code	Plate
1.2	2.27	0.104	2.17
15.2	2.07	0.100	1.97
30.0	1.64	0.100	1.54
90.0	1.67	0.080	1.59

Each datum represents the mean of three determinations.

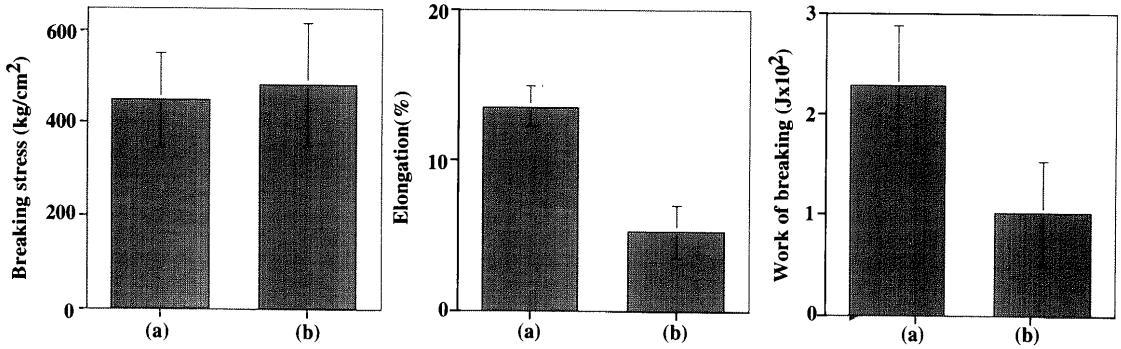

Fig. 6. The Breaking Stress (Left), Elongation (Middle) and Work of Breaking (Right) of Chitosan Films ($n=3$—4)
(a) VF, (b) NVF.

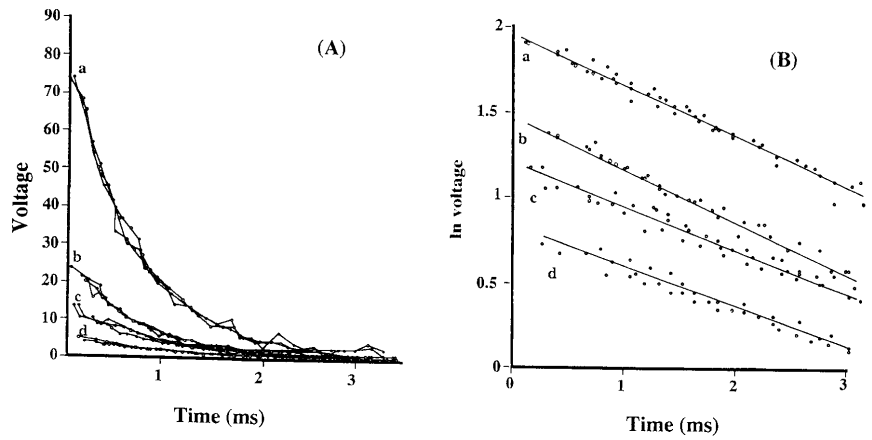

Fig. 7. Decreasing Voltage Curves after Disconnection of the Battery (A), and Relationship between ln Voltage and Time (B)
a, 90 (V); b, 30 (V); c, 15.2 (V); d, 1.2 (V).

marized in Table 1.

The electric capacity in the pair of plates was calculated to be 1.5—2.2 nF, suggesting that the electric charge could exist on the plate, because these values were extremely high compared with the value calculated from the dielectric constant of Teflon film.

Discussion

The differences in morphological change between VF and NVF in water may suggest that the aggregate nature of chitosan molecules or the density of upper and lower surface differ in these films. Such difference was directly confirmed by the microscopic observation of the film cross sectional structure (Fig. 4). The macromolecular orientation in NVF may be comparatively vertical to the film surface, while in VF chitosan molecules may be oriented parallel. This concept is also supported by the result obtained in the measurement of tensile strength. The breaking phenomena accompanying yielding in VF suggest that VF is broken by shearing, although tearing is the dominant factor when NVF is broken by stretching. In VF inter-macromolecular sliding is brought about during elongation[15]; this may cause the appearance of a yielding point. No yielding was obtained in NVF because chitosan molecules were mainly aligned vertical to the film surface.

We thus conclude that the above-described specific structure of chitosan film is primarily due to the electric charge generated on the metal plate. This charge may cause the parallel orientation of chitosan molecules to the plate because of the attraction force between the negative charge on the plate and cationized amino groups of chitosan molecules in the solution.

Much work is still required to understand the macromolecular orientation in chitosan film prepared on a metal plate loaded with an electric charge. Further investigations on this are now in progress, utilizing sophisticated analytical techniques of X-ray diffractometry, Fourier transformed infrared spectroscopy, a microwave molecular orientation analyzer and an optical birefringence analyzer.

Acknowledgments The authors are grateful to Dr. H. Okabe of Lintech Co., Ltd. for his generous support and valuable advice in the tensile strength measurement of chitosan films. They are also grateful to Mr. H. Morita for assistance in the experimental work.

References and Notes

1) Rieder S. V., Buchanan J. M., *J. Biol. Chem.*, **232**, 951 (1958).
2) Arai K., Koromomaki T., Fujita T., *Toukai-Suiken-Hou*, **56**, 89—94 (1986).
3) K. Kifune, "Medical Application of Chitin and Chitosan," Gihodo Shuppan, Tokyo, 1994, p. 163—178.
4) Ohshima Y., Nishino K., Yonekura Y., Maeda M., Horie J., Nonomura K., Kishimoto S., Wakabayashi S., Sotomatshu M., *Nessho*, **12**, 3 (1986).
5) Tokoro A., Kobayashi M., Tatewaki N., Suzuki K., Okawa Y., Mikami T., Suzuki S., Suzuki M., *Microbiol. Immunol.*, **33**, 357—367 (1989).
6) Nishimura K., Ishihara C., Ukei S., Tokura S., Azuma I., *Vaccine*, **4**, 151—156 (1986).
7) Peluso G., Petillo O., Ranieri M., Santin M., Ambrosio L., Calaboro D., Avallone B., Balsamo G., *Biomaterials*, **15**, 1215—1220 (1994).
8) Prudden J. F., Migel P., Hanson P., Friedrich L., Balassa L., *Am. J. Surg.*, **119**, 560 (1970).
9) Kawashima Y., Lin S. Y., Kasai A., Handa T., Takenaka H., *Chem. Pharm. Bull.*, **33**, 2107 (1985).
10) Inoue K., Machida Y., Sannan T., Nagai T., *Drug Design and Delivery*, **2**, 165—175 (1988).
11) Kanke M., Katayama H., Tsuzuki S., Kuramoto H., *Chem. Pharm. Bull.*, **37**, 523—525 (1989).
12) Chandy T., Sharma C. P., *Biomaterials*, **14**, 939—944 (1993).
13) Miyazaki S., Nakayama A., Oda M., Takada M., Attwood D., *Biol. Pharm. Bull.*, **17**, 745—747 (1994).
14) Purcell E. M., "Electricity and Magnetism, Berkeley Physics Course," Vol. 2, ed. by Kittel C., McGraw-Hill Book Co., New York, 1965. Authorized translation from the English-language edition, Maruzen, Tokyo, 1970, p. 168—171.
15) Kawaguchi T., "Structure and Physical Properties of Macromolecules," ed. by Saito N., Kawano H., Kadoto M., Maruzen, Tokyo, 1963, p. 213.