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Effects of chain flexibility of chitosan molecules on the preparation, physical, and release characteristics of the prepared capsule

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The effects of chain flexibility of chitosan molecules on the preparation, physical, and release characteristics of the prepared capsule were studied. The chain flexibility of chitosan molecules in solution was manipulated by using chitosans with different degrees of deacetylation (DD) (67.9%, 81.3%, 90.5%, 92.2%), different pHs (2.0, 3.0, 4.0), and NaCl concentrations (0, 0.3%). The solutions were then used to encapsulate hemoglobin or dextran by the orifice method to make the capsules. Axial ratios and break strengths were used to characterize the appearance and mechanical properties of the prepared capsules. D. S. C. was employed to measure enthalpies and maximum melting temperatures to be used as a quantitative index of hydrogen bond formation in the walls of the capsules. Release rate was used as a pore size indicator. The results show axial ratios of the capsules increased with a decrease in molecular weight and also with an increase in DD of chitosan used. Axial ratios also increased when 0.3% NaCl was added to the solutions used. The effects of solution pH show that capsules prepared from pH 3.0 solutions were smaller than those from pH 2.0 and/or pH 4.0 solutions. Capsules of lower break strengths had higher axial ratios. The enthalpies of the chitosan capsules increased with increasing DD of chitosans used, and also with increasing NaCl concentration in the solutions used. However the maximum melting temperatures showed no linear relationship with the DDs of chitosan used. Maximum melting point temperatures of capsules also increased with an increase in NaCl concentrations in solutions used. The hemoglobin and dextran release percentages from the capsule at 25°C for 24 h increased with an increase of DD of chitosan used. The release rate also increased with the addition of NaCl in the chitosan solution used. The release percentages of capsules prepared from pH 4.0 solutions were higher than those from pH 2.0 and/or pH 3.0 solutions. Release percentages of dextran of different molecular weights from capsules of 81.3% DD chitosan and pH 3 solutions were 52.7% for 19600 Da dextran, 26.3% for 87000 Da dextran and 12.4% for 162000 Da dextran. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Chitin and its deacetylated product, chitosan, are high molecular weight biopolymers and are recognized to be versatile, enviromentally friendly raw materials (Zakaria *et al.*, 1995). The applications of these chitinous materials include agriculture, food processing and biotechnology (Brine *et al.*, 1992; Muzzarelli, 1977; Skjåk-Bræk *et al.*, 1989). The chitinous materials can be used in different forms: as flakes, powders, solutions, gels, membranes, or capsules. Chitinous capsules are prepared for use as control release vectors for application in food processing and for biochemical reagents (Beaumont *et al.*, 1989; Groboillot *et al.*, 1993; Kim & Rha, 1989; Pandya & Knorr, 1991; Shinonaga *et al.*, 1992). Three methods are used to prepare capsules. (1). Complex coacervation is a process in which chitosan is used as a cationic polyelectrolyte to interact with an anionic polyelectrolyte such as alginate, carrageenan, or arabic gum to form the wall material (Daly & Knorr, 1988; Hwang *et al.*, 1986; Knorr & Daly, 1988; Panda & Knorr, 1991; Polk *et al.*, 1994). (2). Adjustment of the solution pH to beyond the isoelectric point (pI) of chitosan causes precipitation and aggregation forming the wall material (Lin, 1991; Rodriguez-Sanchez & Rha, 1981). (3). *In situ* polymerization is used in which opposite charge compounds such as polyphosphate and chitosan are mixed in the solution. Polymerization occurs in the interfacial and forms the wall

material (Beaumont & Knorr, 1987; Vorlop & Klein, 1981). Hwang *et al.* (1986) proposed that the morphology of chitosan plays a significant role in the structure of the capsule membrane. The intrinsic viscosity decreases as the ionic strength or pH increases. This will allow the chains to come close together and cause the chains to form a special network which results in an increase of the average pore size in the matrix. Chen *et al.* (1994) reported on the manipulation of pore size of the membrane by using different chain flexibility solutions prepared from different degrees of deacetylated chitosan, and different pHs and ionic strengths. Persistence length, Smidsrød and Haug parameter, characteristic ratio (C_∞), and Kuhn statistical segment are among parameters which have been used to characterize chain flexibility. The chain flexibility of chitosan was reported to be affected by varying the degrees of deaacetylation (DD) of chitosan, the solution pH, and the ionic strength (Chen *et al.*, 1994; Tsaih *et al.*, 1995). The effects of chain flexibility of chitosan in solution by manipulation of different DD chitosans, solution pHs, and the presence or absence of sodium chloride on the preparation, physical, and release characteristics of capsules prepared by the orifice method were studied.

MATERIALS AND METHODS

Preparation of chitosans with different degrees of deacetylation

Chitin was prepared by the modified method of Stanley *et al.* (1976) and Lai (1979) from shrimp (*Solenocera prominentis*) wastes. Different DD chitosans were prepared by alkali deacetylation of chitin with 50% NaOH at 95, 110 and 140°C for various alkali treatment times (Chen *et al.*, 1994).

Capsule preparation

The orifice method reported by Lin (1991) was followed. Solutions were prepared by dissolving different DD chitosans in solutions with different pHs (2.0, 3.0 and 4.0), and ionic strengths (with or without 0.3% sodium chloride). The chitosan solution flowed down the concentric tube, via the outer tube, whereas the core material (hemoglobin or dextran) flowed down via the inner tube. The flow rate of the two streams was adjusted so as to have the core material be encapsulated then fall into the curing bath to solidify the walls. After curing for 3 min, the capsule was removed and washed with water.

Characteristics of the capsule

Appearance of the capsule
The long axis and short axis of the capsule were measured by a caliper (Mitutoyo, Japan). The axial ratio was calculated from the ratio of the long to the short axes and used as an index of the appearance.

Break force
The break force was measured by a rheometer (Rheometer CR-300, Sun Scientific, Japan) with a flat plunger at a cross-head speed of 1 cm/min. Fifteen capsules were measured for each experiment.

Thermal properties
One capsule was sealed in an aluminum pan and placed in the cell of a differential scanning calorimeter D. S. C. (DuPont TA2000, DSC 10, USA) with the reference and then heated at a heating rate of 10°C /min to 200°C. The energy needed in phase transition was used to calculate the enthalpy, and the maximum temperature on the thermogram was termed as maximum melting point temperature. Measurements on capsules from each experiment were done in triplicate (Lin, 1992; Hwa, 1994).

Release test
About 50 g of capsule was placed in a 1 liter beaker that was filled with distilled water at 25°C. At predetermined time periods, 10 ml of solution was pipetted out to measure the protein content by the Bio-Rad protein assay method, whereas the Somogyi method was used to determine the total glucose content with a spectrophotometer (Hitachi U-2000, Japan). The release percentage was calculated from the change of concentration during the time course and used as an index of pore size. Triplicate samples were tested for each experiment.

RESULTS

Effects of molecular weight and chain flexibility of chitosan on the characteristics of the prepared capsules

Table 1 presents the effect of molecular weight and chain flexibility of chitosan on the preparation and physical characteristics of the capsules. The results show that capsules can not be prepared from 92.2% DD chitosan solutions regardless of the pH or ionic strength of the solution. Capsules can not be prepared from 90.5% DD chitosan with either pH 2.0 or pH 4.0 solutions. However, capsules can be prepared from 90.5% DD chitosan with pH 3.0 solutions regardless of their ionic strength. The results also show that capsules prepared from lower DD chitosan solutions had lower axial ratios than those prepared from higher DD chitosans of the same pH or ionic strength solutions. The capsules prepared from 67.9% or 81.3% DD chitosan and pH 3.0 solutions had lower axial ratios and higher break forces than those prepared from pH 2.0 or pH 4.0 solutions of the same DD chitosan. Capsules prepared

Table 1. The effect of molecular weight and chain flexibility of chitosan on the preparation, axial ratio, and break force of capsules prepared by the orifice method

DD (%)	M_w ($\times 10^5$)	pH	NaCl	Axial ratio	Break force (g)	*B* value[a]	$[\eta]^*$ (dl/g)
67.9	31.8	2	–	1.30	253.8	0.0422	9.148
		3	–	1.21	352.6	0.0552	20.217
		3	+	1.23	308.3	0.0552	5.478
		4	–	1.32	209.8	0.0144	5.668
81.3	20.2	2	–	1.34	170.1	0.0455	10.189
		3	–	1.30	230.7	0.0669	21.839
		3	+	1.32	215.4	0.0669	5.928
		4	–	1.35	158.2	0.0153	6.541
90.5	5.6	2	–	—	—	0.0497	9.273
		3	–	1.32	219.9	0.0743	20.364
		3	+	1.37	159.3	0.0743	5.609
		4	–	—	—	0.0157	5.245
92.2	1.8	2	–	—	—	0.0540	6.014
		3	–	—	—	0.0846	11.588
		3	+	—	—	0.0846	3.566
		4	–	—	—	0.0164	3.603

DD: Degree of deacetylation, determined by IR spectrophotometry (Baxter *et al.*, 1992).
M_w: Weight average molecular weight, determined by light scattering (Anthonsen *et al.*, 1994; Wang *et al.*, 1991).
–: No NaCl added.
+: 0.3% NaCl added.
—: Capsule couldn't form.
[a]These data are cited from Tsaih (1993).
The data of axial ratio and break force are the means of 15 determinations.

from 67.8% or 81.3% DD chitosan and pH 2.0 solutions had smaller axial ratios, but higher break forces than those prepared from their respective pH 4.0 solutions. Capsules prepared from chitosan (67.8%, 81.4%, or 90.5% DD) solutions without sodium chloride had smaller axial ratios but larger break forces than those from their corresponding solutions containing 0.3% sodium chloride.

Effects of chain flexibility of chitosan on the thermal properties of the prepared capsules

Table 2 shows that the enthalpies of the capsules prepared from higher DD chitosans were larger than those prepared from lower DD chitosans. Enthalpies of 250.9 and 290.0 cal/g were measured from capsules prepared from solutions of 67.9% DD chitosan, with 0% and 0.3% sodium chloride, respectively. Whereas, values of 365.5 and 398.0 cal/g were measured for capsules prepared from their corresponding 92.2% DD chitosan solutions, respectively. This indicates that the enthalpies of capsules prepared from solutions containing 0.3% sodium chloride were higher than those solutions free of sodium chloride. The effect of chitosan DD on maximum melting point temperatures of the capsules showed no regular trends. The results show that the maximum melting point temperatures of capsules prepared from 81.3% DD chitosans were higher than those prepared from their counterparts with either 67.9% or 90.5% DD chitosans. However, capsules prepared from solutions containing 0.3% sodium chloride had higher maximum melting point temperatures than those free of sodium chloride. The effect are similar to those for enthalpy.

Effects of chain flexibility of chitosan on the release characteristics of capsules

Release characteristics of hemoglobin

Figure 1 shows the effect of degree of deacetylation of chitosan (a), concentration of sodium chloride (b), and solution pH (c) used in preparing the capsules on the

Table 2. The maximum melting point temperature (°C) and enthalpy of melting (cal/g) of capsules[a] prepared from chitosans with different DD (67.9%, 81.3%, 90.5%) and different concentration (%) of sodium chloride

DD (%)	NaCl (%)	EM[b]	MPT[c]
67.9	0	250.9	139.35
	0.3	290.0	147.26
81.3	0	297.2	146.75
	0.3	343.1	184.27
90.5	0	365.5	138.05
	0.3	398.0	155.26

[a]Capsules were prepared by the orifice method from chitosan solutions of pH 3.0, with different DDs and NaCl concentrations.
[b]Enthalpy of melting.
[c]Maximum melting point temperature.
Enthalpies and maximum melting point temperatures were calculated from an endothermograph. The samples were heated at a heating rate of 10°C/min to 200°C.
Data of EM and MPT are the means of triplicates.

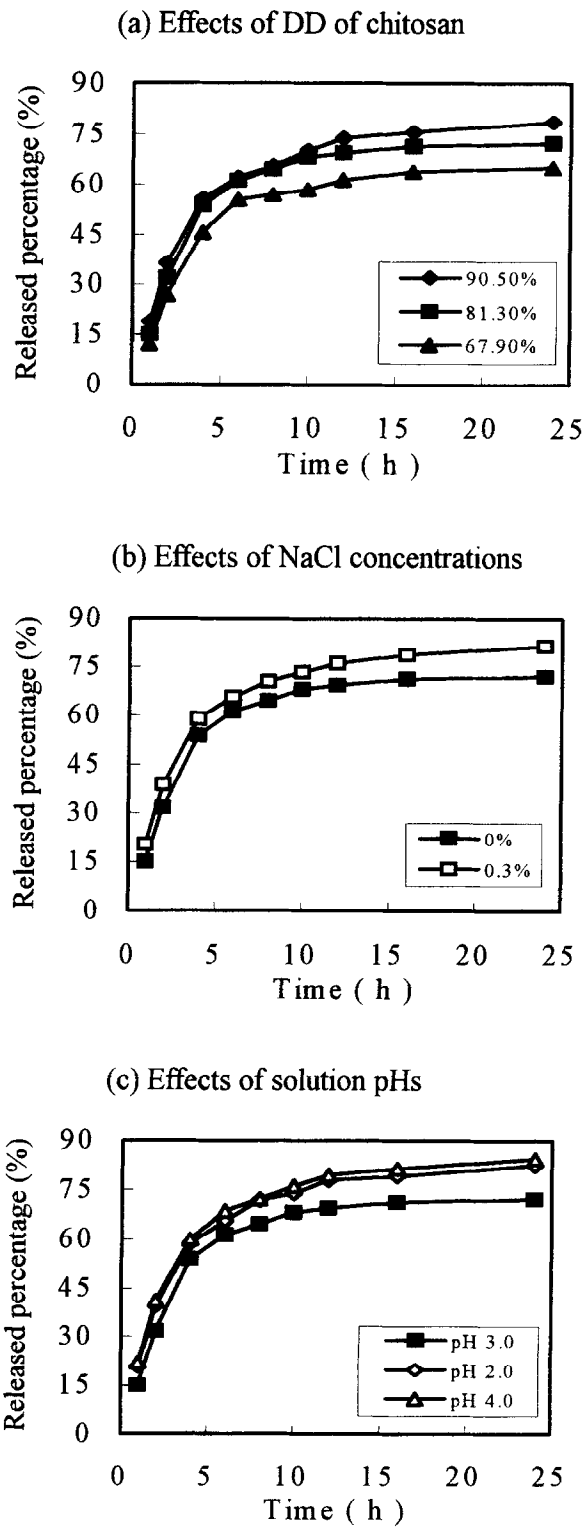


Fig. 1. Effects of DD (a), sodium chloride concentrations (b) and solution pHs (c) on the released percentage of hemoglobin from chitosan capsules prepared by the orifice method. The release percentages were measured at 25±0.1°C. Hemoglobin concentration was measured by the Bio-Rad protein assay method. (a) Capsules prepared from solutions with various DD chitosans in solutions of pH 3.0 without sodium chloride. (b) Capsules prepared from 81.3% DD chitosan in pH 3.0 solutions. (c) Capsules prepared from 81.3% DD chitosan without sodium chloride solutions. The data are means of triplicates.

release percentages of hemoglobin measured at 2.5±0.1°C. The results show that the release percentage of hemoglobin from capsules increased with an increase in DD of chitosan used in capsule preparation (Fig. 1a). Capsules prepared from solutions containing 0.3% sodium chloride had higher hemoglobin release percentages than those from solutions free of sodium chloride (Fig. 1b). However, the release percentages of hemoglobin from capsules prepared from solutions of pH 2.0 and pH 4.0 were higher than those prepared from pH 3.0 solutions (Fig. 1c).

Release characteristics of dextran

Figure 2a shows that the release percentages of dextran from capsules prepared from solutions containing 0.3% sodium chloride were higher than those from solutions free of sodium chloride. Figure 2b shows that the release percentages of dextran from capsules prepared from pH 4.0 solutions were higher than those from pH 2.0 solutions which in turn were higher than those from pH 3.0 solutions.

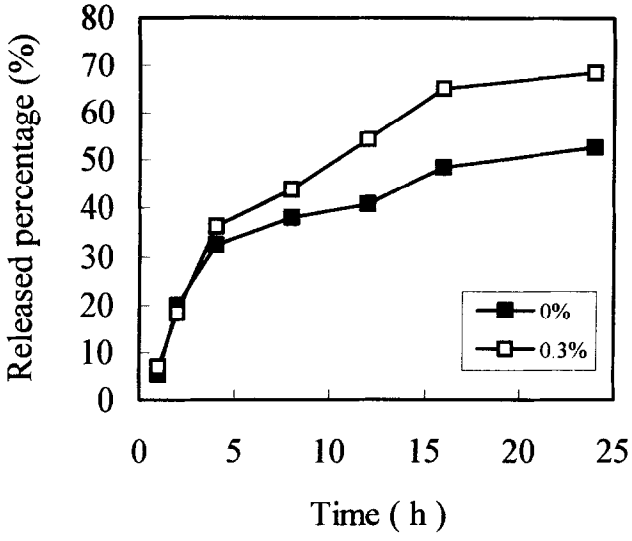
Release percentages of dextrans of different molecular weights from capsules prepared from 81.3% DD chitosan, pH 3.0 solution and without 0.3% sodium chloride are shown in Fig. 3. This figure indicates that the higher the molecular weight of dextran, the lower the release percent which results. The release percentages for 19600, 87000, and 162000 Da dextran were 52.7%, 26.3%, and 12.4% respectively after testing for 24 h at 25±0.1°C.

DISCUSSION

Effects of molecular weight and chain flexibility of chitosan on the preparation of capsules

Results in Table 1 show that capsules cannot be prepared from solutions of 92.2% DD chitosan by the orifice method regardless of the solutions' pH or ionic strength. Capsules can not be prepared from some solutions of 90.5% DD chitosan. However, capsule can be prepared from solutions of either 67.7% or 81.3% DD chitosan regardless of the solutions' pH or ionic strength. Molecular weights of 67.8% and 81.3% DD chitosan are both greater than 10^6 Da, whereas they are 1.8×10^5 and 5.6×10^5 Da for 92.2% and 90.5% chitosan, respectively. This indicates that the molecular weight of chitosan affected the success or failure of capsule preparation. Capsules could be prepared from chitosan of molecular weight 10^6 Da under the conditions studied perhaps because the chain lengths of the molecules were long enough to form hydrogen bonds or entanglements during the precipitation of the chitosan molecule, because the number of hydrogen bonds and entanglements formed should increase proportionally with molecular length. Increased entanglement occur-

(a) Effects of NaCl concentrations



(b) Effects of solution pHs

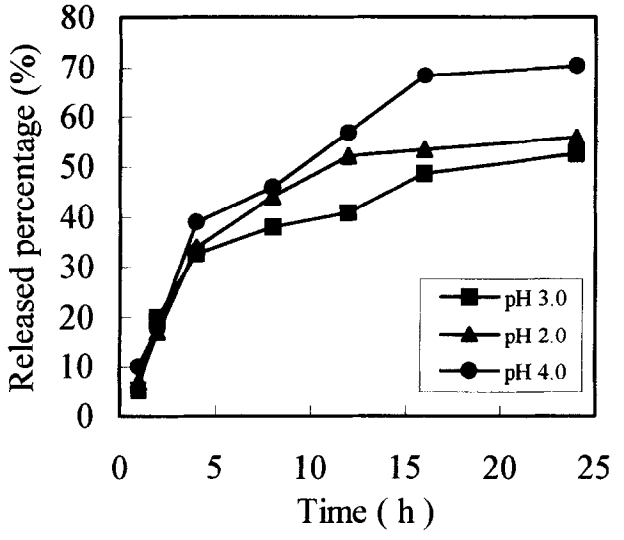


Fig. 2. Effects of sodium chloride concentrations (a) and solution pHs (b) on the release percentages of dextran (M_w: 19 600) from chitosan (DD:81.3%) capsules prepared by the orifice method. The release percentages were measured at 25±0.1 °C. Dextran concentrations were determined by the Somogyi method. (a) Capsules prepared from 81.3% DD chitosan and pH 3.0 solutions. (b) Capsules prepared from 81.3% DD chitosan without sodium chloride solutions. The data are means of triplicates.

ring during capsule formation should reinforce the capsule structure. Therefore, the molecular weight of chitosan affects the capability of capsule formation and its physical properties. However, capsules can be prepared from 90.5% DD chitosan and pH 3.0 solution but not with pH 2.0 or pH 4.0 solutions. This indicates that, in addition to molecular weight, chain flexibility may also affect capsule formation. This may be due to the chitosan molecule in the pH 3.0 solution being more extended and rigid than those in pH 2.0 or pH 4.0 solutions (Table 1). Furthermore, chain flexibilities of higher DD chitosans are higher than those of lower DD chitosans (Tsaih, 1993). Therefore the reasons that 92.2% DD chitosan was unable to form capsules by the orifice method used in this reported may be due to the compounded effects of small molecular weight and flexible molecules. However, the inability to prepare capsules from 90.5% DD chitosan with pH 2.0 or pH 4.0 solutions, but the ability to prepare them with pH 3.0 solutions may mainly be due to the chain flexibility effect.

Effects of molecular weight and chain flexibility of chitosan on the physical characteristics of the prepared capsules

Results in Table 1 show that capsules prepared from lower DD chitosans had smaller axial ratios and higher break forces than those prepared from higher DD counterparts. This may be due to chitosan with a larger molecular weight and a more extended molecule becoming more entangled and resulting in a stronger capsule during capsule formation. The stronger capsule will result in a nearly rounded shape, thus producing a smaller axial ratio, because the shape of the capsule is less affected by outside forces such as gravity. For those less entangled capsules, the shape will be more susceptible to external effects and thus result in a more ellipsoidal shape. This may also be due to the effects of chain flexibility, because the higher the DD of the chitosan, the more flexible the chain will be (Tsaih, 1993). Flexible molecules tend to be randomly coiled in the solution (Chen *et al.*, 1995;

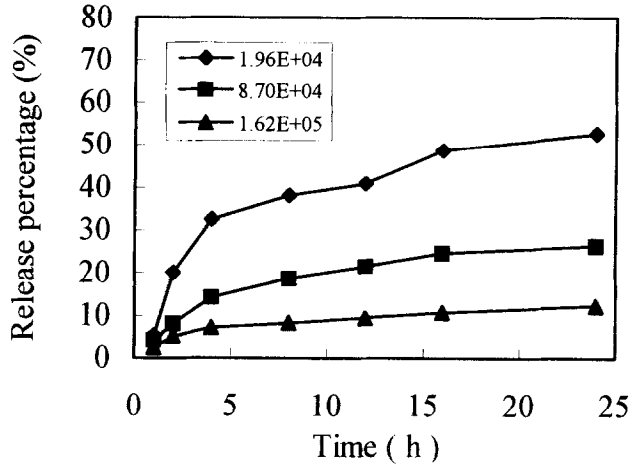


Fig. 3. Release percentages of different molecular weight dextrans from chitosan capsules prepared by the orifice method measured at 25±0.1°C (capsules were prepared from solutions of 81.3% DD chitosan, at pH 3 with no sodium chloride). The data are the means of triplicates.

Wang *et al.*, 1991). The coiled molecule will result in less entanglement, which in turn will result in a weaker structure in which to hold the liquid inside the capsule, and a more ellipsoid shape.

The axial ratios of capsules prepared from solutions of pH 2.0 or pH 4.0 were larger, but their break forces were smaller than those prepared from a solution of pH 3.0. This may due to the intrinsic viscosities of chitosan in different pH solutions being in the order of pH 3.2 > 3.0 > 2.5 > 2.0 > 4.0 > 5.0 (Tsaih, 1993). A higher intrinsic viscosity indicates the molecule will be more extended in the solution. The extended molecule will increase the chances to be involved in inter-molecular entanglements. Therefore, capsules prepared from solutions of pH 3.0 have smaller axial ratios but higher break forces among the pH values studied.

Capsules prepared from solutions containing 0.3% sodium chloride have larger axial ratios and lower break forces than those prepared from solutions free of sodium chloride. This may be due to the counter-ion effect which depresses the third electroviscous effect and renders the chitosan molecules more flexible with randomly coiled shape (Chen *et al.*, 1995; Kienzle-Sterzer *et al.*, 1984; Lin, 1992; Lyubina *et al.*, 1983; Pogodina *et al.*, 1986; Tsaih, 1993; Tsaih *et al.*, 1995). Coiled molecules will result in less inter-molecular entanglements and also in larger axial ratios and smaller break forces as mentioned before.

Effects of chain flexibility on the thermal properties of the prepared capsules

Results in Table 2 show that enthalpies of capsules prepared from higher DD chitosans were higher than those from lower DD chitosans. Enthalpy is an index of energy needed to change the phase of a material. It has been used as an index of the energy needed to break the hydrogen bond, therefore capsules or membranes with higher enthalpy indicate the presence of more hydrogen bonds incorporated in the wall materials (Hwa, 1994). The reason that enthalpies of capsules prepared from higher DD chitosans were higher than those from lower DD chitosans may due to the fact that chains of higher DD chitosans are more flexible than those of lower DD chitosans. Flexible chain molecules facilitate intra-molecular hydrogen bond formation and form more junction zones. More energy is required to break junction zones than regular chains. Therefore, capsules prepared from high DD chitosans have higher enthalpies than those from lower DD chitosans. The reason that capsules prepared from solutions containing 0.3% sodium chloride were higher in enthalpy than those prepared from solutions free of sodium chloride may due to the fact that chloride ions will neutralize the protonated amine groups. The electrostatic shielding effect will result in coiled molecules, thus facilitating intra-molecular hydrogen bonding. This will also result in a higher enthalpy. The effects of the DD of chitosan on maximum melting point temperatures are shown in Table 2 and indicate that capsules prepared from 81.3% DD chitosan solutions had higher maximum melting point temperatures than those prepared from the other two DD chitosan solutions. Hwa (1994) reported that maximum melting point temperatures of membranes prepared from 81.3% DD chitosan were higher than those prepared from lower or higher DD chitosans. The capsules prepared from solutions containing 0.3% sodium chloride had higher maximum melting point temperatures than those prepared from solutions without sodium chloride, which may be due to the electrostatic shielding effect mentioned earlier.

Relationships between the physical and thermal properties of the prepared capsules

The number of hydrogen bonds or entanglements formed in the walls of the capsules may be related to their axial ratios, break forces, enthalpies, and maximum melting point temperatures. Capsules with more hydrogen bonds and entanglements should possess higher break forces, maximum melting point temperatures, and enthalpies but lower axial ratios. Table 3 shows the correlations of axial ratios, break forces, enthalpies, and maximum melting point temperatures of the capsules reported in Tables 1 and 2. Enthalpies of the capsules show a very high correlation with axial ratios (0.93417) and a negative correlation (−0.92229) with break forces. This indicates that capsules with higher enthalpies possessed weak mechanical properties. This may be because capsules prepared from low DD chitosans contain less intra-molecular hydrogen bonds but more inter-molecular entanglements owing to high molecular weights and less flexible chains. In other words, capsules with higher numbers of intermolecular entanglements and less intra-molecular hydrogen bonds will have higher break forces but lower enthalpies. Capsules prepared from higher DD chitosans have less inter-molecular entanglements but more intra-molecular hydrogen bonds owing to higher chain flexibilities and lower molecular weights thus resulting in lower break forces and higher enthalpies.

Table 3. The correlation coefficients of axial ratios, break forces, enthalpies, and maximum melting point temperatures of capsules prepared by the orifice method

	EM[a]	MPT[a]	Axial ratio	Break force
EM	1.0000	0.32445	0.93417	−0.92229
MPT		1.0000	0.39844	−0.41229
Axial ratio			1.00000	−0.99372
Break force				1.00000

[a]Same as Table 2.

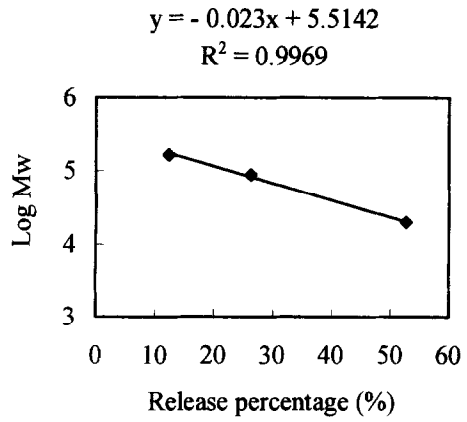


Fig. 4. Release percentages vs logarithm molecular weight of dextrans.

The effects of chitosan chain flexibility on the release characteristics of the prepared capsules

The results in Fig. 2a indicate that capsules prepared from higher DD chitosans have larger pore sizes. This may be due to greater chain flexibilities of higher DD chitosans. The flexible molecules result in larger pore sizes in the walls. The results are consistent with reports by Hwang *et al.* (1986) and Chen *et al.* (1994). The results in Table 1 are also consistent with the above principles. The coiled molecules tend to form more intra-molecular hydrogen bonds and less inter-molecular entanglements. Therefore, capsules prepared from flexible molecules had higher enthalpies and axial ratios but lower break forces. The results in Fig. 2b indicate that the counter-ion effect retarded the third electroviscous effect, thus making the molecules become less extended. The coiled molecules resulted in larger pore sizes of the capsules, Therefore, more hemoglobin was released. Results of Fig. 1c show the percent released was lowest through capsules prepared from pH 3.0 solutions which is consistent with the reports of Lin (1992), Tsaih (1993), Chen *et al.* (1995) and Tsaih *et al.* (1995). The chitosan molecules in pH 3.0 solutions were more extended than in the other two pH solutions, therefore the capsules prepared from pH 3.0 solutions had the smallest pore sizes and also the lowest release percentages.

The percentages of dextran released from the prepared capsules show similar trends to those of hemoglobin except release percentages were lower (highest release percent was 70.2% for dextran compared with 84.5% for hemoglobin). This may be due to dextran being a fibrous molecule whereas hemoglobin is globular. The fibrous molecule may have a greater chance to interact with the wall material, and therefore have a lower release percentages. The logarithmic molecular weights of dextrans and their release percentages were plotted and a linear relation of $Y = -0.023x + 5.5142$ was determined. The relationship indicates that dextran with a molecular weight < 1600 Da may possibly pass through the capsule freely.

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