Application of Chitin and Chitosan to Pharmaceutical Preparations. I. Film Preparation and *in Vitro* Evaluation

Motoko KANKE,* Hirokazu KATAYAMA, Sumi TSUZUKI and Hiromi KURAMOTO

Fukuyama University, Faculty of Pharmacy and Pharmaceutical Sciences, 985 Higashimura-cho, Fukuyama, Hiroshima 729–02, Japan.
Received March 22, 1988

Three types of chitosan films containing prednisolone (PD) were prepared and drug release from the films was studied *in vitro*. The films prepared include 1) a monolayer type (ML) film prepared by evaporating the solvent from a chitosan/drug mixture, 2) a double layer type (DL) film prepared by sticking together two ML films, one of which contained a drug, and 3) N-Ac film which is a DL film with one of the ML films N-acetylated and stuck onto the other ML film, which contained a drug. Release of PD from ML films was retarded as the films became thicker. Release of the drug from N-Ac films was more depressed than from the corresponding DL film and was found to follow zero-order kinetics. Pores were observed in chitosan films by scanning electron microscopy. These results suggested that chitosan and N-acetyl chitosan (chitin) films could be applicable for controlled-release preparations of drugs.

Keywords chitosan; chitin; N-acetyl chitosan; film; controlled release; prednisolone; pore; cover film; SEM; diffusion-controlled

Chitin is one of polysaccharides widely distributed in nature as a principal component of shells of crustaceans and insects and of cell walls of bacteria and mushrooms. Chitin, β-(1—4)-poly-*N*-acetyl-D-glucosamine and chitosan, deacetylated chitin and various of their synthetic derivatives have recently drawn great interest from the standpoint of utilization of natural resources.

It has been reported that chitin and chitosan are biodegradable,[1] have extremely low toxicity, and are biocompatible. These polymers and their derivatives have some pharmacological effects including an anti-infective effect, an immunopotentiating effect, a wound-healing acceleration effect, *etc.*[2] Attempts have been made to apply them in medical fields in the forms of surgical sutures and artificial organ membranes.[3] Recently, the pharmaceutical application of chitosan and its derivatives has been attempted.[4] Release of indomethacin and papaverine hydrochloride from chitosan gels was reported to be more sustained than that from chitin gels.[4a]

In the present study, prednisolone (PD) was chosen as a model drug and 3 types of films containing the drug were prepared in order to investigate the applicability of chitosan and chitin to controlled-release dosage forms and to compare chitosan films with reproduced chitin films as regards drug release.

Experimental

Materials Chitosan (degree of deacetylation, 85%) was supplied by Katakura Tikkarin Co. PD was obtained from Wako Pure Chemical Industries, Ltd. All other chemicals were of the finest grade available.

Preparation of Films a) Monolayer Type (ML) Films: One percent chitosan was dissolved in 0.5% acetic acid. PD fine powder was dispersed in the chitosan solution by the use of an ultrasonic homogenizer (model UR-200P, Tomy Seiko Co., Ltd.) at 115 W for 2 min and the homogeneous suspension was poured into a Petri dish. The dish was kept at 50 °C for several hours in order to remove the dispersion medium, followed by standing at room temperature until no further decrease in film weight was detected. b) Double Layer Type (DL) Films: First, ML film without drug was prepared as described in the previous section (the film thus prepared is referred to as the cover film hereafter). Following the cover film formation, a mixture of PD and chitosan/acetic acid solution was poured onto the film. The former and the latter layers stuck together as the solvent was removed from the latter layer and formed a DL film. c) N-Acetyl DL (N-Ac) Films: The cover film of the DL film was N-acetylated with acetic anhydride in 75% methanol at room temperature for 30 min.[5] The N-acetylated film thus obtained was washed with distilled water and air-dried. Subsequently, the PD-containing layer was formed on top of the cover film by the same technique as described in section b. The film was dried under vacuum at 70 °C. Films used in this study are summarized in Table I.

Release Studies Film-pieces of known surface area (1 × 1 cm) and weight were fixed on a Teflon sheet with synthetic glue in order to expose one side of each film. In the cases of the DL and N-Ac films, the PD-containing layer faced and was stuck to the sheet in order to expose the cover film to the release medium. A film fixed on the sheet was placed in a 200 ml flask containing 100 ml of isotonic phosphate buffer (PB, pH 7.4) prewarmed at 37 °C, and shaken horizontally at a speed of 60 strokes/min in a shaker bath at 37 °C. Aliquots (0.1 ml) of the medium were removed at appropriate intervals and analyzed for drug content. The volume of the medium was maintained constant by adding a sufficient amount of the fresh medium to the container.

Quantitative Determination of Drugs in Films A film-piece of known surface area (1 × 1 cm) and weight was dissolved in 0.1% acetic acid and the amount of drug contained was determined. In the case of N-Ac films, the equilibrium concentrations obtained in the release profiles were adopted as total drug contents since N-Ac films were not dissolved in acetic acid. A Shimadzu LC-6A high performance liquid chromatograph equipped with a Shimadzu SPD-6AV UV-VIS spectrophotometric detector (set at 245 nm) and a Shim-pack CLC-ODS column (15 cm × 6.0 mm i.d.; 5 μm particles size) was employed to determine PD concentrations.

Water Absorption Capability of Films Film-pieces (1 × 1 cm) were immersed in PB (pH 7.4) and incubated at 37 °C. At predetermined times, the films were removed from the medium, blotted to remove excess water and weighed immediately.

Scanning Electron Microscopy (SEM) The films containing no drug were immersed in water, freeze-dried and observed under a scanning electron microscope, Hitachi Akashi ALPHA-25A type.

Results and Discussion

Appearance Change of Films in Release Medium Mean percentages of water absorbed by dry films were 88 and 128% for N-acetylated films and non-treated chitosan films, respectively. An equilibrium was reached within 30 min and no weight increase was observed thereafter up to 24 h. No area change of these films was noticed after immersion. In conclusion, non-treated films had a greater water absorption capacity than N-acetylated ones, while only a slight expansion or swelling was observed for both types of films in PB.

Effects of Film Thickness on PD Release The effect of film thickness on release of PD from ML films is shown in

Fig. 1a. The release of the drug was proportionally retarded as film thickness increased. A zero-order release was observed in the initial stage, particularly with ML-3 films up to 4 h. It is reported that the permeation of drugs through the chitosan membrane is controlled mainly by diffusion through pores.[6] Therefore, release data of this study were evaluated on the assumption that PD release from the films was diffusion-controlled. It has been reported that the diffusion-controlled release of a drug from a monolithic film into an infinite aqueous sink may be described by Eq. 1, when the drug solubility in the polymer is exceeded and the solid drug particles are distributed uniformly in the film and are small relative to the average diffusion distance[7];

$$M_t/M_\infty = (8 \cdot D_e \cdot C_s/C_0)^{1/2} \cdot 1/l \cdot (t)^{1/2} \quad \text{for} \quad C_0 \gg C_s \qquad (1)$$

where M_t is the cumulative drug amount released at time t, M_∞ is the total drug content, D_e is the effective diffusion coefficient, C_s is the drug solubility in the polymer, C_0 is the initial drug concentration and l is the film thickness (Table I). The plots of M_t/M_∞ *vs.* $(t)^{1/2}$ are shown in Fig. 1b; the two parameters correlate well. If C_s equals the saturation concentration of PD in PB at 37 °C (0.37 mg/ml), the D_e values can be calculated from Eq. 1 as follows: 7.83×10^{-8}, 5.53×10^{-8} and 2.16×10^{-8} cm^2/s for ML-1, ML-2 and ML-3, respectively. The decrease of D_e value with increase of film thickness might be attributed mainly to the change of film structure. The increase of chitosan amount might make the film structure denser. Therefore, the release of PD from the films, which contained the same amount of the drug, could be retarded by increasing the film thickness.

Effect of N-Acetylation of Cover Film on PD Release

Hirano *et al.* reported that low molecular-weight compounds (MW < 2900) could permeate through N-acetyl chitosan membranes and the permeabilities of the films prepared from the gels were larger than those in the case of chitosan membranes.[8] Miyazaki *et al.* reported that the release of indomethacin and papaverine hydrochloride from chitosan gel was more sustained than that from chitin gel of various thicknesses.[4a] In the present studies, the release of PD from N-acetylated films was compared with that from native chitosan ones. In order to avoid acetylation of a drug and drug release into the reaction medium, the film was N-acetylated and then stuck to a drug-containing film. The release profile from the N-Ac film

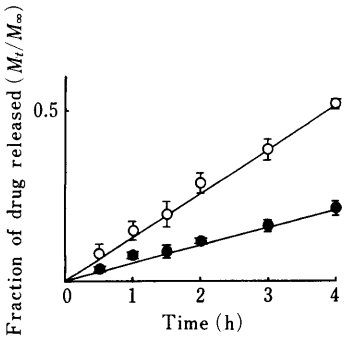

Fig. 2. Effect of N-Acetylation on the Release of Prednisolone from the DL Films

Each point and vertical bar represent the mean ± S.D. of 4 films. ○, DL (51 μm thickness of the cover film, calculated from DL and ML-1 thicknesses); ●, N-Ac (63 μm).

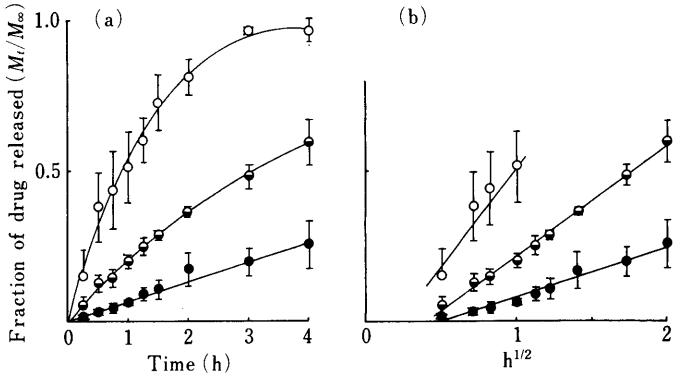

Fig. 1. Effect of ML Film Thickness on the Release of Prednisolone

Each point and vertical bar represent the mean ± S.D. of 4 films. ○, ML-1 (53 μm in thickness); ◒, ML-2 (114 μm); ●, ML-3 (194 μm).

TABLE I. Description of Chitosan/Drug Films

Film I.D.	Chitosan (g)		Thickness (μm)	Film wt.[a] (mg/cm^2)	PD content[b] (μg/mg film)	C_0[c] (mg/cm^3)
ML-1	0.2		53 ± 2	4.2 ± 0.3	100.5 ± 4.2	79.6
ML-2	0.6		114 ± 2	13.5 ± 1.0	31.4 ± 2.3	37.2
ML-3	1.0		194 ± 16	22.6 ± 1.6	18.4 ± 1.0	21.4
	I[d]	II[e]				
DL	0.4	0.2	104 ± 2	12.3 ± 1.3	34.6 ± 2.9	41.0
N-Ac	0.4	0.2	116 ± 4	11.3 ± 1.2	37.7 ± 1.7	36.7

a) Mean ± S.D. (n = 8—10). *b*) Mean ± S.D. (n = 4—6). *c*) Initial drug concentration; (PD content × film wt.)/thickness. *d*) The cover film. *e*) The PD-containing layer.

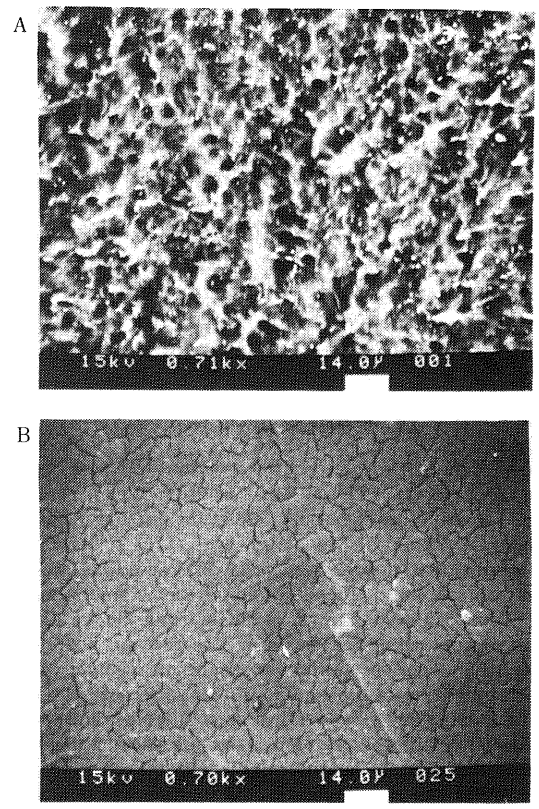

Fig. 3. Scanning Electron Micrographs of Film Surface

A, chitosan film; B, N-acetyl chitosan film.

is shown in Fig. 2, together with that from the corresponding DL film. It is clear from the results that N-acetylation of the films depressed the drug release. In other words, N-Ac films showed slower release than chitosan films for PD, contrary to Miyazaki *et al.*'s findings. The reason might be the difference in the origin of vehicle materials used and of preparation methods of gels and films, *i.e.*, the chitin films used in this study were prepared by N-acetylation of chitosan films, which might be denser than the gels prepared from chitin solution.[4a)]

SEM Scanning electron micrographs of the films prepared by freeze-drying of water-absorbed films are shown in Fig. 3. Pores were observed in chitosan films in the range of 3—4 μm in diameter, but not in N-acetyl chitosan films in this experiment. Pores of N-acetyl chitosan films, if there were any, must be much smaller than those of non-treated chitosan films and could not be detected under the present experimental conditions. Therefore, it was assumed that the sustained release of PD from the N-Ac films was probably due to their extremely small pore sizes.

In conclusion, preparation of chitosan films containing PD was established. Drug release from the films might be diffusion-controlled, and drug release could be controlled by changing the thickness of the films or by the use of an N-acetylated cover film. Following N-acetyl treatment of chitosan films, the release occurred more slowly than from the corresponding DL films, probably due to the presence of extremely small or no pores. The chitosan and chitin films, therefore, could be applicable as controlled-release vehicles for various pharmaceutical agents.

References

1) S. Tokura, "Last Biomass—Chitin, Chitosan," ed. by M. Yabuki, Gihoudou Shuppan, Tokyo, 1988, pp. 61—62.
2) M. Suzuki, Y. Ōkawa, K. Hashimoto, Y. Ōkura and S. Suzuki, Japan. Patent 27827 (1984) [*Chem. Abstr.*, **100**, 180129*p* (1984)]; K. Nishimura, S. Nishimura, N. Nishi, I. Saiki, S. Tokura and I. Azuma, *Vaccine*, **2**, 93 (1984); L. L. Balassa, Ger. Offen. 1906155 (1969) [*Chem. Abstr.*, **72**, 47372*z* (1970)].
3) S. Hirano, *Kagaku Kyouiku*, **34**, 290 (1986); S. Mima, S. Yoshikawa and M. Miya, Japan. Patent 130870 (1975) [*Chem. Abstr.*, **84**, 75239*c* (1976)].
4) *a*) S. Miyazaki, K. Ishii and T. Nadai, *Chem. Pharm. Bull.*, **29**, 3067 (1981); *b*) Y. Sawayanagi, N. Nambu and T. Nagai, *ibid.*, **30**, 2935 (1982); *c*) Y. Kawashima, S. Y. Lin, A. Kasai, T. Handa and H. Takenaka, *ibid.*, **33**, 2107 (1985).
5) M. Miya, R. Iwamoto, K. Ōta and S. Mima, *Kōbunshi Ronbunshu*, **42**, 181 (1985).
6) Y. Sawayanagi, N. Nambu and T. Nagai, *Chem. Pharm. Bull.*, **30**, 3297 (1982).
7) C. G. Pitt, M. M. Gratzl, A. R. Jeffcoat, R. Zweidinger and A. Schindler, *J. Pharm. Sci.*, **68**, 1534 (1979).
8) S. Hirano, K. Tobetto, M. Hasegawa and N. Matsuda, *J. Biomed. Mater. Res.*, **14**, 477 (1980).