Effect of Preparation Method and Characteristics of Chitosan on the Mechanical and Release Properties of the Prepared Capsule

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ABSTRACT: The objective of this study is to elucidate the effect of preparation method and the characteristics of chitosan used on the mechanical and release properties of the prepared capsule. The characteristics of the chitosan explored include molecular weight (1.8, 5.6, 20.2, and 31.8 $\times$ 10^5 Dalton) and chain flexibility parameter (B), which was manipulated by a varying degree of deacetylation (DD, 67.9, 81.3, 90.5, and 92.2%), and sodium chloride concentration (0 or 0.3%). The orifice method was used to encapsulate hemoglobin, whereas complex coacervation was used to encapsulate the bovine serum albumin (BSA). The axial ratio was measured to characterize the appearance of the capsule. Break strength was used as an index of mechanical strength. Release percent of protein was used as a pore size indicator. The results show axial ratio and hemoglobin release percent of the capsule prepared by the orifice method increased with the increase of the chain flexibility parameter (B), but decreased with the increase of the chitosan molecular weight. However, break force behaved just opposite from that of the axial ratio and release percent of hemoglobin. The capsule cannot be prepared from 92.2% DD chitosan. Break strength and BSA release percent of the capsule prepared by complex coacervation did not vary with different DD, molecular weight of chitosan, and sodium chloride concentration. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **66:** 161–169, 1997

Key words: chitosan; capsule; release; mechanical properties; complex coacervation; orifice method

INTRODUCTION

Chitosan is a biopolymer and can be used as the wall material of the capsule. Chitosan capsule can be applied in food, drug, and biochemical areas. Methods to prepare the capsule include (1) complex coacervation[1–5]; (2) phase separation, for example, adjusting the solution pH to higher than the isoelectric point and then to precipitate, aggregate, and to form the wall material[6,7]; and (3) *in situ* polymerization in which oppositely charge compounds are mixed in solution. Polymerization occurs at the interface and forms the wall materials.[8,9] Hwang et al.[2] proposed that the morphology of the chitosan plays a significant role in the structure of the capsule membrane. The morphology of the chitosan molecules can be changed to form a special network that results in increasing the pore size in the wall. Shioya and Rha[10] reported the release rate of the chitosan–carboxymethylcellulose capsule increased by increasing the concentration of sodium chloride or phosphate. The release rate increase by adding sodium chloride is greater than by adding phosphate. However, the

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release rate of the capsule prepared from lower molecular weight chitosan is lower than that from higher molecular weight ones. Kim and Rha[11] reported that the release rate of chitosan alginate capsule increased by increasing pH or sodium chloride concentration of chitosan solutions. McKnight et al.[12] and Polk et al.[5] reported mechanical properties and release rates are better if the chitosan–alginate capsule was prepared from lower molecular weight chitosans than from higher molecular weight ones. Chen et al.[13] reported the effect of chain flexibility of chitosan on the preparation, physical, and release characteristics of the capsule prepared by the orifice method. The effect of different preparation methods (orifice or complex coacervation), molecular weight, and chain flexibility of chitosan on the mechanical and release properties of the prepared capsule are studied in this report.

MATERIALS AND METHODS

Preparation of Chitosan with Different Degrees of Deacetylation

Chitin was prepared by the method of Lin[14] and Chen et al.[15] from shrimp (*Solemocera prominenitis*) waste. Different DD chitosans were preparation by alkali deacetylation with 50% NaOH at 99°C for 1, 3, 6, and 9 h. During alkali deacetylation, nitrogen was flushed into the reaction chamber to alleviate the depolymerization effect.[16]

Weight-Average Molecular Weight

A light-scattering method was used to measure the weight-average molecular weight of the chitosan prepared. Different concentrations (0.01–0.1 g/L) of chitosan in pH 4, 0.2*M* acetic acid–sodium acetate solution were prepared. The solution was filtered through a 0.02 μm filter and measured the scattered light intensity between 30 to 140 degrees by a Malvern light-scattering photometer (Malvern 4700, UK) with 632.8 nm at 25 ± 0.1°C. The weight-average molecular weight was calculated from the Zimm plot[17–19] processed by Malvern software. The refractive index increment (dn/dc) of the solution and solvent was determined by an Interferometric refractometer (Wyatt/Optilab 903, USA).

Degree of Deacetylation

The infrared spectroscopy method[20] was followed. Chitosan and KBr at a ratio of 1 : 100 was mixed well, dried, and then made into a disc. The spectrum was measured by an IR spectrometer (Hitachi, 260–30, Japan), and the degree of deacetylation was calculated by the following equation:

$$\text{Degree of deacetylation} = 100 - (A_{1655}/A_{3450}) \times 115$$

where A_{1655} is absorbance of the amide 1 band at 1655 cm^{-1}, and A_{3450} is the absorbance of the O—H band at 3450 cm^{-1}.

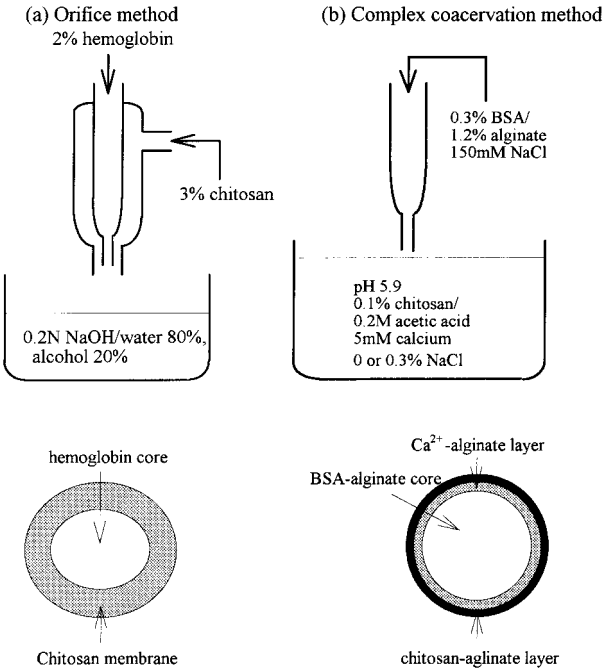


Figure 1 Encapsulation apparatus and hypothetic structure of capsules.

Capsule Preparation

Orifice Method

The orifice method[6] was modified. Three percent chitosan solutions were prepared by dissolving different DD chitosans in different pH (2, 3, and 4), and ionic strength (without or with 0.3% sodium chloride) solutions. The chitosan solution flowed down via the outer tube, whereas the core material (2% hemoglobin) flowed down by the inner tube. Adjusting the flow rate of both solutions was used to encapsulate the core material. The capsule was solidified in the cure bath containing 0.2*N* NaOH/water : alcohol = 8 : 2 for 3 min. The capsule was removed and washed to neutrality [Fig. 1(a)].

Table I Effect of Characteristics of Chitosan and Concentration of Sodium Chloride on the Axial Ratio, Break Force, and Released Percentage of Capsules for 24 h (R24)

DD (%)	M_w ($\times 10^5$ dalton)	B	NaCl (%)	Axial Ratio	Break Force (g)	R24 (%)	T60 (h)
67.9	31.8	0.0552	0	1.21	352.6 ± 16.4	65.3	12.0
			0.3	1.23	308.3 ± 14.5	69.8	8.0
81.3	20.2	0.0669	0	1.30	230.7 ± 15.3	72.1	6.0
			0.3	1.32	215.4 ± 17.6	81.6	5.0
90.5	5.6	0.0743	0	1.32	219.9 ± 16.1	78.5	5.5
			0.3	1.37	159.3 ± 13.8	91.1	3.5
92.2	1.8	0.0846	0	—	—	—	—
			0.3	—	—	—	—

DD: Degree of deacetylation, determined by infrared spectrophotometry.[20]
M_w: Weight average molecular weight, determined by light scattering.[17,19]
R24: The maximum released percentage of hemoglobin for 24 h from chitosan capsules prepared by orifice method at 25 ± 0.1°C.
T60: The time to release 60% of hemoglobin from chitosan capsules at 25 ± 0.1°C.
B: Chain flexibility parameter are cited from Tsaih.[22]
—: Capsule can't form.
The data of axial ratio, break force, and R24 are the mean of 15, 15, and 3 determinations, respectively.

Complex Coacervation

The method of Hwang et al.[2] was followed. Different DD chitosans (0.1%) (67.9, 81.3, 90.5, and 92.2%) were dissolved in 0.2*M* acetic acid, sodium chloride (0 or 0.3%), and 5 m*M* calcium acetate, and adjusted pH to 5.9. The core material, 0.3% bovine serum albumin, was mixed with 1.2% sodium alginate containing 150 m*M* NaCl. The BSA alginate solution flowed through a glass tube (0.9 mm i.d.) and dropped into the chitosan acetic acid solution to form the capsule. The droplet, capsule, was cured for 5 min before being collected and washed [Fig. 1(b)].

Characteristics of the Capsule

Appearance of the Capsule

The long- and short-axial dimensions of the capsule were measured by a caliper (Mitutoyo, Japan). The axial ratio was calculated from the ratio of the long to the short axial and used as an index of the appearance. Fifteen measurements were determined for each experiment.

Break Strength

A flat plunger was used to measure the break strength by a rheometer (Rheometer CR-300, Sun Scientific, Japan). The plunger speed was 1 cm/min. Fifteen measurements were performed for each experiment.

Release Test

About 50 g of the capsule were placed in a 1 L beaker filled with distilled water at 25 ± 0.1°C. A 10 mL solution was piped out at predetermined time periods. The Bio-Rad protein assay method was employed to measure the protein content by a spectrophotometer (Hitachi U-2000, Japan). The release percent was calculated from the change of protein concentration during the time course and used as an index of capsule pore size.

RESULTS

Effect of Molecular Weight, Chain Flexibility of Chitosan on the Axial Ratio, and Break Strength of the Capsule Prepared by the Orifice Method

Table I shows the axial ratio of the capsule increased with the increase of chain flexibility parameter (B) of the chitosan used. An Axial ratio of 1.21 was measured from the capsule prepared from chitosan with a chain flexibility parameter (B) equal to 0.0552, compared to 1.32 from chitosan with a B value of 0.0743. However, break strength decreased with the increase of chain flexibility parameter (B) of the chitosan used, with break strengths of 352.6 g compared to 219.9 g for the same two capsules. The capsules prepared from higher molecular weight chitosans were higher in break strength and lower in axial

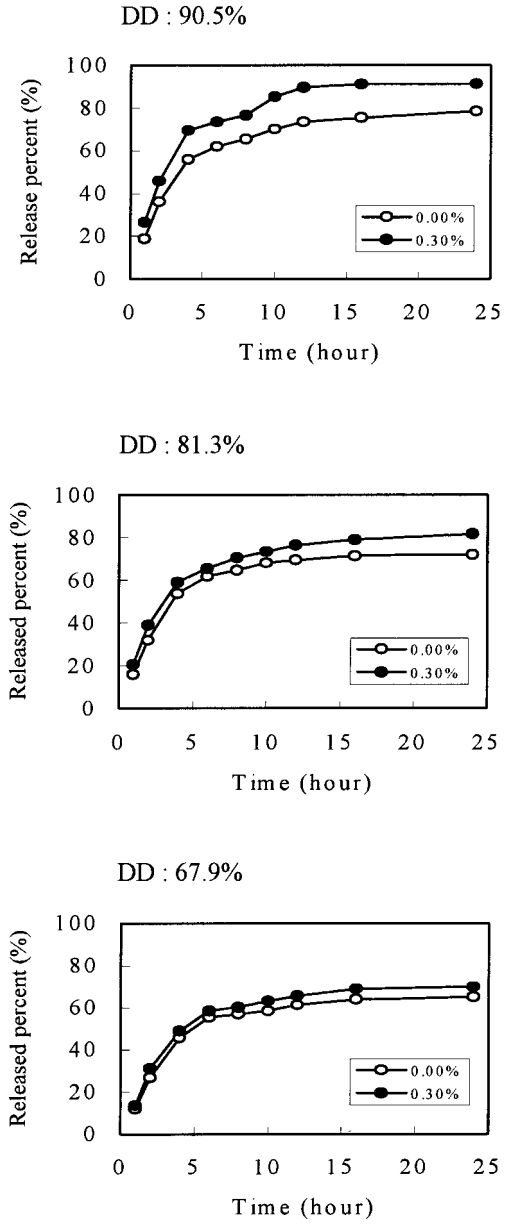


Figure 2 Effect of degree of deacetylation (90.5, 81.3, 67.9%) and concentration of sodium chloride (0, 0.3%) on release percent of hemoglobin from chitosan capsules prepared by the orifice method. Hollow circles: no sodium chloride added; solid circles: 0.3% soldium chloride added. The release percent was measured at 25 ± 0.1°C.

ratio than those prepared from lower molecular weight chitosans. An axial ratio of 1.21 and break strength of 352.6 g was measured from the capsule prepared from 31.8×10^5 Dalton chitosan compared to an axial ratio of 1.32 and break strength of 219.9 g prepared from 5.6×10^5 Dalton chitosan.

Effect of Molecular Weight and Chain Flexibility of Chitosan on the Release Characteristic of the Capsule Prepared by the Orifice Method

Figure 2 shows that the release percent of hemoglobin increased with the increase of DD of chitosan used in capsule preparation. The release percent for 24 h was 65.3% for the capsule prepared from 67.9% DD chitosan compared to 78.5% for the capsule prepared from 90.5% DD chitosan. The chain flexibility parameter (B) of chitosans were higher for those higher DD chitosans than that of the lower DD chitosans (Table I). Table I show the time needed to release 60% of hemoglobin (T60) was 12 h and 5.5 h for capsules prepared from 67.9 and 90.5% DD chitosan, respectively. Both results indicate that the capsules prepared from higher DD chitosans have larger pore sizes or higher permeability than those prepared from lower DD chitosans. The capsules prepared from solutions contained 0.3% sodium chloride have a higher release percent (Fig. 2), lower T60 (Table I) than those prepared from solutions containing no sodium chloride.

Break Strength of the Capsule Prepared by Complex Coacervation

Table II shows the break strength of the capsules did not vary with the differences of the molecular weight or chain flexibility parameter (B) of the chitosans used. The break strengths are near 406 ± 6 g, regardless of the ionic strength difference achieved by vary the sodium chloride concentration in chitosan–acetic acid solution.

Release Characteristics of the Capsule Prepared by Complex Coacervation

Release percent and release percent for 24 h of BSA from capsules prepared by complex coacervation were shown in Figure 3 and Table II, respectively. The results show release characteristics did not vary with the variation of the molecular weight or chain flexibility parameter (B) of chitosans used or the variation of sodium chloride concentration in chitosan–acetic acid solutions. The release percentages for 24 h are around 17 ± 1%.

DISCUSSION

Effect of Molecular Weight, Chain Flexibility of Chitosan on the Axial Ratio, and Break Strength of the Capsule Prepared by the Orifice Method

Results in Table I show the capsules prepared from higher molecular weight chitosans were

Table II Effect of Characteristics of Chitosan and Concentration of Sodium Chloride on Break Force and the Maximum Released Percentage for 24 h (R24) of Chitosan–Alginate Capsules Prepared by Complex Coacervation

DD (%)	M_w ($\times 10^5$ dalton)	B	NaCl (%)	Break Force (g)	R24 (%)
67.9	31.8	0.0552	0	402.6 ± 18.6	16.1
			0.3	398.7 ± 16.3	16.9
81.3	20.2	0.0669	0	406.2 ± 15.8	18.0
			0.3	411.8 ± 20.9	16.2
90.5	5.6	0.0743	0	409.4 ± 17.4	17.6
			0.3	399.5 ± 15.1	17.5
92.2	1.8	0.0846	0	406.4 ± 19.6	15.6
			0.3	407.2 ± 14.2	16.3

DD, M_w, B, R24: Same as Table I.
R24: The maximum released percentage of BSA for 24 h from chitosan–alginate capsules prepared by complex coacervation at 25 ± 0.1°C.
The data of break force, and R24 are the mean of 15 and 3 determinations, respectively.

higher in break strength and lower in axial ratio than those prepared from lower molecular weight chitosans. This may be due to the fact that during capsule formation larger molecules will form more entanglements and result in stronger capsules than those of smaller molecules. Therefore, the capsules prepared from higher molecular weight chitosans were higher in break strength and lower in axial ratio.

Table I also shows that the axial ratio of the capsules increased, however, break strength decreased with the increase of chain flexibility parameter (B) of the chitosan used. The molecule with a higher chain flexibility parameter (B) indicates that the molecule is more flexible than that of the lower flexible parameter (B). This may be due to the fact that the flexible molecule tends to be a random coil in the solution.[15,19,21] The coil molecule will result in less entanglement, which in turn, weaken the structure to hold the core material. So the capsule will be higher in axial ratio and lower in break strength.

The relationship between the chain flexibility parameter (B), molecular weight of chitosan used, and the axial ratio and break strength of the capsules prepared were shown in Figures 4 and 5, respectively. Their linear relationship and correlation coefficient were also listed. These relationships indicate that the control of the chain flexibility and molecular weight of chitosan used are the key factors to control the appearance and mechanical properties of the prepared capsule. Higher axial ratio capsules can be prepared from more flexible or lower molecular weight chitosans but give lower break strengths.

Table I also show capsules prepared from solutions containing 0.3% sodium chloride were lower in break strength and larger in axial ratio than those from solutions free of sodium chloride. This may be due to the counterion effect, which depressed the third electroviscous effect and resulted in the chitosan molecules to be more flexible and random coil in solution.[14,21–23] The coil molecule will result in less intermolecular entanglement; therefore, will be larger in axial ratio and smaller in break strength.

Effect of Molecular Weight and Chain Flexibility of Chitosan on the Release Characteristics of the Capsule Prepared by the Orifice Method

The results in Figure 2 show release percent of hemoglobin increased with the increase of DD of chitosan used in the capsule prepared. It indicated that the capsules prepared from higher DD chitosans have larger pore sizes. This may be due to the fact that chain flexibility of higher DD chitosan are higher than lower DDs.[15,21,24] The flexible molecule tends to be the random coil in the solution.[19,21] Coil molecules tends to form more intramolecular hydrogen bond but less intermolecular entanglement. Therefore, the capsules prepared from higher DD chitosans were higher in enthalpy,[13] a higher axial ratio, but lower break strength and higher release percent than those prepared from lower DDs (Table I).

Figure 2 also show the capsules prepared from solutions containing 0.3% sodium chloride were higher in release percent than those from sodium chloride-free solutions. It also indicated that the

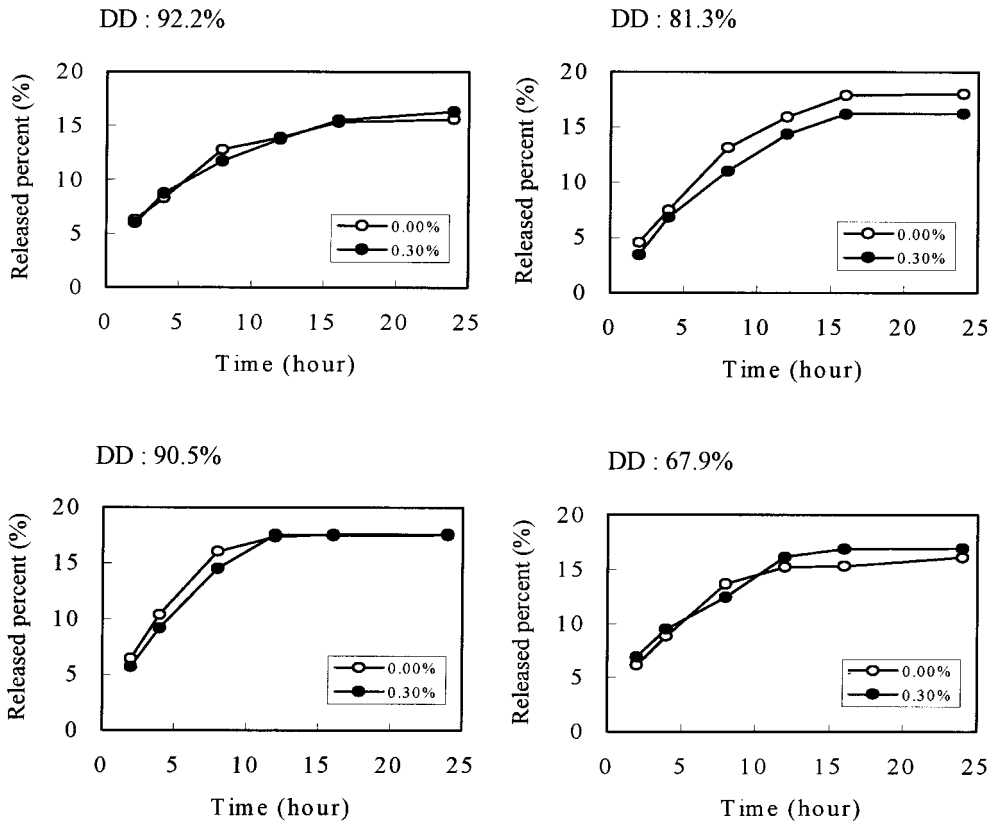


Figure 3 Effect of degree of deacetylation (92.2, 90.5, 81.3, 67.9%) and concentration of sodium chloride (0, 0.3%) on release percent of BSA from chitosan capsules prepared by the complex coacervate method. Hollow circles: no sodium chloride added; solid circles: 0.3% sodium chloride added. The release percent was measured at 25 ± 0.1°C.

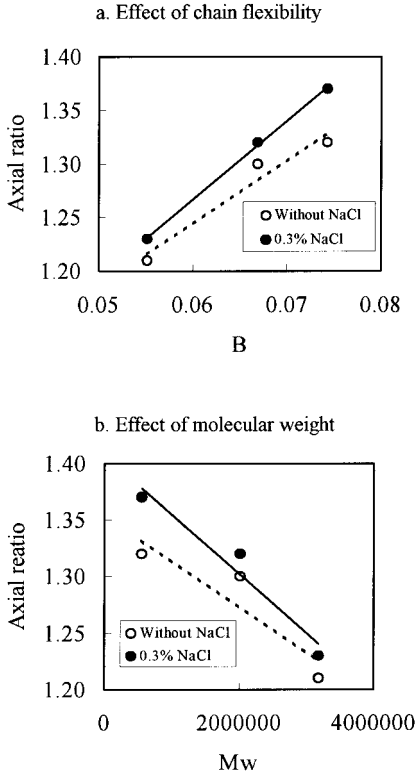
counterion effect retarded the third electroviscous effect that rendered the molecules to become less extended. The coil molecule resulted in larger pore sizes in the matrix mentioned, therefore more hemoglobin released.

The relationship between chain flexibility parameter (B), molecular weight of chitosan used, and the R24 of the prepared capsules were shown in Figure 6. Their linear relationship and correlation coefficient were also listed. These good relationships indicate the control of chain flexibility or molecular weight of chitosan used is the key factor to control the released properties (R24) of the capsules prepared. Higher release capsules can be prepared from more flexible or lower molecular weight chitosans.

Break Strength and Release Characteristic of the Capsule Prepared by Complex Coacervation

Results in Table II and Figure 3 show break strength and release characteristics of the capsules prepared by complex coacervation did not vary with the variation of the molecular weight or chain flexibility parameter (B) of chitosan used. It may due to the fact that the conditions used did not result in different chain flexibility needed to have different pore size, as mentioned previously.

Daly and Knorr,[1] and Knorr and Daly[3] reported that mechanical properties of capsule prepared by complex coacervation cannot be manipulated via changing the properties of chitosan used. It may due to the fact that the properties of the membrane are dominated by the Ca^{2+}–alginate layer and not by the chitosan–alginate complex layer shown in Figure 1(b). The mechanical properties of the membrane can be varied by changing the concentration of alginate,[1,5] degree of esterification, type of alginate,[3] plasticizer such as calcium chloride, glucose and its concentration,[1,3] or adjust the pH of release solutions.[5] However, McKnight et al.[12] and Polk et al.[5] reported mechanical properties of the complex coacervation capsule prepared by using lower molecular weight



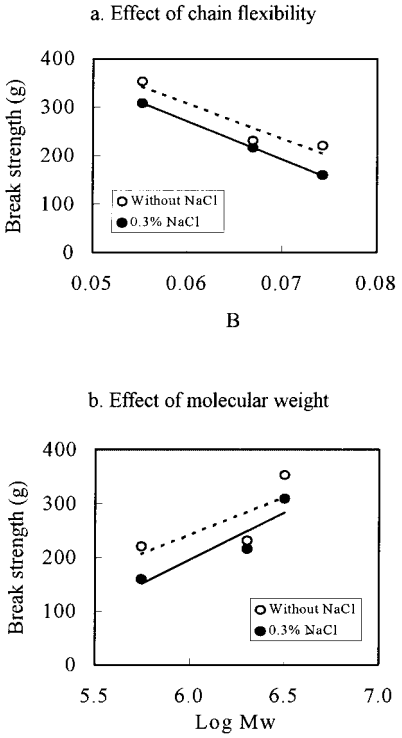
	Effect of chain flexibility	Effect of molecular weight
Without NaCl	y = 7.3626x+0.8247	y = -4E-08x+1.355
	R^2 = 0.9988	R^2 = 0.8351
0.3% NaCl	y = 5.9339x+0.8882	y = -5E-08x+1.4077
	R^2 = 0.9512	R^2 = 0.9482

Figure 4 Effect of chain flexibility parameter (B) (a) and molecular weight (b) on the axial ratio of chitosan capsules prepared by the orifice method.

chitosan and alginate is larger than that prepared from higher molecular weight chitosan.

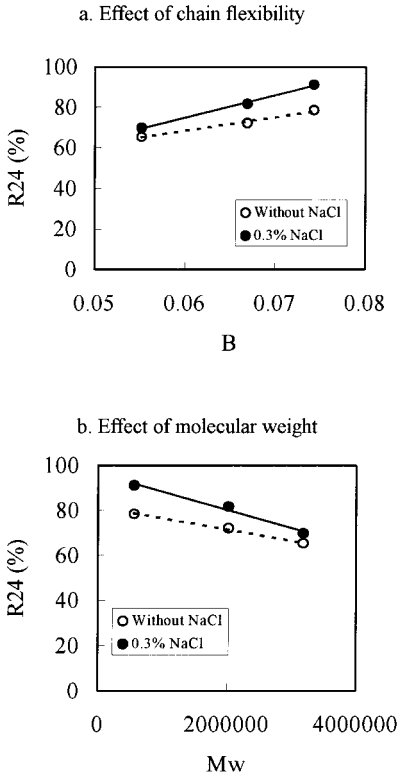
The release properties of the complex coacervation capsule can be enhanced by increasing the degree of esterification of alginate.[3] Polk et al.[5] reported the release rate of the complex coacervation capsule prepared by larger or mixture of larger and small molecular weight chitosan and alginate is larger than that prepared from smaller molecular weight chitosan. It may due to the fact that the larger molecular weight chitosan and alginate will result in stronger ionic interaction. Furthermore, smaller molecular weight chitosan will penetrate into the network, thus resulting in a smaller pore size in the capsule membrane. The discrepancy between various literature sources and the inconsistency with the results reported in Table II and Figure 3 may be due to the fact that the release characteristics of the capsule membrane are dominated by the Ca^{2+}–alginate layer not by the chitosan–alginate complex layer shown in Figure 1(b). The release properties of the capsule can be enhanced by increasing the degree of esterification of alginate[3] owing to minimizing the charge effect or increaseing the steric effect.

If the release characteristic of the capsule membrane is dominated by the chitosan–alginate complex layer; however, the conditions used did not result in significance different chain flexibility needed to have different pore size. This may be due to the effect of calcium ion or chloride ion contained in the system. In regard to the effect of chloride ion, because it is a counterion of chitosan, chloride ion will neutralize the protonated amine group needed to interact ionic with alginate in complex coacervation processes. At pH 5.9, the degree of ionization is quite low (pI of chitosan $\fallingdotseq$ pH 6.3[25]). Furthermore, the chitosan solution used contained 52 m*M* chloride ion (0.3%). The



	Effect of chain flexibility	Effect of molecular weight
Without NaCl	y = -7261.5x+743.12	y = 5E-05x+174
	R^2 = 0.9004	R^2 = 0.7563
0.3% NaCl	y = -7813.6x+739.2	y = 6E-05x+119.98
	R^2 = 0.9998	R^2 = 0.9575

Figure 5 Effect of chain flexibility parameter (B) (a) and molecular weight (b) on break strength of chitosan capsules prepared by the orifice method.



	Effect of chain flexibility	Effect of molecular weight
Without NaCl	y = 681.16x+27.373	y = -8E-06x+96.311
	R^2 = 0.9876	R^2 = 0.9836
0.3% NaCl	y = 1105.5x+8.4572	y = -5E-06x+81.587
	R^2 = 0.9955	R^2 = 0.993

Figure 6 Effect of the chain flexibility parameter (B) (a) and molecular weight (b) on maximum rleased percentage for 24 h (R24) of hemoglobin from chitosan capsules prepared by the orifice method at 25 ± 0.1°C.

chloride ion will shield the protonated amine groups existing on the backbone of the chitosan and will result in very low ionic interaction between chitosan and alginate. Therefore, the conditions used did not result in different chain flexibility, which is needed to give differences in pore size in the capsule matrix. In regard to the effect of calcium ion, it will interact with alginate stronger than with chitosan. During curing, chitosan will be replaced gradually by calcium ion and will result in calcium alginate layer, especially at the conditions where the degree of ionization of chitosan is very low. Base on the above analysis, the properties of the complex coacervation capsule shall be dominated by the Ca^{2+}–alginate layer, not by the chitosan–alginate complex layer. Therefore, the properties of the complex coacervation capsule did not vary with the variation of molecular weight or chain flexibility parameter (B) of chitosan used.

Methods to improve the release properties of the complex coacervation capsule includes (1) varying the pH of chitosan–acetic acid solution[2]; (2) reverse the procedure of Figure 1(b) by dropping the chitosan solution into the alginate curing solution such as reported previously[1,3]; (3) the two-stage process of Pandya and Knorr.[4] Stage one is to form the bead of alginate or kappa-carrageenanin with calcium chloride or potassium chloride solution. Stage two is to remove the bead and then to solidify the bead in the chitosan solution.

CONCLUSIONS

The axial ratio and hemoglobin release percent of the capsule prepared by the orifice method increased with the increase of chain flexibility of chitoasan but decreased with the increase of chitosan molecular weight. However, the break force behaved just opposite from that of the axial ratio and release percent of hemoglobin.

Break strength and BSA release percent of the capsule prepared by complex coacervation did not vary with chain flexibility or molecular weight of chitosan.

The capsule cannot be prepared from 92.2% DD chitosan may be due to too small a molecular weight and/or too flexibility of the molecules.

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