June, 1993] © 1993 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 66, 1807—1812(1993) 1807

Molecular Weight Dependence of the Properties of Chitosan and Chitosan Hydrogel for Use in Sustained-Release Drug

Naoji KUBOTA
Department of Applied Chemistry, Faculty of Engineering, Oita University, Dannoharu, Oita 870-11

(Received December 17, 1992)

Chitosan was treated with $NaBO_3$ under a homogeneous condition in which it was previously dissolved in an aqueous acetic acid solution. Different molecular weight chitosan samples were obtained, depending upon the reaction conditions, $NaBO_3$ concentration, reaction temperature, and reaction time. The performance of chitosan hydrogel for use in a sustained-release drug was investigated while focusing on the swelling properties of chitosan coated on a drug. Theophylline was used as a model drug to be released. Theophylline tablets were coated with respective chitosan samples, and then tested for the drug release rate. Each drug release obeyed zero-order or near zero-order kinetics, due to a reservoir system comprising a saturated theophylline solution and a chitosan hydrogel wall. The release rate of theophylline decreased with increasing the amount of coated chitosan, which affects the permeability of the drug, and increased with decreasing the molecular weight of coated chitosan, which affects the degree of hydration of the gel. A lag time effect was observed when higher molecular weight chitosan was coated. These experimental results were interpreted in terms of the degree of hydration of chitosan hydrogel and Fick's law of diffusion.

Chitin (poly-$\beta(1\rightarrow4)$-*N*-acetyl-D-glucosamine) has been regarded as being a potential resource, since it is a useful aminopolysaccharide analogous to cellulose structurally, and is naturally abundant, especially in the cuticle of marine crustacean. Deacetylation of chitin by alkaline hydrolysis yields chitosan, which is one of the few natural cationic polyelectrolytes. Chitin, chitosan, and their derivatives have found a wide variety of applications in both industrial and medical fields.[1,2] In particular, they are suitable for biomedical and pharmaceutical applications, since chitin and chitosan have bio-compatibilities and bio-degradabilities.[3—6] Furthermore, the control of drug delivery has recently been of interest in basic and applied fields as a new approach to achieve improved drug therapies.[7—9] The author has previously reported on the transport of alkali metal ions through membranes made of chitosan derivatives,[10—12] and also reported on membranes that change the permeability of ions and low molecular weight molecules according to stimulation, such as pH and redox reagents.[13—15]

In the present study chitosan was adopted as a coating material for drugs on account of its excellent biocompatibilities, membrane-forming abilities, and water permeabilities. Chitosan was degraded by a treatment with $NaBO_3$, and each degradation product was characterized prior to the drug release experiment. The effects of the molecular weight, amount, and acetylation of coated chitosan upon the sustained-release of theophylline were investigated. Further, the drug release rate could be explained in terms of the degree of hydration of chitosan hydrogel and Fick's law of diffusion.

Experimental

Materials. Chitosan was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo and used after purification (M_η: 1.7×10^6; degree of acetylation: 14%). Methanol, acetone, acetic anhydride, and pyridine were distilled just before use. The other reagents were used as received.

Degradation of Chitosan. Purified chitosan (1.5 g) was dissolved in 5% aqueous acetic acid (60 cm^3) in a round-bottom flask; aqueous $NaBO_3$ solution in a predetermined concentration (100 cm^3) was then added. After stirring for a given time and at a given temperature, the reaction mixture was cooled and 1 $mol\,dm^{-3}$ KOH solution was added up to pH 10—11. The obtained precipitate was centrifuged, and washed well with a water–methanol (1 : 1) mixture. Finally, it was washed with acetone and dried under vacuum at room temperature.

Characterization of Chitosan. The molecular weight was determined by viscometry,[16] and the degree of acetylation was determined by spectrophotometry.[17] Each chitosan sample with a different molecular weight was partially acetylated and its water-solubility was tested according to a method reported by Kurita et al.[18] IR spectra were recorded on a Hitachi 270-50 spectrophotometer.

Preparation of Theophylline Tablet Coated with Chitosan. Two w/v% solutions of untreated chitosan, chitosan treated with 0.01 w/v% $NaBO_3$ at 30 °C, and chitosan treated with 0.01 w/v% $NaBO_3$ at 50 °C were made by dissolution in 5% acetic acid solutions under agitation. The resulting solutions were allowed to filter with a sintered glass filter. The theophylline tablets, which were made from the theophylline powder (10 mg) in a pellet molding apparatus (Hitachi PD-2, 2.5 ϕ) under a vacuum at 0.6 ton (ram area: 33.18 cm^2), were dipped into these chitosan solutions and coated. The theophylline tablets coated with chitosan were dried at 30 °C. After washing with acetone, they were further dried under vacuum at 40 °C for 1 d. The constancy of the thickness of coated chitosan was checked by measuring the dimensions. The acetylation of chitosan coated on a theophylline tablet was attempted with acetic anhydride in acetone.

Measurement of Drug Release Rate. Drug release experiments were carried out by the rotating-basket method in 400 cm^3 of a phosphate buffer solution (pH 7.4) containing 0.9 w/v% NaCl at 37 °C. To monitor the theophylline released at the desired intervals a programmed measurement system was used which comprised a spectrophotometer (Hitachi U-2000) connected to a flow cell and a computer (Fig. 1). The theophylline concentration was determined based on the absorbance at 272 nm. After measuring the release rate, hollow, cylindrical chitosan hydrogel that remained intact was recovered. It was weighed, dried under vacuum at 40 °C, and weighed again; these weights were used to determine the degree of hydration.

Determination of the Diffusion Coefficient of Theophylline. A 2 w/v% solution of chitosan was made by dissolving in a 5% aqueous acetic acid. The resulting solution was poured onto a clean, flat PTFE plate. The poured chitosan solution and plate were put in an incubator thermostated at 30 °C. The obtained chitosan membrane was washed with acetone and dried under vacuum at 40 °C for 1 d. The permeation of theophylline was measured at 37 °C using a diffusion cell with two chambers. Each chamber had a volume of 25.0 cm^3 and an effective permeation area of 4.0 cm^2. Both chamber solutions were well-stirred. At proper intervals, the absorbance at 272 nm of the donor and receiver side solutions were measured and the permeability coefficient was evaluated from Fick's first law of diffusion.[15)] The diffusion coefficient was determined by dividing the permeability coefficient by the degree of hydration.

Results and Discussion

Chitosan was adopted as a coating material for drugs by considering its excellent bio-compatibilities, membrane-forming abilities, and water permeabilities. Because raw chitosan contained impurities, it was purified three times and characterized with regard to the molecular weight and degree of acetylation. As a result, the viscosity-average molecular weight was 1.7×10^6, and the degree of acetylation was 14%.

A number of reports concerning the degradation of chitosan are available; $NaBO_3$ was used in this study.[19)] However, degradation was not performed smoothly under the heterogeneous condition in which pulverized chitosan was only suspended in an aqueous $NaBO_3$ solution. It was then conducted under a homogeneous condition using aqueous acetic acid in which chitosan had been previously dissolved. Under this homogeneous condition, the chitosan main chain is thought to be cleaved randomly. Table 1 shows the dependence of the molecular weight of the degradation products on the reaction conditions, along with the degree of acetylation and water-solubility of partially acetylated derivatives at pH 8.9. Chitosan was degraded ranging in molecular weight from 1.2×10^6 to 2.4×10^4. It is strongly dependent on the $NaBO_3$ concentration, reaction temperature, and reaction time. The IR spectra of the degradation products are similar to that of untreated chitosan, except for absorption at 1710 cm^{-1}, which is based on the carboxyl group, as shown in Fig. 2. The absorption at 1710 cm^{-1} is clearly observed in the IR spectrum of a highly degraded product, suggesting that the chitosan main chain is oxidatively cleaved at the acetal moiety.

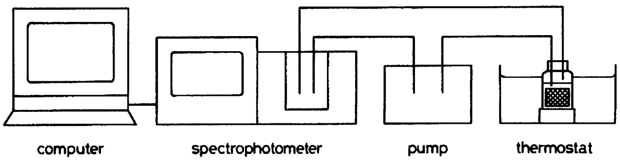

Fig. 1. Flow diagram of a programmed sampling system for the determination of the amount of theophylline released.

As for the solubility of partially acetylated chitosan, no water-soluble derivative was obtained from chitosan with a molecular weight above 1×10^6. This is attributable to the fact that high molecular weight chitosan became gelled when poured into pyridine to acetylate with acetic anhydride. On the contrary, chitosan which was degraded to a molecular weight below 5×10^5 was randomly acetylated up to about 50% to produce water-soluble chitosan,[20)] though it was acetylated under the same reaction condition as mentioned above.

Membranes were fabricated from different molecular weight chitosan samples thus obtained, and the degree of hydration was measured. The results are summarized in Table 2. As can be seen, the lower was the molecular weight, the higher was the degree of hydration. It is probable that the lower is the molecular weight, the stronger is the electrostatic repulsion, since degraded chitosan has a carboxyl group, as described above. In addition, acetylation decreases the degree of hydration of each chitosan membrane, though it is reported that highly *N*-acetylated chitosan was more swollen than low *N*-acetylated chitosan.[21)] This result can be due to a reduction of the hydrophilicity by acetylation of the hydroxyl group as well as the amino group, since in this study acetylation was carried out in nonaqueous acetone.[21)] Figure 3 shows the IR spectra of acetylated chitosan derivatives. Absorption of the ester carbonyl group at 1740 cm^{-1} was observed in addition to the amide group at 1660 and 1560 cm^{-1} for each spectrum. Changes in the degree of hydration of chitosan membranes suggest that the permeability properties of chitosan are dependent on its molecular weight and the degree of acetylation, since the diffusion coefficient of solute, D, is related to the degree of hydration, H, as follows:[22)]

$$\ln D = \ln D_0 - a\left(\frac{1}{H} - 1\right). \tag{1}$$

Here, D_0 is the diffusion coefficient at $H=1$ and a is a constant concerning the free volume, respectively.

Tanaka et al. previously reported that in a spherical gel the surface adjacent to water swells and relaxes immediately, and inside the polymer chains are slowly hydrated.[23)] The swelling of a theophylline tablet coated with chitosan in this study may be assumed to be of a spherical shape, since the difference in the relaxation time between a spherical gel and a cylindrical gel of equal length and diameter is small.[24)] Figure 4 shows swelling curves of theophylline tablets coated with 2.4,

Table 1. Degradation Conditions and Properties of Degradation Products

Degradation conditions			M_η	Degree of acetylation %	Absorbance at 600 nm[a]	Acetylated derivative	
$NaBO_3$ concentration w/v%	Temperature °C	Time h				Degree of acetylation %	Absorbance at 600 nm[a]
0.01	r.t.	1	1.2×10^6	15	0.231	—[b]	—[b]
0.01	30	1	1.1×10^6	14	0.097	—[b]	—[b]
0.01	50	1	4.9×10^5	15	0.075	41	0.010
0.05	50	1	3.4×10^5	14	0.082	40	0.007
0.2	50	1	2.5×10^5	14	0.113	43	0.009
0.5	50	1	2.2×10^5	14	0.125	40	0.006
1.0	50	1	1.8×10^5	16	0.147	44	0.007
2.0	50	1	1.6×10^5	16	0.139	51	0.007
Sat.	50	1	6.9×10^4	15	0.175	47	0.005
Sat.	50	2	3.3×10^4	14	0.236	46	0.006
Sat.	50	4	2.4×10^4	12	0.212	44	0.007

a) Concentrations were 0.025 w/v% at pH 8.9. b) Sample was insoluble in aqueous acetic acid.

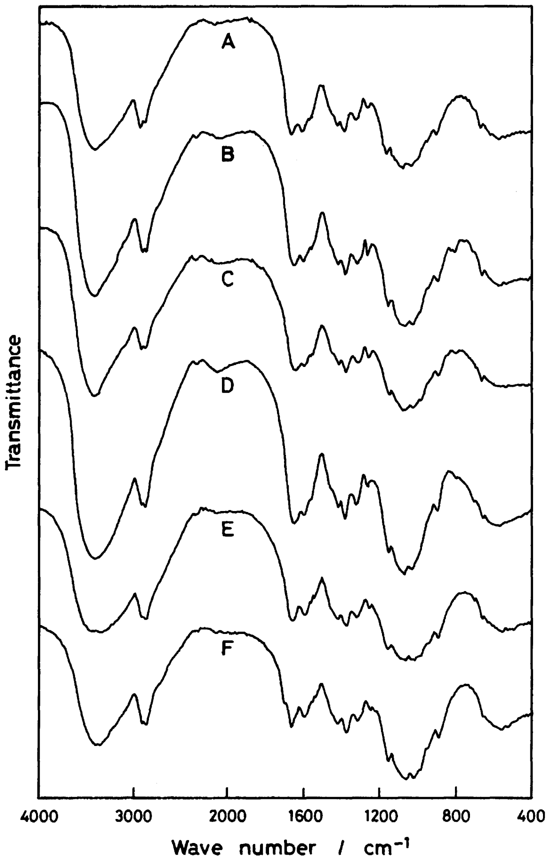

Fig. 2. IR spectra of untreated chitosan and degradation products. A: Untreated chitosan, B: chitosan treated with 0.01 w/v% $NaBO_3$ for 1 h at 30 °C, C: chitosan treated with 0.01 w/v% $NaBO_3$ for 1 h at 50 °C, D: chitosan treated with 0.2 w/v% $NaBO_3$ for 1 h at 50 °C, E: chitosan treated with 2.0 w/v% $NaBO_3$ for 1 h at 50 °C, F: chitosan treated with saturated $NaBO_3$ solution for 1 h at 50 °C.

Table 2. Degree of Hydration of Chitosan and Acetylated Chitosan

Sample code	Chitosan	Acetylated chitosan
A	0.78	0.69
B	0.80	0.71
C	0.82	0.65

Sample codes correspond to those in Figs. 2 and 3.

6.6, and 8.0 mg of chitosan, respectively, in a phosphate buffer solution. The swelling of chitosan gel readily reaches equilibrium. Chitosan gel seems to have a rather small relaxation time. Therefore, the relaxation of chitosan coated on drugs is not expected to cause any latent period of drug release.

Figures 5, 6, and 7 show the drug release profiles of theophylline tablets coated with different molecular weight chitosan. All of the cases required a long time to release completely, as compared to an uncoated tablet; the larger was the amount of coated chitosan, the longer was the complete release time. The slope of the curve in the case of low molecular weight chitosan is larger than that in the case of high molecular weight chitosan. This can be due to a larger hydration degree of low molecular weight chitosan than high molecular weight chitosan. In addition, when untreated chitosan was used, the lag time effect could be clearly observed. This lag time seems to result from the low dissolution rate of a theophylline tablet, since the relaxation of the chitosan gel network is too rapid to govern the change in the permeability of theophylline, as described above.

Accordingly, the flux of theophylline can be estimated on the basis of Fick's second law of diffusion, assuming the diffusion coefficient of theophylline to be independent of its concentration:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}. \tag{2}$$

The internal theophylline concentration gradually increases and reaches the saturated concentration (0.0108 g cm^{-3}). To simplify the discussion, we assume that

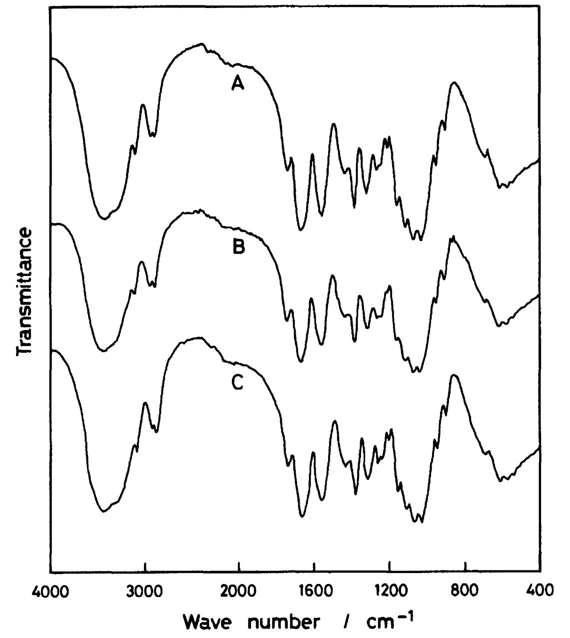

Fig. 3. IR spectra of acetylated chitosan. A: Acetylated derivative of untreated chitosan, B: acetylated derivative of chitosan treated with 0.01 w/v% $NaBO_3$ for 1 h at 30 °C, C: acetylated derivative of chitosan treated with 0.01 w/v% $NaBO_3$ for 1 h at 50 °C.

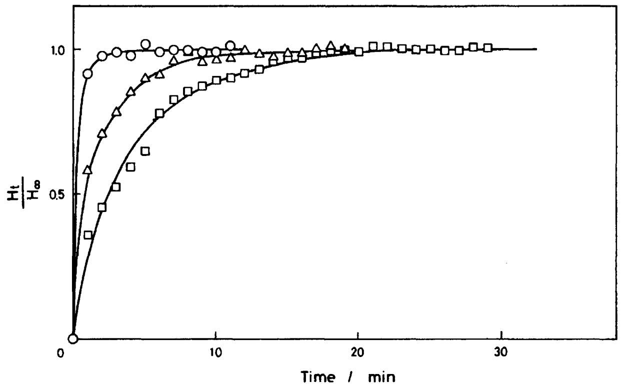

Fig. 4. Swelling curves of chitosan coated on theophylline tablets. ○: 2.5 mg, △: 6.6 mg, □: 8.0 mg.

the theophylline concentration in the internal solution increases linearly with time as $c_{in}=Kt$, and remains constant, $c_{in}=c_{sat}$, at $t>t_{sat}$, where c_{in} is the theophylline concentration in the internal solution, K is the constant, and t is the time, respectively. At the initial condition, $\bar{c}(x,0)=0$, and the boundary conditions, $\bar{c}(0,t)=SKt$ and $\bar{c}(l,t)=0$ (Fig. 8), we obtain

$$\bar{c}(x,t) = \frac{SK(l-x)}{l}\left\{t - \frac{(2l-x)x}{6D}\right\} + \frac{2SKl^2}{D\pi^3}\sum_{n=1}^{\infty}\frac{1}{n^3}\sin\left(\frac{n\pi x}{l}\right)\exp\left(-\frac{n^2\pi^2}{l^2}Dt\right) \quad (0<t<t_{sat}) \qquad (3)$$

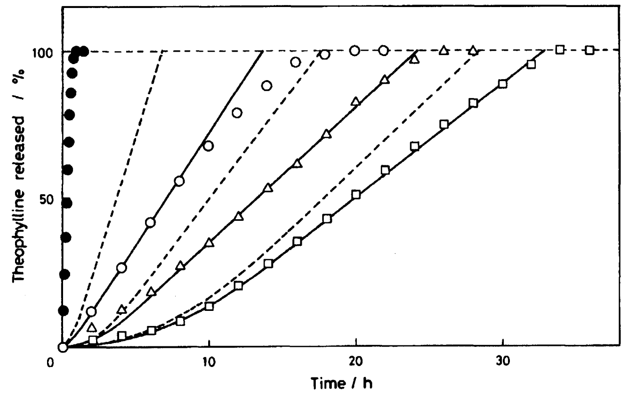

Fig. 5. Release profiles of theophylline tablets coated with untreated chitosan. ●: Uncoated, ○: 1.2 mg, △: 3.1 mg, □: 4.5 mg. The broken lines represent simulation curves using $c_{in}=0.0108\ g\,cm^{-3}$; the solid lines represent simulation curves using the maximum flux of theophylline.

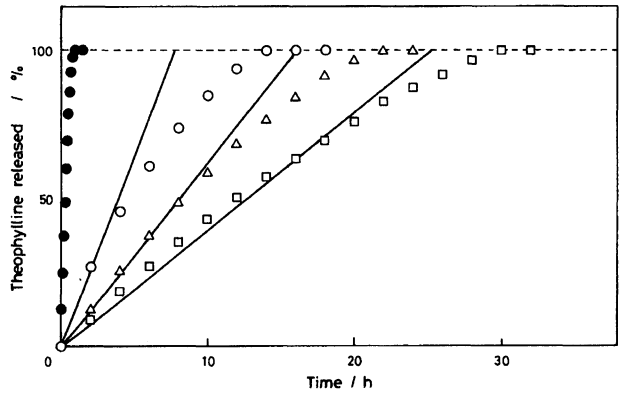

Fig. 6. Release profiles of theophylline tablets coated with chitosan treated with 0.01 w/v% $NaBO_3$ for 1 h at 30 °C. ●: Uncoated, ○: 1.6 mg, △: 3.0 mg, □: 6.0 mg. The solid lines represent simulation curves using the maximum flux of theophylline.

$$= Sc_{sat}\left\{1 - \frac{x}{l} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{l}\right) \times \exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\} \quad (t_{sat}<t), \qquad (4)$$

$$l = l_0X,$$

and $$X^3 = \frac{1}{1-H}.$$

Here, $\bar{c}$ is the theophylline concentration in the membrane, S the partition coefficient of theophylline between the chitosan hydrogel and solution (approximately $S=H^{22)}$), l the thickness of swollen chitosan hydrogel, l_0 the thickness of chitosan before swelling, and X^3 the expansion coefficient of chitosan, respectively. The flux of theophylline at the gel surface, $J_{x=l}$, is written as follows:

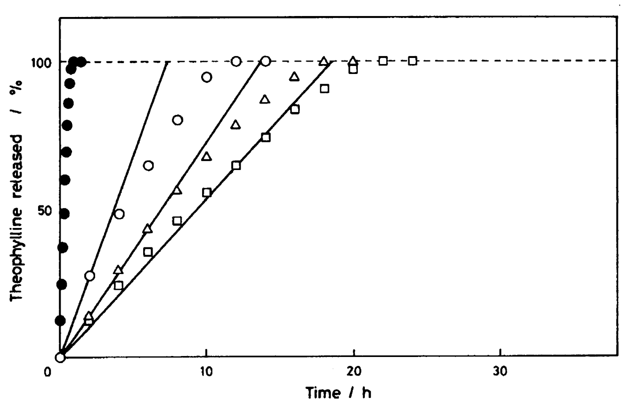

Fig. 7. Release profiles of theophylline tablets coated with chitosan treated with 0.01 w/v% NaBO$_3$ for 1 h at 50 °C. ●: Uncoated, ○: 1.0 mg, △: 3.1 mg, □: 6.0 mg. The solid lines represent simulation curves using the maximum flux of theophylline.

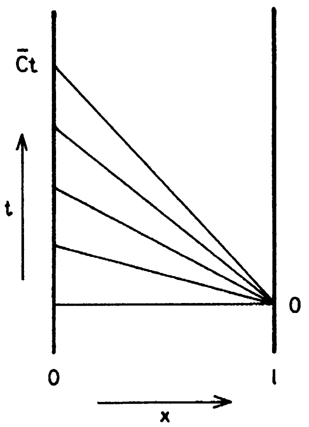

Fig. 8. Schematic representation of the concentration of theophylline in the membrane.

$$
\begin{aligned}
J_{x=l} &= -D\left(\frac{\partial c}{\partial x}\right)_{x=l} \\
&= \frac{DSK}{l}\left\{t - \frac{l^2}{6D} - \frac{2l^2}{D\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\right. \\
&\qquad \left.\times \exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\} \qquad (0 < t < t_{\mathrm{sat}}) \qquad (5) \\
&= \frac{DSc_{\mathrm{sat}}}{l}\left\{1 + 2\sum_{n=1}^{\infty}(-1)^n \exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\} \\
&\qquad (t_{\mathrm{sat}} < t). \qquad (6)
\end{aligned}
$$

D_0 and a of Eq. 1 were experimentally estimated with a flat membrane, according to a previously reported method.[15] As a result, D_0=4.2×10^{-6} cm^2 s^{-1} and a= 2.38. Finally, the mass of theophylline released, M_t, is expressed as follows:

$$
\begin{aligned}
M_t &= \frac{ADSK}{l}\left\{\frac{1}{2}t^2 - \frac{l^2}{6D}t + \frac{7l^4}{360D}\right. \\
&\quad \left. + \frac{2l^4}{D\pi^4}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^4}\exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\} \\
&\qquad (0 < t < t_{\mathrm{sat}}) \qquad (7) \\
&= \frac{ADSc_{\mathrm{sat}}}{l}\left\{t - \frac{l^2}{6D} - \frac{2l^2}{D\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\right. \\
&\quad \left.\times \exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\} \qquad (t_{\mathrm{sat}} < t), \qquad (8)
\end{aligned}
$$

and $A = A_0X^2$,

where A is the diffusion area of swollen chitosan hydrogel, and A_0 is the inner surface area before swelling (0.21 cm^2), respectively. Therefore,

$$
\begin{aligned}
M_t &= \frac{ADSc_{\mathrm{sat}}}{l}\left\{t - \frac{1}{2}t_{\mathrm{sat}} - \frac{l^2}{6D} + \frac{7l^4}{360Dt_{\mathrm{sat}}}\right. \\
&\quad + \frac{2l^4}{D\pi^4 t_{\mathrm{sat}}}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^4}\exp\left(-\frac{n^2\pi^2}{l^2}Dt_{\mathrm{sat}}\right) \\
&\quad + \frac{2l^2}{D\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{n^2\pi^2}{l^2}Dt_{\mathrm{sat}}\right) \\
&\quad \left. - \frac{2l^2}{D\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{n^2\pi^2}{l^2}Dt\right)\right\}. \qquad (9)
\end{aligned}
$$

To determine the value of K, t_{sat} should be estimated. When t_{sat} is large, the fourth, fifth, sixth, and seventh terms on the right-hand side are negligible, and we obtain

$$M_t = \frac{ADSc_{\mathrm{sat}}}{l}\left(t - \frac{1}{2}t_{\mathrm{sat}} - \frac{l^2}{6D}\right). \qquad (10)$$

Extrapolation of the linear plot of Eq. 10 to the intercept shows the lag time, t_{lag}, is

$$t_{\mathrm{sat}} = 2\left(t_{\mathrm{lag}} - \frac{l^2}{6D}\right). \qquad (11)$$

Substituting the lag time in Eq. 11 yields t_{sat}. Thus, the theophylline release profile can be simulated by a numerical method using Eqs. 9 and 11. Figure 5 shows a simulation of the theophylline release profiles in the case of untreated chitosan. The slope of the calculated curve is somewhat larger than that of the experimental data. The internal theophylline concentration seems to be greatly affected by the dissolution rate. This point should be further investigated using another drug form with a high dissolution rate. In Figs. 5, 6, and 7, curves calculated from the maximum flux of theophylline are also plotted. They are linear at the latter half of drug release, since the internal theophylline concentrations are supposed to be saturated and constant up to the end of release. Experimental data are apart from the linear plots at the latter half of drug release, particularly in the case of low molecular weight chitosan. It is suggested that in the case of low molecular weight chitosan the rate-determining step is not permeation, but the dissolution of theophylline, due to the low dissolution rate of a theophylline tablet in a calm environment wrapped into chitosan hydrogel. Then, to decrease the diffusion coefficient further, acetylation of coated chitosan was attempted. However, the drug release rate and release behavior remained unchanged, since acetylation was not successfully achieved, but differed from the membrane case.

In conclusion, chitosan hydrogel seems to be suitable for sustained-release drug. Untreated high molecular weight chitosan has a rather low water permeability, and exhibits a lag time effect in drug release. On the other hand, $NaBO_3$-treated chitosan is highly swollen, and has a high degree of hydration and high permeability of the drug. In this case, however, dissolution of drug is the rate-determining step, and release of the drug does not obey zero-order kinetics. Therefore, appropriately $NaBO_3$-treated chitosan has a favorable degree of hydration and a favorable permeability of the drug; its use meets the requirement for zero-order drug release.

References

1) "Chitin in Nature and Technology," ed by R. A. A. Muzzarelli, C. Jeuniaux, and G. W. Gooday, Plenum Press, New York (1986).

2) E. R. Pariser and D. P. Lombardi, "Chitin Sourcebook," Wiley, New York (1989).

3) Y. Kawashima, T. Handa, A. Kasai, H. Takenaka, and S. -Y. Lin, *Chem. Pharm. Bull.*, **33**, 2469 (1985).

4) S. Miyazaki, H. Yamaguchi, C. Yokouchi, M. Takada, and W. -M. Hou, *J. Pharm. Pharmacol.*, **40**, 642 (1988).

5) R. Bodimeier, H. Chen, and O. Paeratakul, *Pharm. Res.*, **6**, 413 (1989).

6) Y. Nishioka, S. Kyotani, H. Masui, M. Okamura, M. Miyazaki, K. Okazaki, S. Ohnishi, Y. Yamamoto, and K. Ito, *Chem. Pharm. Bull.*, **37**, 3074 (1989).

7) T. Okano, M. Miyajima, F. Komada, G. Imanidis, S. Nishiyama, S. W. Kim, and W. I. Higuchi, *J. Controlled Release*, **6**, 99 (1987).

8) R. A. Siegel, M. Falamarzian, B. A. Firestone, and B. C. Moxley, *J. Controlled Release*, **8**, 179 (1988).

9) L. Brannon-Peppas and N. A. Peppas, *J. Controlled Release*, **8**, 276 (1989).

10) Y. Kikuchi and N. Kubota, *Bull. Chem. Soc. Jpn.*, **60**, 375 (1987).

11) Y. Kikuchi, N. Kubota, K. Maruo, and Y. Goto, *Makromol. Chem.*, **188**, 2631 (1987).

12) Y. Kikuchi, N. Kubota, and H. Mitsuishi, *J. Appl. Polym. Sci.*, **35**, 259 (1988).

13) Y. Kikuchi and N. Kubota, *Bull. Chem. Soc. Jpn.*, **61**, 2943 (1988).

14) Y. Kikuchi and N. Kubota, *Makromol. Chem., Rapid Commun.*, **9**, 727 (1988).

15) N. Kubota, K. Ohga, and M. Moriguchi, *J. Appl. Polym. Sci.*, **42**, 495 (1991).

16) G. A. F. Roberts and J. G. Domszy, *Int. J. Biol. Macromol.*, **4**, 374 (1982).

17) R. A. A. Muzzarelli and R. Rocchetti, in "Chitin in Nature and Technology," ed by R. A. A. Muzzarelli, C. Jeuniaux, and G. W. Gooday, Plenum Press, New York (1986), p. 385.

18) K. Kurita, Y. Koyama, S. Nishimura, and M. Kamiya, *Chem. Lett.*, **1989**, 1597.

19) Y. Kawamura, H. Ikebe, I. Kurahashi, H. Seo, and S. Nakajima, Kokai Tokkyo Koho, 61-40337 (1986).

20) S. Aiba, *Int. J. Biol. Macromol.*, **11**, 249 (1989).

21) M. Miya, R. Iwamoto, K. Ohta, and S. Mima, *Kobunshi Ronbunshu*, **42**, 181 (1985).

22) H. Yasuda, C. E. Lamaze, and L. D. Ikenberry, *Makromol. Chem.*, **118**, 19 (1968).

23) T. Tanaka and D. J. Fillmore, *J. Chem. Phys.*, **70**, 1214 (1979).

24) Y. Li and T. Tanaka, *J. Chem. Phys.*, **92**, 1365 (1990).