Polyelectrolyte Complexes of Sodium Alginate with Chitosan or Its Derivatives for Microcapsules

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ABSTRACT: Chitosan, a cationic polysaccharide, was heterogeneously deacetylated with a 47% sodium hydroxide solution and followed by a homogeneous reacetylation with acetic anhydrides to control the N-acetyl content of the chitosan having a similar molecular weight. The chitosans having different degrees of N-acetylation were complexed with sodium alginate, an anionic polysaccharide, and the formation behavior of polyelectrolyte complexes (PECs) was examined by the viscometry in various pH ranges. The maximum mixing ratio (R_{max}) increased with a decrease in the degree of N-acetylation of the chitosan at the same pH, and with a decrease in pH at the same degree of N-acetylation. Similarly, N-acylated chitosans were also prepared. The N-acyl chitosans scarcely affected the formation behavior of PECs with sodium alginates. For the application of the PECs produced, the microencapsulation of a drug was performed and the release property of drug was tested. The microcapsules were prepared in one step by the extrusion of a solution of guaifenesin and sodium alginate into a solution containing calcium chloride and chitosan through interpolymeric ionic interactions. The drug release during the drug-loaded microcapsules storage in saline was found to depend on the pH where the microcapsules were formed and the kind of N-acyl groups introduced to the chitosan. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **63:** 425–432, 1997

Key words: N-acyl chitosan; alginate; polyelectrolyte complex (PEC); microcapsule; drug release

INTRODUCTION

Chitosan is a (1,4) linked 2-amino-2-deoxy-β-D-glucan and can be prepared by the N-deacetylation of chitin. The chitosan has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions. Therefore, there have been many interesting chitosan derivatives, especially for biomedical applications.[1–3] Alginate comprises a linear, unbranched chain of (1,4) linked β-D-mannuronate and α-L-guluronate residues arranged in a blockwise fashion. The alginate has many applications in food technology and its gelation by divalent cation has been widely used.[4]

Polyelectrolyte complexes (PECs) are formed by the reaction of a polyelectrolyte with an oppositely charged polyelctrolyte in an aqueous solution. Polysaccharides, which have bulky pyranose rings and highly stereoregular configuration in their rigid, linear backbone chains, have been frequently studied.[5–8] PECs have numerous applications such as membranes, antistatic coatings, en-

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vironmental sensors, and chemical detectors, medical prosthetic materials, etc.[9] Among these, their wide uses as membranes for dialysis, ultrafiltration, and other solute separation processes are of special interest and also make it possible for the use in microcapsule membranes.

Microcapsules can be used for mammalian cell culture and the controlled release of drugs, vaccines, antibiotics, and hormones.[10–13] To prevent the loss of encapsulated materials, the microcapsules should be coated with another polymer that forms a membrane at the bead surface. The most well-known and promising system is the encapsulation of alginate beads with poly-L-lysine (PLL). Because this system has a limitation due to the high cost of PLL, another systems such as alginate beads coated with chitosan or its derivative have been developed.[14–17]

The objective of this work is to examine the formation behavior of PECs of alginate with the chitosan or its derivatives having N-acyl groups, and to apply these PECs for the preparation of microcapsules. Few results have been reported about the formation of PECs of alginate with the chitosan under acidic conditions.[8] Although alginate/chitosan microcapsules have been studied a lot, the studies have been limited in a narrow pH region due to the solubility of chitosan. In this work, the chitosan was modified to dissolve at various pHs and to examine the effect of pH and derivatized groups on the complex formation with alginate. The dependences of release rate of encapsulated drug from alginate/chitosan microcapsules on the pH where the microcapsules formed and on the kind of derivatized groups introduced to the chitosan were also examined. The control of the release rate by changing the pH or the derivatization of chitosan may bring some improvements in designing the microcapsules.

EXPERIMENTAL

Materials

Chitosan was purchased from Sigma Co. and purified with the reprecipitation method.[18] Sodium alginate (Sigma Co., $\bar{M}_w$ = 236,000) was dissolved in distilled water and dialyzed for 3 days by using cellulose tubular membranes in deionized water.[19] The dialyzed solution was precipitated with ethanol, followed by filtering, washing with methanol and then ether, finally drying in a vacuum at room temperature. Acetic anhydride, *n*-butyric anhydride, and *n*-hexanoic anhydride were used as carboxylic anhydrides without further purification. Guaifenesin (Tokyo Kasei Co.), an expectorant, was used as an encapsulated drug.

Selective N-Acylation of Chitosan

Chitosan, having N-acetyl content of about 20%, was heterogeneously deacetylated with a 47% sodium hydroxide (NaOH) solution at 110°C, followed by washing it thoroughly with distilled water at about 80°C. The alkali treatment and washing in water were repeated two times. For further deacetylation, thread-like chitosan was prepared by pouring a 5% chitosan solution into an 1 *N* NaOH solution. The thread-like chitosan was treated with alkali and washed with water as in the above method.[20] The deacetylated chitosan having less than 5% of degree of N-acetylation was homogeneously acylated with carboxylic anhydrides by the method of Domszy and Roberts.[3,21] A chitosan solution having the same volume of methanol was prepared and different volumes of carboxylic anhydride were added. After 15 min, the solution was poured into the methanol/ammonia solution (7/3, v/v). The precipitated chitosans were filtered off, washed thoroughly with methanol and ether, followed by drying in a vacuum at 50°C.

Characterization of N-Acyl Chitosans

Degree of N-acylation was determined by the titrimetric method.[22] About 0.05*M* chitosan in a 0.3 *N* HCl solution was titrated with a standard solution of 0.1 *N* NaOH solution by using a pH meter (HI8418, Hanna instrument Co.) equipped with a glass electrode. ^{1}H NMR measurements were performed on a Varian SUN UNITY 300 NMR spectrometer at room temperature. The chitosan sample was dissolved in a 2% CD_3COOD/D_2O (v/v) solution. A 30° single-pulse sequence was used for FID accumulation. The pulse repetition delay was 6 s. Chemical shifts (δ) were expressed in ppm downfield from the signal for sodium 3-(trimethyl silyl) propane sulfonate (TSP) as an internal reference.

Formation of Polyelectrolyte Complexes

Each polyelectrolyte was dissolved at 0.8×10^{-2} g/dL concentration in a 0.01*M* sodium chloride (NaCl) solution, followed by adjusting the desired

pH (2.05, 2.80, 4.80, 6.80, 8.80, and 9.50 ± 0.01). The polycation solution with a fixed pH was added to the polyanion solution having the same pH under slow stirring condition, and the mixing time was 30 min. The viscosity of each component polyelectrolyte or complex solution, which was filtered with a filtering paper (pore size: 0.45 μm, Millipore Co.), was measured by a Schott–Gerate automatic viscometer (AVS440 system) equipped with an Ubbelohde type viscometer at 25°C.

Solubility of Polyelectrolyte Complexes

Solubility tests of PECs were performed on formic acid, acetic acid, trifluoroacetic acid, dimethyl sulfoxide, hexafluoroisopropanol, lithium chloride/dimethyl acetamide (5/95 in weight ratio), ternary solvent mixtures (water/acetone/sodium bromide = 50/20/30, water/dioxane/hydrochloric acid = 5/50/45, water/acetic acid/potassium chloride = 70/10/20 in weight ratio), and a quaternary solvent mixture (water/dimethyl formamide/dimethyl sulfoxide/sodium bromide = 33/20/37/10 in weight ratio) with vigorous stirring and occasional heating.

Preparation of Microcapsules

A 1.5% solution of sodium alginate (3 mL) containing 0.01*M* NaCl and 0.5% guaifenesin was prepared and extruded through an 1 mL disposable syringe with a 26 1/2 gauge needle. The droplets were pulled off into 50 mL of a 0.5% chitosan solution containing 0.01*M* NaCl and 0.05*M* calcium chloride whose pH was adjusted to a given value (2.80, 4.50, 4.80, 6.80, and 8.80 ± 0.01) by rapid flow of air stream, then allowed to harden for 30 min. Conditions for pumping rate and air flow were adjusted such that microcapsules had about 0.5 mm diameter. After hardening, the microcapsules were rinsed with distilled water and transferred into saline for examination of drug release.

Drug Release

Guaifenesin release from microcapsules was determined by direct measuring the absorbance of supernatants of storage solutions at 272 nm using an UV/VIS scanning spectrometer (UV-2101PC, Shimadzu Co.) as a function of time. At the end of measurement, 30 mL of a 0.05*M* sodium citrate solution was added and a final absorbance was recorded. The value of the final absorbance was corrected by considering the dilution.

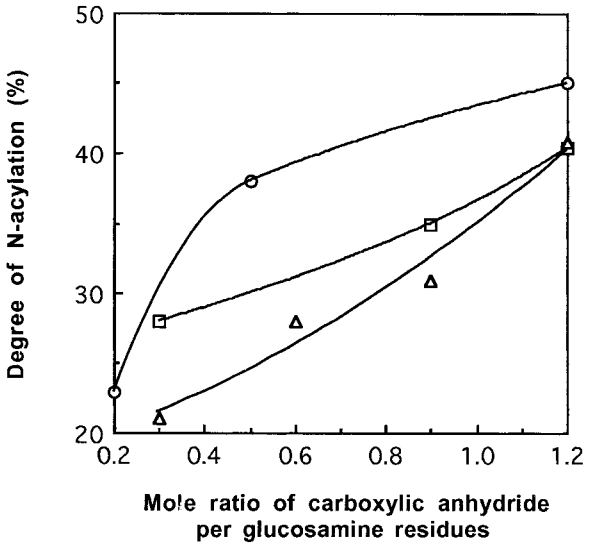


Figure 1 Change in degree of N-acylation of chitosan after N-acylation with various carboxylic anhydrides. ○, N-acetyl; □, N-butyryl; △, N-hexanoyl chitosan.

RESULTS AND DISCUSSION

Selective N-Acylation of Chitosan

The addition of carboxylic anhydrides to the solutions of chitosan in aqueous methanolic acetic acid produced a series of selectively N-acylated chitosans (Fig. 1). Although heterogeneous deacetylation of chitin also makes it possible to prepare the chitosans having different degrees of N-acetylation, their molecular weights decrease as the deacetylation time increases. For this reason, the deacetylated chitosan was used as a starting material to minimize the variation of molecular weights among N-acylated chitosans. The degree of N-acylation was determined by the titrimetric method and calculated from the amount of NaOH (the amount between two points of inflection in the titration curve) consumed by the free amine groups in the solution.[3] From infrared spectra, it was confirmed that the additional O-acylation did not occur (data not shown). The degrees of N-acylation and molecular weights of chitosan samples are summarized in Table I.

Table II lists the chemical shifts of protons of N-acyl chitosans. The signals at 3.5–4.0 ppm were attributed to H3, 4, 5, 6, 6′ of the pyranose ring.

Table I *N*-Acylated Chitosan Samples Used

Sample	*N*-Acyl Group	Degree of *N*-Acylation (%)[a]	Molecular Weight
A-05	*N*-acetyl	4.9	440,000[b]
A-23	*N*-acetyl	23.2	391,000[b]
A-45	*N*-acetyl	45.2	410,000[b]
B-42	*N*-butyryl	41.7	781,000[c]
H-41	*N*-hexanoyl	40.8	—

[a] Measured by titrimetric method.
[b] Determined by intrinsic viscosity measurement (ref. 23).
[c] Determined by gel permeation chromatography with pullulan standard (ref. 3).

In the vicinity of 2.0 ppm, the resonance band due to CD_2H residue of CD_3COOD overlapped with that due to CH_3 residues of N-acetyl. The H1 signal of the pyranose ring overlapped with HOD peak near 4.8 ppm. Though each characteristic methylene protons appeared qualitatively as chitosan was N-acylated with various carboxylic anhydrides, the H2 signal had no significant changes.

Formation of Polyelectrolyte Complexes and Their Solubility

The variation of reduced viscosities of PECs of sodium alginate with chitosans having different degrees of N-acetylation is shown in Figure 2. A mixing ratio (R) was defined as following when a polycation solution was added to a polyanion solution:

$$R = \frac{\text{PA}}{\text{PA} + \text{PC}}$$

where PA and PC are moles of polyanion and polycation, respectively. The viscosity decreased as the two polyelectrolyte solutions were mixed together and a maximum mixing ratio (R_{max}), where the maximum amount of complexes were formed existed. R_{max} was dependent on the degree of N-acetylation and shifted to a lower value as the degree of N-acetylation increased. This is because the number of free amine groups that can participate in a complex formation decreases as the N-acetyl groups are introduced to the chitosan. The viscosity usually decreases by the drastic reduction of the total hydrodynamic volume of the two polyelectrolytes through complexation, and the formation of compact complexes. But in the case of the formation of gel-like, expanded complexes, the viscosity may increase.[24] The gel-like PECs can be prepared by the addition of a certain amount of electrolytes to reduce the electrostatic interaction between two polyions and to slow down the PEC formation. In case of PEC gels,

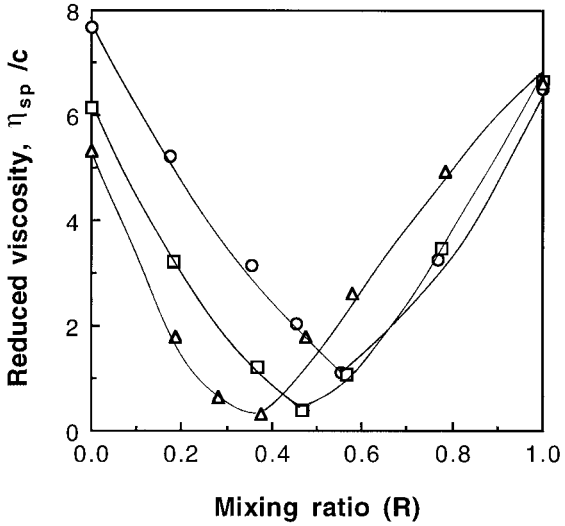
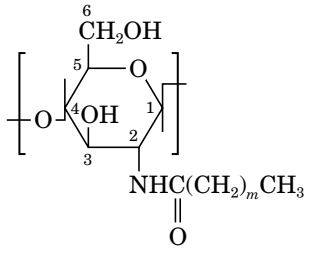


Figure 2 Variation of reduced viscosity of polyelectrolyte complexes of alginate with chitosans having different degrees of N-acetylation (pH 4.50, 25°C). ○, A-05; □, A-23; △, A-45.

Table II Chemical Shifts of Some Protons of N-Acyl Chitosans



Sample	*m*	H2	Chemical shift, δ (ppm) —CH_2—	—CH_3
A-05	0	3.17	—	2.08
A-45	0	3.17	—	2.10
B-42	2	3.16	2.30, 1.61	0.93
H-41	4	3.17	2.32, 1.60, 1.28	0.88

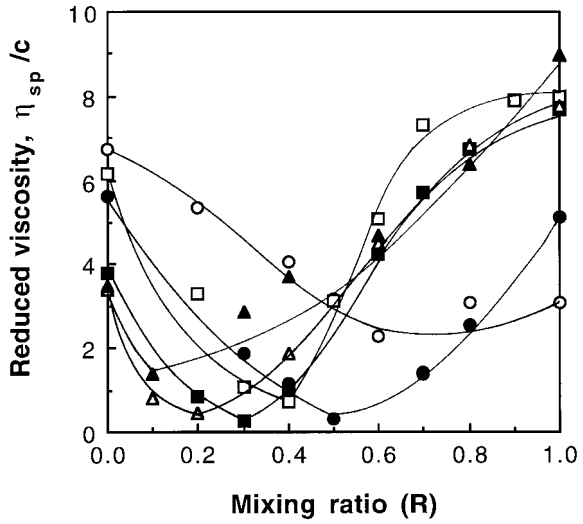


Figure 3 Variation of reduced viscosity of polyelectrolyte complexes of alginate with A-45 at various pHs, 25°C. ○, pH 2.05; ●, pH 2.80; □, pH 4.80; ■, pH 6.80; △, pH 8.80; ▲, pH 9.50.

R_{max} may be depenendent on the swelling of PECs or pore size.[25,26]

In general, chitosan has the limited solubility in the higher pH region, i.e., gelation or precipitation occurs where the pH exceeds 6. But the solution of partially N-acetylated chitosan (A-45) maintained its homogeneity under neutral or even alkaline conditions and the gelation or precipitation did not occur. The stability of chitosan solution was examined by Aiba[27] and reported that chitosan, having about 50% of the degree of N-acetylation, maintained its homogeneous solution state at alkaline conditions. This makes it possible to set up a hypothesis of the random-type copolymers of N-acetyl glucosamine and glucosamine units rather than the block-type copolymers.[28] A-45 was complexed with alginate at various pH ranges, from 2.05 to 9.50 (Fig. 3). As the pH increased, R_{max} decreased because the number of dissociated carboxylic groups in alginate increased and reversely the number of protonated amine groups in chitosan decreased. Figure 4 shows the change of maximum mixing ratio with the variation of the pH. At the extremely lower pH region, most carboxylic groups of alginate are in the form of COOH. In contrast, at the extremely higher pH region, most amine groups of chitosan are in the form of NH_2. However, PECs can be obtained by the induced dissociation of carboxylic or amine groups with coexisting polyions of opposite charge.[29]

Hydrophobic interactions as well as Coulombic forces are concerned with the formation of PECs. The hydrophobicity of polycations and its effect on the formation of complexes were studied by Tsuchida et al.[30,31] with fluorescence measurements. In the case of N-acyl chitosan films, the hydrophobic long-chain acyl groups existed on the film surface to balance the hydrophobic and hydrophilic properties and to play a key role to improve the antithrombogenicity.[3] However, in the case of solution states, the N-acyl groups used in this work were not long enough to enhance the hydrophobic interactions, so R_{max} of the PECs were very similar, regardless of the N-acyl groups (Fig. 5). In order to introduce the longer acyl or aromatic groups to the chitosan, excess amounts of anhydrides are required because of the solubility of the anhydrides in an aqueous solution. Because these derivatization accompanies the gelation of chitosan and it is very hard to dissolve the derivatives in organic solvents under mild conditions, these carboxylic anhydrides were excluded in this work.

PECs, prepared with polysaccharides having rigid chain conformations, are hardly soluble in most organic solvents, even on heating. Chitosan/sodium carboxymethyl cellulose, chitosan/carboxylmethyl dextran, or sodium dextran sulfate complexes have a limited solubility in formic acid on heating.[7] The PECs of chitosan/alginate were insoluble in most organic solvents, even in ternary or quaternary solvent mixtures that were known

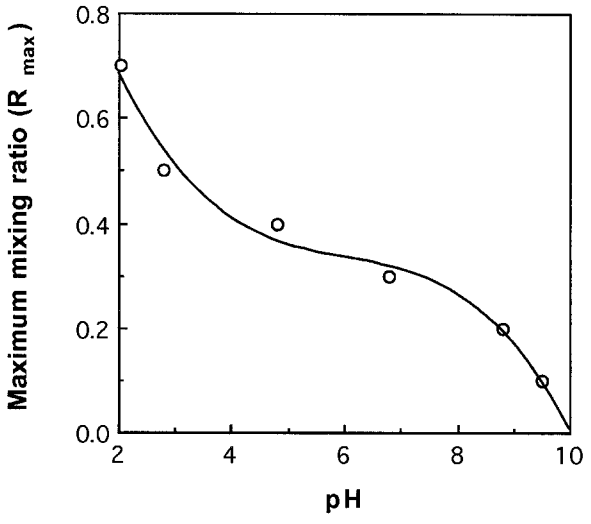


Figure 4 Change of maximum mixing ratio (R_{max}) of PECs of alginate and A-45 as a function of pH at 25°C.

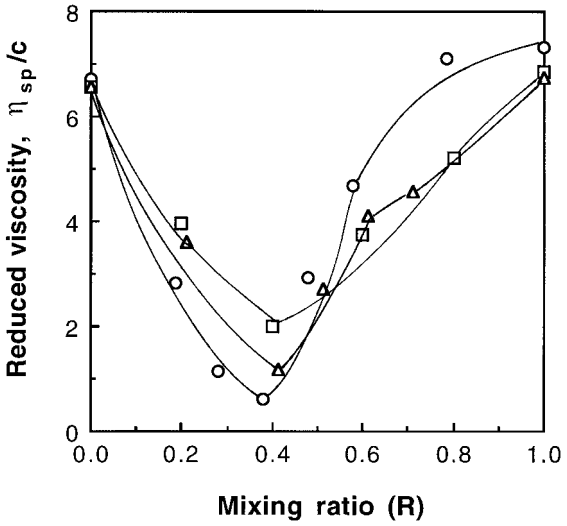


Figure 5 Variation of reduced viscosity of polyelectrolyte complexes of alginate with N-acyl chitosans at pH 4.50, 25°C. ○, A-45; □, B-42; △, H-41.

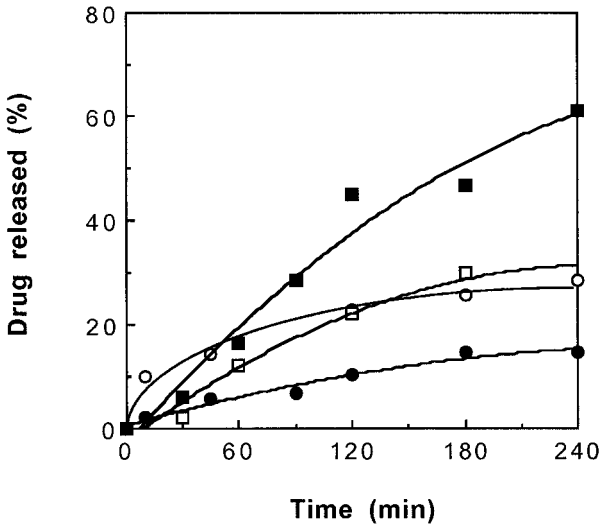


Figure 6 Release rate of guaifenesin from microcapsules of A-45/alginate prepared at various pHs. ○, pH 2.80; ●, pH 4.80; □, pH 6.80; ■, pH 8.80.

as solvents for complexes between polysaccharides.

Microcapsule Formation and Its Release Properties

When an alginate drop falls into a chitosan solution, the interphasic membrane is formed by complexation between two polyelectrolytes of opposite charge through electrostatic interactions. Calcium chloride in a chitosan solution diffuses into the alginate core more rapidly than chitosan because of its low molecular weight and forms a gel core. Subsequently, a microcapsule of an alginate gel core coated with alginate/chitosan interphasic membrane is formed.[15]

The release rates of guaifenesin in saline from microcapsules prepared with A-45/ALG at various pHs are shown in Figure 6. The microcapsules prepared at pH 4.80 showed a minimum release rate and the release rate increased as the pH increased. Because both amine or carboxylic groups in both polyelectrolytes have about 70–80% of the degree of dissociation near pH 5.0, each polysaccharide may sustain the rigid, linear conformation to result in a dense membrane formation [Fig. 7(b)].[17] Above pH 5.0, the degree of dissociation of chitosan is suppressed and the chitosan may form some kinds of loops. This loop formation makes chitosan/alginate membranes less dense and increases the rate of release [Fig. 7(c) and (d)]. In the case of pH 2.80, the release rate is similar to that of pH 6.80 and this also can be explained as a schematic representation (a) in Figure 7.

Figure 8 shows the release rate of guaifenesin from N-acyl chitosan/alginate microcapsules prepared at pH 4.50. Although R_{max} was independent of the N-acyl groups used (Fig. 5), it seemed that the long-chain acyl groups affected the structure of the membrane to hinder the dense membrane

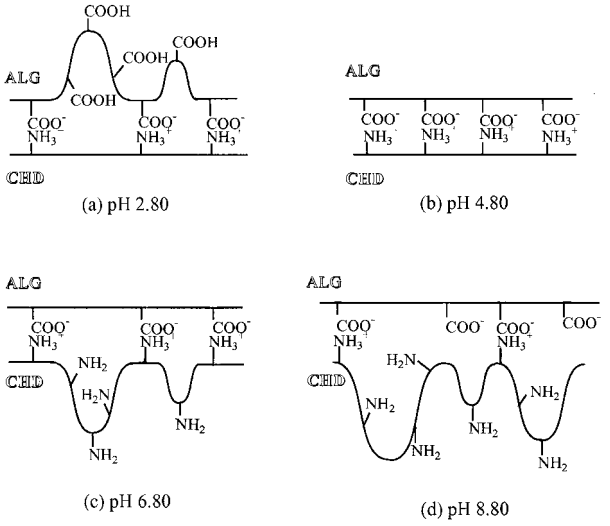


Figure 7 Schematic representation of membrane formation of PECs between chitosan and sodium alginate (ALG, sodium alginate; CHD, chitosan).

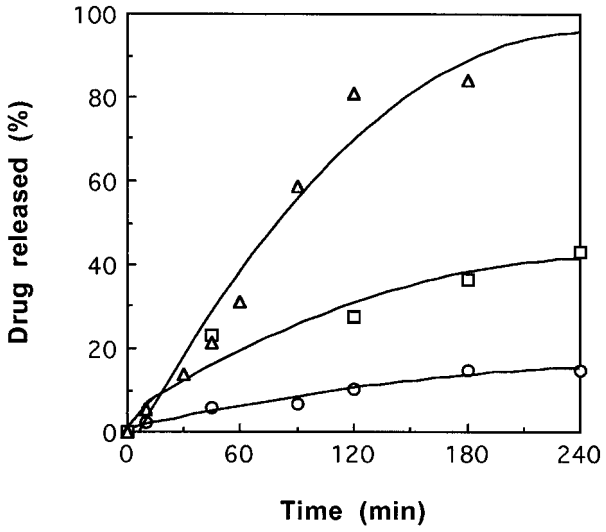


Figure 8 Release rate of guaifenesin from microcapsules of N-acyl chitosan/alginate prepared at pH 4.50. ○, A-45; □, B-42; △, H-41.

formation. The longer the acyl groups were, the more effectively the release rate enhanced.

The release rate of drug from microcapsules may depend mainly on two factors. One is the thickness, and the other is the compactness of the membrane that forms the outer shell of the microcapsule. The thickness of the membrane is related to the amount of PECs formed and that can be confirmed from the decreasing amount of viscosity through complexation. From Figure 3, the amount of PECs formed looks similar to each other, irrespective of the variation of pH. Therefore, it can be suggested that the release rate of drug from A-45/ALG microcapsules prepared at various pHs may dominantly depend on the compactness of the membrane due to the formation of the loops of backbone chains (Fig. 7). In the case of the N-acyl chitosan/ALG microcapsules, it seems that the two factors compete with each other and, consequently, the compactness of the membrane may be more dominant in determining the release properties of microcapsules owing to the introduction of the long N-acyl groups to the chitosan (Figs. 5 and 8).

CONCLUSION

Selectively, N-acylated chitosans were obtained by homogeneous acylation in the presence of methanol. They were used for the formation of PECs with the sodium alginate and the preparation of microcapsules. Alginate/chitosan or chitosan derivative mixtures made the nonstoichiometric PECs with the pH. R_{max} varied with the pH and the amount of N-acetyl groups in the chitosan. However, the kind of N-acyl groups used in this work scarcely affected the formation behavior of PECs. These PECs were hardly soluble in most organic solvents and usable to form membranes coating on alginate beads. The release rate of drug from microcapsules was able to be controlled by the pH where the capsules were formed and the kind of N-acyl groups in the chitosan. The microcapsules prepared at pH 4.80 showed a minimum release rate and the release rate varied with the pH due to the loop formation of backbone chains of polyelectrolytes. The long N-acyl groups introduced to the chitosan enhanced the release rate remarkably.

REFERENCES

1. R. A. A. Muzzarelli, *Carbohydr. Polym.,* **3,** 53 (1983).
2. Q. Li, E. T. Dunn, E. W. Grandmaison, and M. F. A. Goosen, *J. Bioact. Compat. Polym.,* **7,** 370 (1992).
3. K. Y. Lee, W. S. Ha, and W. H. Park, *Biomaterials,* **16,** 1211 (1995).
4. V. J. Morris, in *Functional Properties of Food Macromolecules,* J. R. Mitchell and D. A. Ledward, Eds., Elsevier Applied Science Publishers, London, 1986.
5. A. Nakajima and K. Shinoda, *J. Colloid Interface Sci.,* **55,** 126 (1976).
6. S. Hirano, C. Mizutani, R. Yamaguchi, and O. Miura, *Biopolymers,* **17,** 805 (1978).
7. H. Fukuda, *Bull. Chem. Soc. Jpn.,* **53,** 837 (1980).
8. T. Takahashi, K. Takayama, Y. Machida, and T. Nagai, *Int. J. Pharm.,* **61,** 35 (1990).
9. A. S. Michaels, *Ind. Eng. Chem.,* **57,** 32 (1965).
10. T. Yoshika, R. Hirano, T. Shioya, and M. Kako, *Biotech. Bioeng.,* **35,** 66 (1990).
11. M. A. Wheatley, M. Chang, E. Park., and R. Langer, *J. Appl. Polym. Sci.,* **43,** 2123 (1991).
12. R. Bodmeier and J. Wang, *J. Pharm. Sci.,* **82,** 191 (1993).
13. S. Benita, J. P. Benoit, F. Puisieux, and C. Thies, *J. Pharm. Sci.,* **73,** 1721 (1984).
14. C. A. McKnight, A. Ku, M. F. A. Goosen, D. Sun, and C. Penney, *J. Bioact. Compat. Polym.,* **3,** 334 (1988).
15. D. Knorr and M. Daly, *Process Biochem.,* **23,** 48 (1988).

16. E. J. Dunn, X. Zhang, D. Sun, and M. F. A. Goosen, *J. Appl. Polym. Sci.,* **50,** 353 (1993).
17. M. L. Hughet, A. Grogoillot, R. J. Neufeld, D. Poncelet, and E. Dellacherie, *J. Appl. Polym. Sci.,* **51,** 1427 (1994).
18. S. Aiba, *Int. J. Biol. Macromol.,* **14,** 225 (1992).
19. O. Smidsrod, *Carbohydr. Res.,* **13,** 359 (1970).
20. S. Mima, M. Miya, R. Iwamoto, and S. Yoshikawa, *J. Appl. Polym. Sci.,* **28,** 1909 (1983).
21. J. G. Domszy and G. A. F. Roberts, *Makromol. Chem.,* **186,** 1671 (1985).
22. T. Sannan, K. Kurita, and Y. Iwakura, *Makromol. Chem.,* **177,** 3589 (1976).
23. G. G. Maghami and G. A. F. Roberts, *Makromol. Chem.,* **189,** 195 (1988).
24. G. Staikos, G. Bokias, and C. Tsitsilianis, *J. Appl. Polym. Sci.,* **48,** 215 (1993).
25. T. Sakiyama, C. Chu, T. Fujii, and T. Yano, *J. Appl. Polym. Sci.,* **50,** 2021(1993).
26. C. Chu, H. Kumagai, and K. Nakamura, *J. Appl. Polym. Sci.,* **60,** 1041 (1996).
27. S. Aiba, *Int. J. Biol. Macromol.,* **11,** 249 (1989).
28. Idem, *Ibid,* **13,** 40 (1991).
29. V. Chavasit, C. Kienzle–Sterzer, and J. A. Torres, *Polym. Bull.,* **19,** 223 (1988).
30. K. Abe, M. Koide, and E. Tsuchida, *J. Polym. Sci., Polym. Chem. Ed.,* **15,** 2469 (1977).
31. H. Ohno and E. Tsuchida, *Makromol. Chem., Rapid Commun.,* **1,** 585 (1980).