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Sustained Release of Indomethacin from Chitosan Granules[1)]

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The use of chitosan granules as a means to achieve sustained release of indomethacin was examined. In contrast with the rapid dissolution of indomethacin in powdered form, sustained but almost complete release from chitosan granules was observed. A unique characteristic of the chitosan granules was that they gradually swelled and floated on the acid medium at pH 1.2. This floating property of the granules on the acid medium can be applied to the formulation of sustained-release preparations of various drugs.

The effect of cross-linking procedure on the drug release patterns from chitosan granules was also examined. The release rate of the drug from the granules could be controlled by varying the cross-linking procedure. Thus, chitosan might be useful as a vehicle for a sustained-release preparation of indomethacin in granular form.

Keywords——chitosan; cross-linked chitosan; drug vehicle; sustained release; granule; indomethacin

The possible use of chitosan as a new vehicle for sustained-release preparations has been examined.[3)] Chitosan is a natural polysaccharide prepared from chitin of crabs and lobsters by *N*-deacetylation with alkali.

Chitosan has been reported to have some useful medical and pharmaceutical applications.[4)] For example, chitosan membranes have been proposed as an artificial kidney membrane because of their suitable permeability and high tensile strength.[5)] Chitosan also has some pharmacological activities such as antacid and antiulcer activities[6)] and hypocholesterolemic activity,[7)] and it causes a depression of cholesterol and fatty acid absorptions.[8)] It was also reported that chitosan selectively aggregated L1210 leukemia cells and inhibited the transplantability of the cells.[9)] Despite these biomedical applications, there are still few reports concerning the utilization of chitosan in the pharmaceutical field. Recently, Sawayanagi *et al.* reported the usefulness of chitosan as a vehicle for direct compressed tablets[10)] and ground mixtures[11)] of drugs. Since chitosan has good biocompatibility and is inexpensive, it might be suitable for use in the preparation of dosage forms of commercial drugs.

Indomethacin has been widely used as a non-steroidal drug having anti-inflammatory and anti-pyretic effects.[12)] However, when given by the usual oral administration route, indomethacin may produce gastrointestinal side effects. In order to minimize its side effects on the gastrointestinal tract and to provide an effective blood level for a reasonably long time, indomethacin has been formulated as sustained-release preparations (*e.g.*, Spansule).

The purpose of this study was to develop sustained-release granules of indomethacin as a part of our studies on the pharmaceutical application of chitosan.

Experimental

Materials——Chitosan, Flonac N®, was kindly supplied by Kyowa Yushi Co., Tokyo, and used after being

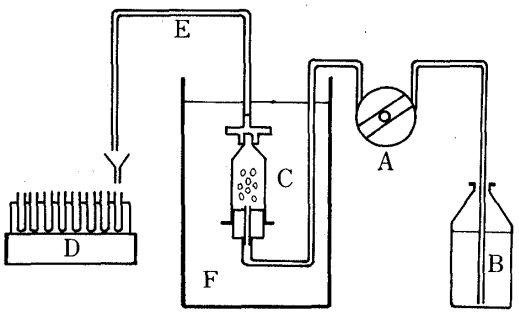

Fig. 1. Apparatus for Determination of Drug Release from Chitosan Granules
See the text for letter identifications.

passed through a 40 mesh screen. Indomethacin was obtained from Sigma Chemical Co., St. Louis. Glutaraldehyde was a 25% aqueous product (Wako Pure Chemical Co., Osaka).

Preparation of Chitosan Granules——Dried chitosan granules, in which indomethacin was dispersed in the form of fine crystals, were prepared by the following procedures. Indomethacin (0.5 g) was dissolved in 5 ml of methanol at 60 °C and chitosan (0.5 g) was added to the drug solution. After evaporation of the solvent at 60 °C, the residue was dissolved in 10% acetic acid (5 ml). The gelatinous chitosan–drug mixture was allowed to stand at room temperature for 1 h, then sucked into a glass syringe, and extruded onto a glass plate. After drying for 1 h at room temperature, the chitosan gel cord was cut into pieces and dried for an additional 8 h at 50 °C *in vacuo*. The final chitosan granules were 0.5—0.7 mm in diameter and 1—3 mm in length, and contained 50% drug by weight.

Preparation of Cross-Linked Chitosan Granules——The gelatinous chitosan–drug mixture was prepared as mentioned above, and allowed to stand at room temperature for 1 h, then 4.8% glutaraldehyde solution in water (0.525 ml) was added.[13] Dried, cross-linked chitosan granules were produced by the same granulation method as mentioned above. The product was washed with distilled water to remove glutaraldehyde and dried *in vacuo* at 50 °C for 8 h. The final cross-linked chitosan granules were 0.5—0.7 mm in diameter and 1—2 mm in length, and contained 50% drug by weight.

Release Study by the Open Elution Method——Apparatus: An appratus to provide a simple and reproducible method of determining the rate of drug release from granules was constructed from apparatus that should be readily available in most laboratories. A schematic diagram of the release test apparatus used in the present study is shown in Fig. 1. It consisted of a micro-tube pump (A, MP-3A Tokyo Rikakikai Co.), a reservoir (B) for the release medium, a release cell (C) constructed from the cylinder of a 5 ml plastic syringe and a membrane filter holder (Millipore catalog number SX-25) with a 0.45 μm membrane filter, a fraction collector (D, SF-160K, Tokyo Seisakusho Co.), sufficient lengths of silicon tubing (E, 1.15 mm i.d., 3 mm o.d.) to connect the components, and a water bath (F) in which the release cell and silicon tubing are immersed. With this appearatus, release medium continuously flowing over the granules could be collected by a fraction collector.

General Procedure: The release medium was poured into the reservoir and the pump was primed by allowing release fluid to flow through the pump into the release cell. This procedure expelled air from the pump and tubing. A granule sample (20 mg) was placed in the release cell. The cell was then immersed in a water bath equilibrated at 37 °C, and as much connecting tubing as possible was also placed in the bath. As soon as the cell had reached the bath temperature, the pump was turned on and regulated to the desired flow rate. The effluent was continuously collected in test tubes placed in a fraction collector at specified time intervals and assayed for drug content by a spectrophotometric procedure.

Simulated intestinal fluid (USP) at pH 7.5 was used as a release medium. In certain studies, granules in a net were immersed in 100 ml of simulated gastric fluid (USP), pH 1.2, at 37 °C for 2 h in a beaker, then removed and placed in the release cell with simulated intestinal fluid (USP) at pH 7.5.

A JP X dissolution test apparatus (method I) was used for comparison. All tests were made at a temperature of 37 °C.

Results

Application of the Open Elution Method for Measuring Drug Release from the Granules

Preliminary release determinations were performed on chitosan granules in an attempt to ascertain the usefulness of the open elution method for evaluating sustained-release dosage forms.

The release patterns of indomethacin from the chitosan granules in simulated intestinal fluid (USP), pH 7.5, at flow rates of 50 and 100 ml/h are shown in Fig. 2. Standard deviations of percent released at a flow rate of 100 ml/h ($n=6$) were within 10% of the respective means,

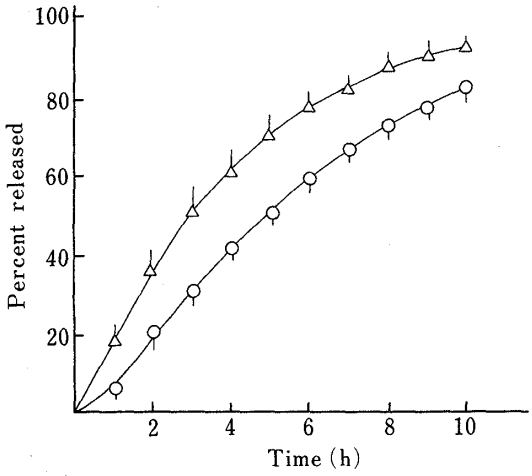

Fig. 2. Release Pattern of Indomethacin from Chitosan Granules in the Release Medium, pH 7.5, at Flow Rates of 50 (○) and 100 (△) ml/h

Each value represents the mean±S.D. of 3 (50 ml/h) or 6 (100 ml/h) experiments.

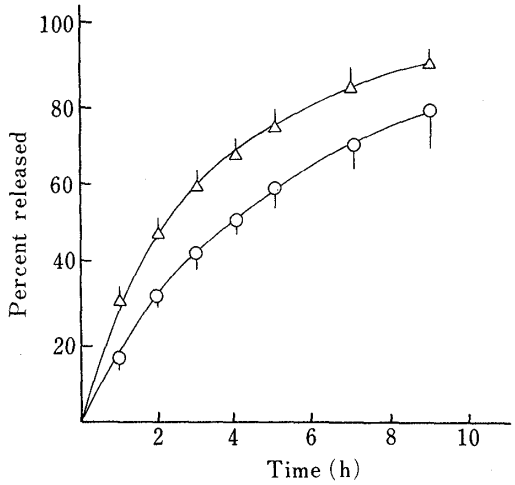

Fig. 3. Release Pattern of Indomethacin from Chitosan Granules in the Release Medium, pH 7.5, at Agitation Speeds of 50 (○) and 100 (△) rpm, as Determined by the JP X Rotating Basket Method

Each value represents the mean±S.D. of 3 (50 rpm) or 6 (100 rpm) experiments.

except during the early release period (in which the standard deviations were within 20%). Thus, the method of measurement was found to give reproducible results.

Further, the drug release profile from chitosan containing indomethacin was compared with that obtained by the JP X dissolution test (method I) (Fig. 3). Both types of *in vitro* elution systems gave similar release patterns and similar magnitudes of release rates. However, no quantitative comparison of the two should be made, since different experimental conditions were used. When these granules were tested by the open elution method (Fig. 3) the total drug release after 9 h was 78.7 (50 ml/h) or 91.2% (100 ml/h); in the JP X dissolution test, the value was 78.6 (50 rpm) or 90.5% (100 rpm).

These findings suggest that the open elution method is useful for drug release determinations of sustained-release granules. The apparatus involved in this test is standard equipment in most laboratories, and sample collection is simple. There are also other advantages with this system.[14] However, the present experiments were performed primarily to test the usefulness of this apparatus and method. More complete and detailed studies aimed at fully evaluating the method are in progress.

Release of Indomethacin from Chitosan Granules

Release profiles as well as dissolution profiles of indomethancin in the release medium at pH 7.5 are shown in Fig. 4. In contrast with the rapid dissolution of indomethancin in powdered form, sustained but almost complete release from the chitosan granules was observed. Chitosan gels were thus demonstrated to serve as barriers to the liberation of indomethacin. Similar observations have been made earlier in studies on drug release from a film-type dosage form.[3]

Granule samples were kept in the acid medium at pH 1.2 for 2 h in the beaker and transferred to the release cell; the results are also shown in Fig. 4. The release rate of the drug from the chitosan granules in the release medium at pH 7.5 after exposure to the acid medium at pH 1.2 was larger than that in the release medium at pH 7.5 alone. This faster release was attributed to the extent of swelling and gel-layer formation, which are significantly dependent on the pH of the medium. Chitosan granules have greater swelling and gel layer forming abilities at low pH than at high pH. The sizes of the chitosan granules before and after

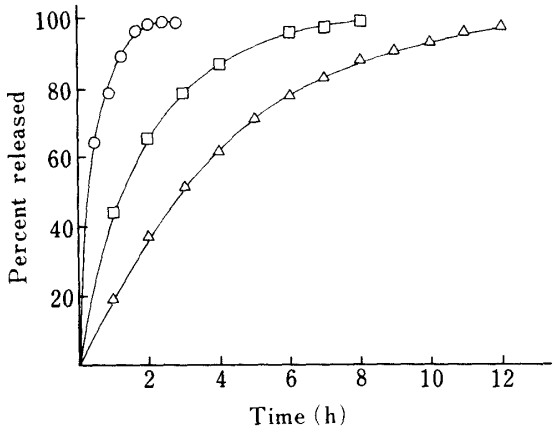

Fig. 4. Release Pattern of Indomethacin from Chitosan Granules (△) and Dissolution Pattern of the Drug Powder (○) in Release Medium, pH 7.5, and Release Pattern of Indomethacin from Chitosan Granules in Release Medium, pH 7.5, after Exposure to the Acid Medium, pH 1.2, for 2 h (□) at a Flow Rate of 100 ml/h

Each value represents the mean of 3—6 experiments.

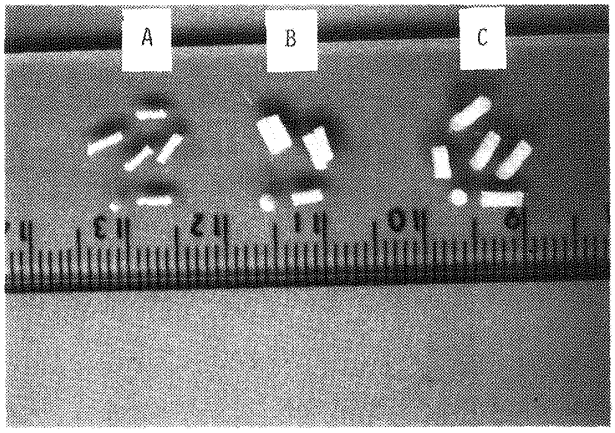

Fig. 5. The Sizes of Chitosan Granules before Release (A), and after Release for >10 h (Complete Drug Release) in the Release Medium at pH 7.5 (B) and in the Release Medium at pH 7.5 after Exposure to the Acid Medium at pH 1.2 for 2 h (C)

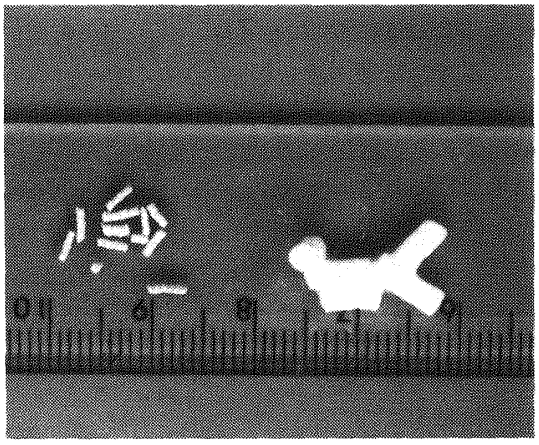

Fig. 6. The Sizes of Chitosan Granules before (Left) and after (Right) Exposure to the Acid Medium at pH 1.2 for 1 h

swelling are shown in Fig. 5. Chitosan granules were significantly swollen by exposure to the acid medium (Fig. 6). Increased swelling at the low pH would facilitate the movement of drug molecules out of the chitosan granules.

Release of Indomethacin from Cross-Linked Chitosan Granules

The release pattern of indomethacin from the cross-linked chitosan granules is compared with the dissolution of the drug powder in the release medium at pH 7.5 in Fig. 7. Sustained release was obtained from the cross-linked chitosan granules, whereas the drug powder dissolved completely within 2 h.

The release profile of the drug from the cross-linked chitosan granules in the release medium at pH 7.5 was not very different from that in the release medium at pH 7.5 after exposure to the acid medium at pH 1.2. Therefore no sudden change in the release pattern is expected to occur during the passage of chitosan granules through the gastrointestinal tract.

Cross-linked chitosan granules have rather weak swelling and gel-layer forming properties even at low pH. The sizes of the granules before and after swelling are shown in Fig. 8. The difference in size after swelling in the acid medium was not significant (Fig. 9). This observation suggests that the cross-linking procedure gives a more sustained release in the release medium because of the denser gel structure after the cross-linking process. Aldehyde is

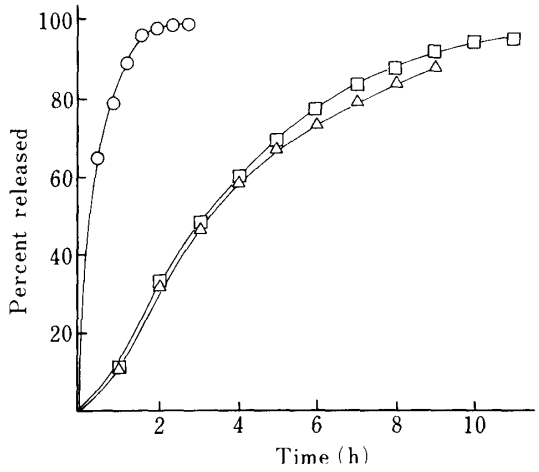

Fig. 7. Release Pattern of Indomethacin from Cross-Linked Chitosan Granules (△), Dissolution Pattern of the Drug Powder (○) in Release Medium, pH 7.5, and Release Pattern of Indomethacin from Cross-Linked Chitosan Granules in Release Medium, pH 7.5, after Exposure to the Acid Medium, pH 1.2, for 2 h (□) at a Flow Rate of 100 ml/h

Each value represents the mean of 3—6 experiments.

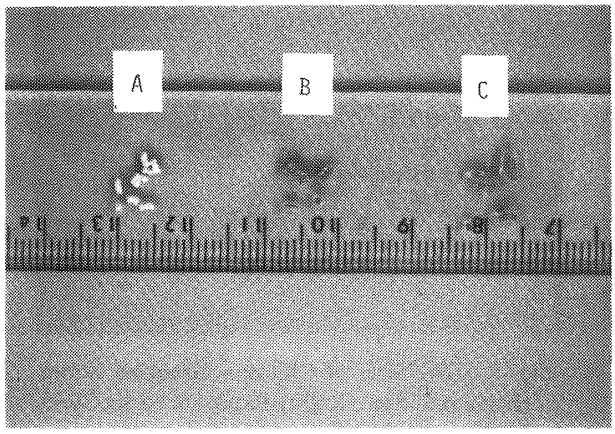

Fig. 8. The Sizes of Cross-Linked Chitosan Granules before Release (A), and after Release for >10 h (Complete Drug Release) in the Release Medium at pH 7.5 (B) and in the Release Medium at pH 7.5 after Exposure to the Acid Medium at pH 1.2 for 2 h (C)

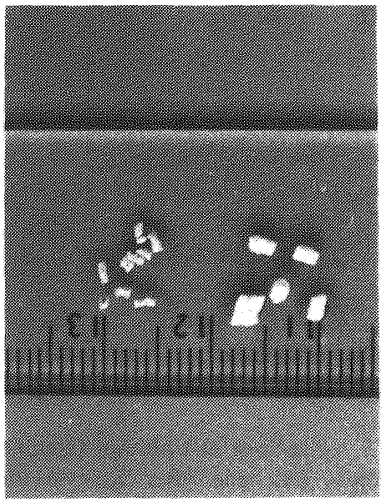

Fig. 9. The Sizes of Cross-Linked Chitosan Granules before (Left) and after (Right) Exposure to the Acid Medium at pH 1.2 for 1 h

known as a reagent for cross-linking and Schiff's base formation. Therefore, a part of the hydroxyl and amino groups of chitosan is presumably involved.[13]

Discussion

The present results indicate that chitosan is useful for the preparation of granules which exhibit sustained release of drugs. One major advantage of chitosan from a commercial point of view is its low cost, since commercial sustained-release preparations are more expensive than ordinary dosage forms. Another advantage of chitosan granules over conventional sustained-release formulations is the complete release of the drug from the granules, since some commercial sustained-release preparations have been reported to fail to release all of the drug in the preparation.[15]

A unique advantage of the chitosan granules is that they gradually swelled and floated on the acid medium at pH 1.2, as shown in Fig. 10. Many pharmaceutical techniques have been developed to achieve sustained release of drugs, and recently, a sustained-release orally administered product was achieved by using a formulation which floats on the gastric

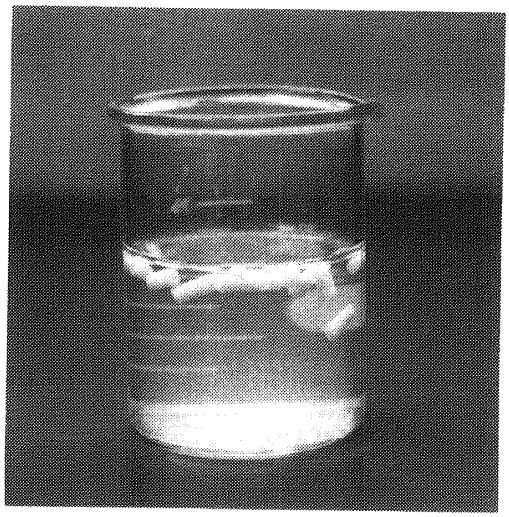

Fig. 10. Floating Behavior of Chitosan Granules in the Acid Medium at pH 1.2 after 1 h

medium.[16] The ability of the chitosan granules to float on acid medium may permit extensive use of chitosan in the formulation of sustained-release preparations of various drugs.

Furthermore, chitosan granules have a gel-layer forming property in the low pH range, and chitosan has antacid and antiulcer activities.[6] These characteristics may be useful for preventing drug irritation in the stomach, since the clinical usefulness of indomethacin is severely restricted by gastrointestinal side effects. Most gel-forming polymers examined for sustained-release preparations, such as pectin,[17] konjac,[18] and polyacrylic acid,[19] form gels or viscous solution at high pH.

The significant difference between the dissolution rates of indomethacin from powders and the release rates from chitosan granules (Fig. 4) or cross-linked chitosan granules (Fig. 7) suggested that the drug absorption following oral administration of the granules may be prolonged without adverse effects on the stomach. An *in vivo* study on indomethacin release after oral administration of a capsulated product containing the granules is in progress.

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References and Notes

1) Pharmaceutical Application of Biomedical Polymers, Part XVI. Part XV: S. Miyazaki, W.-M. Hou, and M. Takada, *Chem. Pharm. Bull.*, **33**, 428 (1985).
2) Present address: *Department of Dosage Form, Shanghai Institute of Pharmaceutical Industry, 1320 Beijing Road West, Shanghai, China.*
3) S. Miyazaki, K. Ishii, and T. Nadai, *Chem. Pharm. Bull.*, **29**, 3067 (1981).
4) R. A. A. Muzzarelli, "Chitin," Pergamon Press, Oxford, 1977, pp. 255—265.
5) S. Mima, S. Yoshikawa, and M. Mima, Japan. Patent 130870 (1975) [*Chem. Abstr.*, **84**, 75239 (1975)].
6) I. W. Hillyard, J. Doczi, and P. B. Kiernan, *Proc. Soc. Exp. Biol. Med.*, **115**, 1108 (1964).
7) M. Sugano, T. Fujikawa, Y. Hiratsuji, K. Nakashima, N. Fukuda, and Y. Hasegawa, *Am. J. Clin. Nutr.*, **33**, 787 (1980).
8) G. V. Vahouny, S. Satchithanandam, M. M. Cassidy, F. B. Lightfoot, and I. Furda, *Am. J. Clin. Nutr.*, **38**, 278 (1983).
9) A. E. Sirica and R. J. Woodman, *J. Natl. Cancer Inst.*, **47**, 377 (1971).
10) Y. Sawayanagi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **30**, 2982 (1982).
11) Y. Sawayanagi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **30**, 4464 (1982).
12) L. Helleberg, *Clin. Pharmacokinet.*, **6**, 245 (1981).
13) S. Hirano, R. Yamaguchi, N. Matsuda, O. Miura, and Y. Kondo, *Agric. Biol. Chem.*, **41**, 1547 (1977).
14) P. L. Gould, *Br. Polym. J.*, **15**, 172 (1983).

15) L. E. Hollister, S. H. Curry, J. E. Derr, and S. L. Kanter, *Clin. Pharmacol. Ther.*, **11**, 49 (1970).
16) J. C. Johnson (ed.), "Sustained Release Medications," Noyes Data Co., Park Ridge, 1980, pp. 199—230.
17) Y. Takahashi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **26**, 3836 (1978).
18) M. Nakano, K. Takikawa, and T. Arita, *Membrane*, **3**, 141 (1978).
19) K. Morimoto, I. Hama, Y. Nakamoto, T. Takeeda, E. Hirano, and K. Morisaka, *J. Pharmacobio-Dyn.*, **3**, 24 (1980).