Alginate−Oligochitosan Microcapsules. II. Control of Mechanical Resistance and Permeability of the Membrane

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The simultaneous regulation of mechanical properties and permeability, for a new microcapsule based on the complexation of oligochitosan and alginate, is possible through control of the reaction conditions. Specifically, the results of mechanistic study have shown that the molar mass of the polyanion influences primarily the capsule mechanical resistance, without changing its permeability. Furthermore, both a reduction in membrane permeability and an improvement in mechanical strength can be achieved by raising the polyanion concentration. Alginate coatings were also found to be an effective means to reduce the membrane permeability and cutoff without altering the microcapsule's mechanical resistance. It has been shown that the size of the capsules does not effect the morphological characteristic of the capsule membrane. However, due to the differences in the membrane thickness, some changes in mechanical and permeability characteristic were observed. Additionally, a linear correlation between internal bursting pressure and the capsule wall thickness was found with a mechanical resistance of the alginate−chitosan membrane of approximately 360 MPa/m. The high microcapsule strengths, and its tunable permeability, are ideally suited for, and have been tested in, applications such as microencapsulation of living cells and control delivery systems.

Introduction

The immobilization of living cells in microcapsules has been reported by a number of investigators, with potential applications ranging from the bioartificial pancreas, the encapsulation of hepatocytes for the treatment of the liver failure, to high-density cell growth for immunotheraphy. Microencapsulation can also be used in the controlled release of drugs, vaccines, antibodies, and hormones. Therefore, over the past two decades, several research groups have been involved in the development of polymeric biomaterials for the immobilization of various biologically active species.[1−3] The principal issues involved in the evaluation of polymers for bioapplications as well as their stability and biocompatibility have been reviewed[4] and tested.[5]

Microcapsules Based on Chitosan. Chitosan, as a cationic polysaccharide derived from the deacetylation of chitin, is the second most abundant biopolymer. It is commonly employed in the clinic owing to its nontoxicity and bioactivity, which have led to applications in wound healing acceleration, the reduction of blood cholesterol levels, and immune system stimulation.[6] Several papers and patents concerning chitosan microcapsules and microspheres, which are summarized in two recent reviews by Yao et al.[7] and Kas,[8] focus on the preparation, properties, and applications of such microparticulate systems. One of the most promising preparation method of stable microcapsules, which has attracted considerable attention over the past two decades, is the creation of polyelectrolyte complexes by interpolymer ionic interaction between chitosan and negatively charged polysaccharides, such as alginate,[9,10] carboxymethyl cellulose,[11] chondroitin sulfate,[12] gellan,[13] hyaluronic acid,[14] and carrageenans.[15] In most cases, the reactions have stoichiometric character and strongly depend on charge density and the degree of ionization of the ionizable sites on the polyanion and chitosan chains. The degree of swelling and its strength depend on both the chitosan concentration and reaction time. In general, chitosan with a molar mass greater than 10 kDa is

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soluble only under nonphysiological conditions (below a pH of 6.6).

Recently, the authors have shown that the control of the molar mass (MM) of the chitosan is a key parameter in the formation of stable, elastic capsules with high moduli. Specifically, oligomers within the MMs of 2 and 20 kDa are favored if one seeks to obtain capsules with mechanical resistance approaching 1 N and with permeabilities in the 40−110 kDa range.[16] The novel chemistry forms a membrane directly between two oppositely charged polyelectrolytes (alginate−oligochitosan) solutions in the absence of simple electrolyte and involves a single-step process to generate the microcapsules.[17] The method does not require either high-temperature changes or multivalent metal ions to promote gelling of the polymer solution nor the use of organic counterions in the precipitation bath. Furthermore, the selection of the molar mass provides an additional degree of freedom permitting the simultaneous regulation of mechanical properties and permeability. Specifically, the use of low-molar-mass chitosan (<3 kDa) permits the formation of capsules with good mechanical properties at a physiological pH, which represents a strong advantage over existing chitosan-based microcapsules.

Some other features of the oligochitosan-alginate system include the following: (1) The ionic strength and the pH of the solution utilized during the capsule formation process strongly influence the structure of the alginate−oligochitosan membrane.[18] (2) The relative number of interchain ionic bonds, which determine the interpolymer complex density and membrane properties, can be controlled by either the pH or ionic strength. (3) Specific binding with respect to the molar mass of chitosan occurs during capsule formation. (4) With increasing ionic strength and pH, a shift toward higher chitosan molar masses involved in membrane formation is observed. (5) The presence of a low-MM salt (0.9% NaCl) accelerates the diffusion of the oligocations and leads to thicker capsule walls, although with lower relative cross-linking density.

We have shown that sodium chloride diminishes the effect of chitosan charge density on the permeability and the mechanical properties of capsules and shifts the cutoff of prepared membranes toward higher MM values.[18] The permeability of the new alginate−oligochitosan microcapsules, therefore, depends primarily on two factors: (1) the density of the membrane that forms the outer shell influences the cutoff, which is particularly important for capsules prepared in water, and (2) the final membrane thickness controls the kinetics of solute diffusion and is more important for capsules synthesized in 0.9% NaCl. The mechanical properties of capsules prepared at physiological conditions (saline, pH = 7.0) are primarily influenced by membrane thickness and can be controlled by parameters such as reaction time, oligochitosan MM, and concentration. On the other hand, the alginate concentration significantly affects both mechanical and porosity characteristics of the capsule membrane.

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Table 1. Samples of Alginate Used for Capsule Preparation

sample	degrad.[a] (mmol/g)	intrin. visc. [η] (mL/g)	$M\eta$[b] (kDa)
Keltone HV		880	440
KEL-HV-D-6	0.5	716	358
Keltone LV		532	266
KEL-HV-D-3	2.0	220	110

[a] The degradation procedure is described in Experimental Section. Values are given in millimoles of hydrogen peroxide per gram of starting material. [b] Molar mass was calculated using the Mark−Houwink equation proposed by Smidsrød.[10]

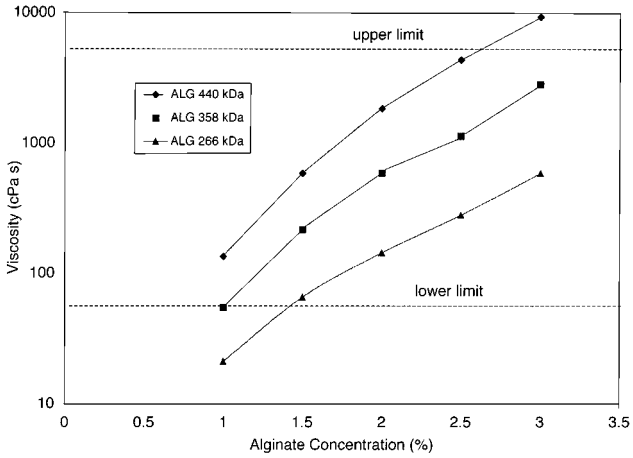

Figure 1. Viscosity of alginate solutions within a 1−3% concentration range. Solutions were prepared in 0.9% NaCl.

The present investigation involves the elucidation of the influence of the alginate MM and concentration on oligochitosan microcapsule formation under physiological conditions. The effect of microcapsule size as well as the application of additional coating are also discussed.

Experimental Section

Materials. *Alginate.* Sodium alginate samples, Keltone HV (lot 54650A) and Keltone LV (lot 46198A), were purchased from Kelco/NutraSweet (San Diego, CA). Samples with varying molar masses were obtained via the controlled radical degradation of Keltone HV by continuous addition of 30% aq hydrogen peroxide (0.5−2.0 mmol/g of polysaccharide) to 400 mL of 1.0% alginate solution of pH 7.0 at 80 °C. After a 1 h degradation process, samples were purified by ultrafiltration (Hollow-Fiber Concentartor CH2A, Amicon, Switzerland) and ultimately concentrated and freeze-dried (Beta 1−16, Christ, Germany). Intrinsic viscosities, [η], of the alginate samples were measured in 0.1 M NaCl at 20 °C with a capillary viscometer (Viscologic TI 1, SEMA Tech, France). The concentrations were corrected by sample dry content, and MMs were calculated according to Mark−Houwink equation proposed by Smidsrød[19] (Table 1).

The viscosities of the alginate solutions as a function of molar mass and concentration were measured using a CS Bohlin rheometer (Muhlacker, Germany) equipped with a CP 40 cone and plate system at 20 °C with temperature control of ±0.2 °C. The viscosity values were determined in Newtonian flow region at low shear rates (Figure 1).

Chitosan. Oligochitosan with a molar mass M_n = 2.9 kDa and a degree of deacetylation >95% was obtained by a radical degradation method previously described,[20,21] where chitosan of MM 50 kDa was used as the starting material (Hutchinson/

(19) Smidsrød, O. *Carbohydr. Res.* **1970**, *13*, 359.

McNeil Int., Philadelphia, product E−055). All other reagents were of analytical grade.

In this study, oligochitosans of MM 2.9 kDa were employed because previous investigations revealed an optimum in mechanical resistance without compromising permeability.[18] However, due to the partial precipitation of 1% solutions adjusted to pH 7.0, all of the capsule preparations were carried out at pH 6.8, where chitosan of MM 2.9 kDa was fully soluble.

Microcapsule Preparation. Capsules within diameter of 2.9−3.2 mm were produced from a pair of oppositely charged polysaccharides, where 1 mL of a 1−2.5% aqueous sodium alginate was added dropwise using a syringe pump into 20 mL of 1% oligochitosan solution. Both polysaccharides were dissolved in 0.9% NaCl, and the pH of the final solutions were adjusted to 6.8. Following a 20 min reaction, capsules were collected, washed three times with saline solution, and stored at 4 °C in 0.9% NaCl/0.01% sodium azide. To study the effect of capsule size, the spherical droplets of 1.2% alginate solution within a diameter of 0.8−3.5 mm were formed by atomization using an air-jet droplet generator. All experiments were performed at ambient temperature.

Chitosan Conversion Determination. Samples of 400 μL of chitosan solutions were withdrawn from the reaction bath every 5 min during capsule formation and characterized by using the aforementioned chromatographic method.[18] Briefly, molar masses of chitosan samples were estimated by GPC at a flow rate of 0.5 mL/min. The liquid chromatograph consisted of an isocratic pump (LaChrom L-7110, Merck, Darmstadt, Germany) equipped with a refractometric detector (LaChrom RI detector L-7490, Merck). A Shodex OHpak SB-803 HQ column (Showa-Denko Company, Tokyo, Japan) was employed as the stationary phase, using 0.5 M acetic acid/0.5 M sodium acetate as an eluent, as recommended.[22] Poly(ethylene glycol) standards (PSS, Mainz, Germany) were used for column calibration and as a relative reference for MM calculation. By assuming that the total concentration of the solute is proportional to the GPC peak area (Beers' Law), the conversion and MM of chitosan were calculated from the respective chromatogram derivatives.

Permeability Measurements. A 0.1% polymer standard solution (2 mL, dextran 70 and 110 kDa in 0.9% NaCl) was added under gentle agitation to 1 mL of microcapsules placed in a 10 mL vial. Aliquots were withdrawn after 3 h and injected into a liquid chromatograph equipped with Shodex protein KW-G and KW-804 columns. The eluent, 0.9% NaCl/0.01% sodium azide, was applied at a flow rate of 0.5 mL/min. The dextran concentrations were proportional to the maximum heights of the detected chromatographic peaks (Beers' Law) and calculated with respect to the initial polymer standard concentration, which is the concentration of the dextran standard with the defined MM at time 0 (immediately following the addition of 2 mL of standard solution into 1 mL of capsules). The membrane permeability at the point of equilibrium, calculated from the decrease in dextran concentration for ideal mixing, was maximal at a 33.3% change in polymer standard, which diffuses into the capsule. The cutoff of the microcapsules was defined as the lowest MM of dextran for which diffusion was smaller than 2% after 3 h.

Mechanical Characterization. The mechanical resistance of microcapsules was determined on a texture analyzer (TA-2xi, Stable Micro Systems, Godalming, U.K.). The mobile probe was driven at constant speed of 0.1 mm/s during the microcapsule compression. The imposed force (N) and the displacement of the squeezed capsule were automatically recorded until bursting occurred. Twenty capsules per batch were analyzed in order to obtain statistically relevant data.

Microscopic Observation. Capsule size and membrane thickness were visually examined under a standard inverted-light microscope (Axiovert 100, Carl Zeiss Jena GmbH, Jena, Germany).

Results and Discussion

1. Influence of Alginate Molar Mass and Concentration. *Viscosity Regimes.* The ability to control solution rheology is critical if one wishes to regulate capsule sphericity, and reproducibility, during scale-up. Alginates of three different molar masses were used in this study (Table 1). The viscosities of the samples, as a function of their concentration (range 1−3%), are presented in Figure 1 and varied between 20 and 10 000 cPa s. One can observe a significant growth of viscosity by increasing either MM or the concentration of the polyanion. For different molar masses of alginate, approximately linear relations as a function of concentration are observed with similar slopes. During capsule formation in binary systems, the viscosity of polyanion solution is one of the most important factors. When the viscosity is too low, the alginate droplets tend to deform or fracture during the contact with surface of polycation solution. The scale of deformation during extrusion is primary related to differences in viscosity between both solutions although the kinetics of membrane formation also plays a role.[23] The preferable viscosity range of alginate solutions is between 60 and 5000 cPa s, since below the lower limit droplets cannot resist the impact into oligochitosan solution. The upper value limits the preparation of homogeneous polyanion solutions as well as the formation of uniform spherical droplets during atomization process. All capsules prepared from alginate solutions, within aforementioned viscosity range, during and after the 20 min reaction, have a spherical shape without any surface deformations.

Capsule Mechanical Resistance. The predominant factor that influences capsule mechanical strength is the molar mass of the polyanion. For example, capsules obtained from solutions of alginates of MM 440 kDa have a deformation force 6 times higher than that of capsules prepared of MM 266 kDa, at an alginate concentration of 2.5% (Figure 2). The mechanical resistance of capsules also increases with concentration of alginate solution.

For higher MM of alginate, which translates to longer polymer chains with higher total number of charges along the backbone, the number of the cross-linkages on one polyanion chain within the membrane is higher. Therefore, higher molar mass chains are more immobilized, increasing the resistance and mechanical stability of the network. A higher charge density is obtained with higher polyanion concentrations, and as a consequence, more chitosan is bound during capsule formation, also contributing to an increased network density.

Oligochitosan Conversion. Generally, an increase in the carboxyl group density has a moderate effect on oligocation diffusion, which leads to a slower membrane growth rate. A change in alginate MM, within a solution family of the same polyanion concentration, does not effect the total number of $-COO^-$ groups, and therefore,

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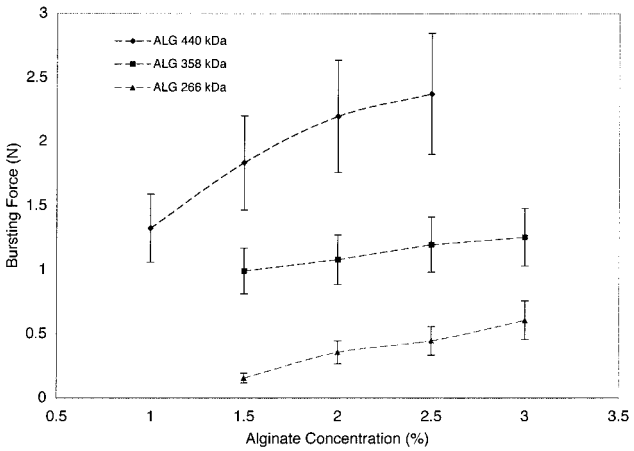

Figure 2. Bursting force of the capsules prepared from alginate solutions varying in concentration and molar mass.

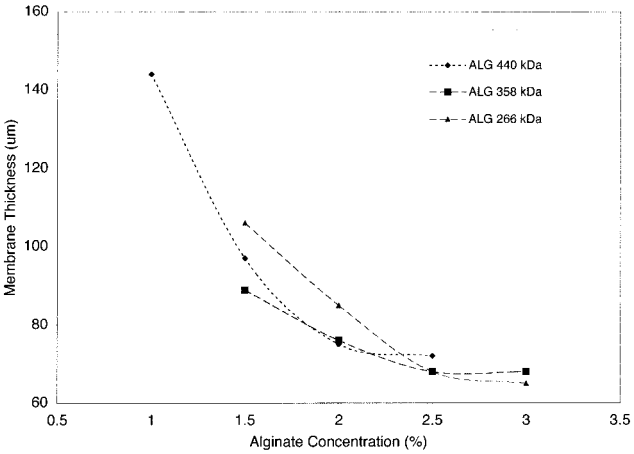

Figure 3. Membrane thickness of capsules prepared from alginate solutions varying in concentration and molar mass.

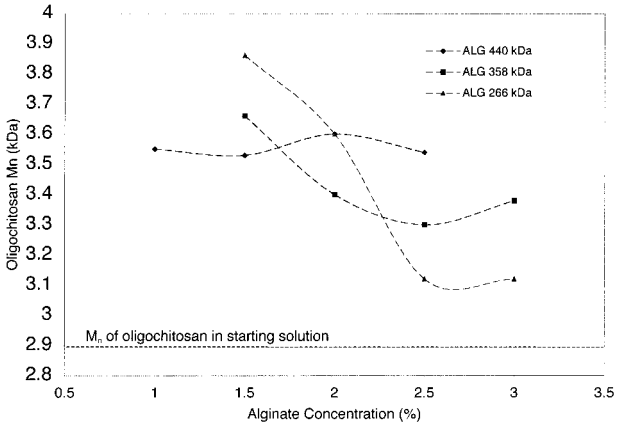

Figure 4. Molar masses of oligochitosan bounded during capsule formation. The oligocation in starting solution had molar mass of 2.9 kDa.

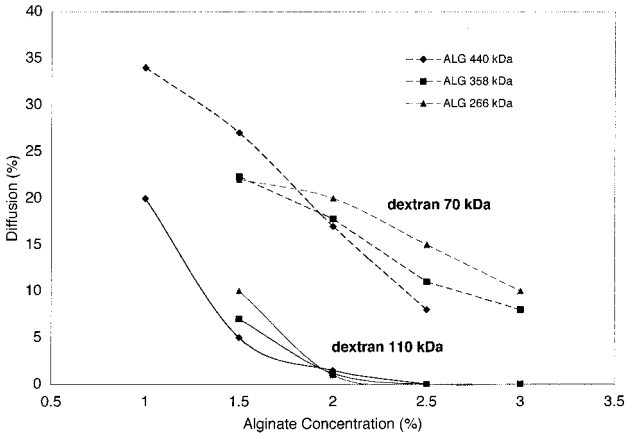

Figure 5. Diffusion of dextran of 70 and 110 kDa after 3 h through capsules prepared from different alginate concentrations and MM.

there is no significant difference in membrane thickness of capsules obtained in reactions with the same alginate concentration but different MMs (Figure 3). Furthermore, neither the alginate molar mass nor concentration influence the conversion of oligochitosan during capsule formation, over the ranges investigated. However, very selective binding with respect to chitosan MM takes place during capsule formation (Figure 4). Clearly, the higher MM portion of the distributions preferentially forms a membrane.

Recently, we have reported that the presence of sodium chloride in the reaction media during the alginate−oligochitosan capsule formation, especially at pHs close to neutral, causes a pronounced shift of the complexed oligocations into the direction of higher molar masses. Herein, it has been observed that final MMs of oligochitosan involved in membrane formation (M_n between 3.1 and 3.9 kDa; Figure 4) are above the M_n of oligochitosan in the starting receiving bath solution (M_n = 2.9 kDa). Generally, one can observe a decrease in the reacted oligochitosan MMs, increasing alginate concentration where this relation is more pronounced for alginate samples with smaller MM. In the case of alginate 440 kDa, the oligochitosan of M_n around 3.5−3.6 was preferentially bound independently on the polyanion concentration. For 1.5% alginate solutions, one can observe that alginates with higher MMs, which have higher thermodynamic volumes in solution, react preferentially with lower oligochitosan MM. With concentrations above 2.0%, this dependence is reversed. This implies that the selective binding of oligocations is related to both the length of polyanion chains and its size in solution, as would be reasonably expected because both increase the number of functional groups on the coil's reactive surface.

Capsule Permeability. From diffusion experiments for dextran at 70 and 110 kDa, one can conclude that the alginate concentration has significant influence on capsule membrane porosity (Figure 5). These differences in permeability can be correlated with cross-linking density of the membrane. As was mentioned in the previous section, no significant difference in the oligochitosan conversion was observed with an increase of alginate concentration, although the thickness of the capsule membrane decreases. As a consequence, a thinner and less permeable capsule wall is formed at higher polyanion levels. On the basis of these results, we can conclude that the alginate MM can be used as a parameter to tune the mechanical resistance, with the manipulation of the concentration of polyanion permitting a concomitant change in mechanical resistance and permeability. Therefore, the alginate MM and concentration, together, permit simultaneous control of permeability and mechanical resistance over a broad practical range. This is not possible with traditional microcapsule chemistries such as alginate−poly-L-lysine.

Table 2. Mechanical Properties of Different Size Capsules[a]

caps. rad. (mm)	membr. thick. (mm)	force at burst. F_B (N)	strain at burst. d_f (%)	surface at burst. S (mm^2)	pressure at burst. (kPa)
0.80	0.170	0.79	89.6	12.9	61.2
0.97	0.145	1.11	90.2	20.3	54.7
1.39	0.122	1.54	88.1	33.7	45.7
1.57	0.115	1.67	87.3	40.9	40.8

[a] Reaction of 1.2% sodium alginate (Keltone HV) solution and a 1% chitosan solution (M_n = 2.9 kDa) at pH 6.8.

Table 3. Conversion of Oligochitosan during Preparation of Different Size Capsules

caps. rad. (mm)	caps. surf. (mm^2)	conver. (%)	conver. (mg)	no. of caps.[a]	conver./caps. (mg)
0.44	2.4	16.1	32.2	2800	0.011
0.80	8.0	10.1	20.2	470	0.043
0.97	11.8	8.0	16.0	260	0.063
1.39	24.3	6.5	13.0	90	0.145
1.57	31.0	5.5	11.0	62	0.179

[a] Calculatation based on assumption that the total volume of capsules was 1 mL.

Table 4. Permeability of Capsules of Different Size

caps. rad. (mm)	memb. thick. (mm)	diffus. of dextran 110 kDa (%)	diffus./caps. (μg)	diffus.[a] (mg/m)
0.44	-	41	0.29	-
0.80	0.170	30	1.29	27.2
0.97	0.145	27	2.06	25.3
1.39	0.122	23	5.22	26.2

[a] Diffusion of dextran 110 kDa standard correlated to surface and thickness of the capsule membrane.

2. Influence of Capsule Size. *Membrane Thickness.* Size control and the production of very small capsules have been major concerns in cell and bioactive agent immobilization given the need to minimize the dead volume and the mass transfer resistance. The properties of capsules with diameters varying from 0.88 to 3.5 mm, prepared under the same reaction conditions, are given in Tables 2−4. One can observe that the membrane thins with increase of capsule diameter (Table 2). In the case of the smallest radius, 0.44 mm (not shown in Table 2), the membrane was very thick and it was difficult to identify a sharp internal boundary between the interior membrane wall and the capsule center. Small capsules have a large surface-to-volume ratio compared to larger capsules. This has an influence on kinetics of membrane formation as well as final properties of the capsules. However, the mechanism of alginate−chitosan complex formation and the membrane morphology should not be affected by the capsule radius. Therefore, one would anticipate that it should be possible to correlate most capsule properties by taking into the account the changes in capsule surface/volume ratio. The conversion of chitosan, after 20 min, increases from 6 to 16% with decrease of the capsule size (Table 2). One can calculate the penetration of oligocation through a single capsule and present it as the function of the capsule surface S_{cap} (Table 3, Figure 6). The linear correlation indicates that the kinetics of diffusion through the capsule membrane, prepared at a given conditions, is constant and does not depend on the size of the capsule. On the basis of the assumption that the diffusion of chitosan through the capsule surface, as well as relative cross-linking within the membrane, do not change with size of the capsule, one can attempt to correlate membrane thickness as well as mechanical resistance to the capsule size. If the quantity of diffusing oligochitosan is proportional to the surface of the capsule (Figure 6) and the relative density of oligochitosan within the membrane is assumed to be constant, the volume of membrane V_m should be proportional to capsule surface S_{cap}:

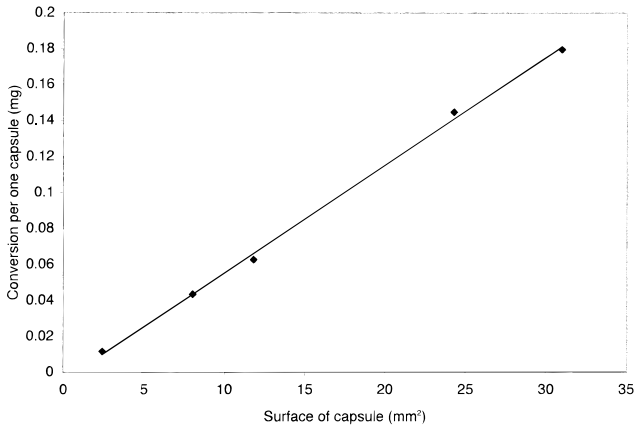

Figure 6. Conversion of chitosan during capsule formation as a function of capsule external surface area. Reaction between a 1.2% alginate (Keltone HV) solution and a 1% chitosan solution (M_n = 2.9 kDa) at pH 6.8. The correlation fit to a curve $y = 0.006x$.

$$V_m = f(S_{cap})$$

in other words,

$$\frac{4}{3}\Pi r^3 - \frac{4}{3}\Pi(r^3 - d)^3 = f(4\Pi r^2)$$

where r is radius of capsule and d is the thickness of the membrane.

This linear correlation is verified in Figure 7 and indicates that there is no influence of capsule size on the mechanism of chitosan−alginate membrane formation. As a consequence, one should also observe no difference in deformation or relative mechanical resistance of capsules. This will be examined in the following section.

Mechanical Resistance. There are several models proposed in the literature to describe the deformation of a liquid-filled spherical elastic membrane microcapsule with either a Mooney−Rivlin law or a neo-Hookean constitutive equation.[24] However, all of these describe the systems that are "elastic" in throughout their deformation range. It has been observed that alginate−oligochitosan capsules have "plasto-elastic" characteristic and start to behave elastically only after 50% of deformation (Figure 8). The experimental technique used in this paper permits one directly to measure the force imposed on a single microcapsule and its compressive displacement. All capsules tested have high deformability with the maximum resistance (force at bursting point F_B) increasing with capsule size (Table 2). Only the smallest capsule does not burst, which is probably due to its high membrane-to-capsule ratio. At strains higher than 60−80%, the internal volume of

(24) Liu, K. K.; Williams, D. R.; Briscoe, B. J. *Phys. Rev. E* **1996**, *54* (6), 6673.

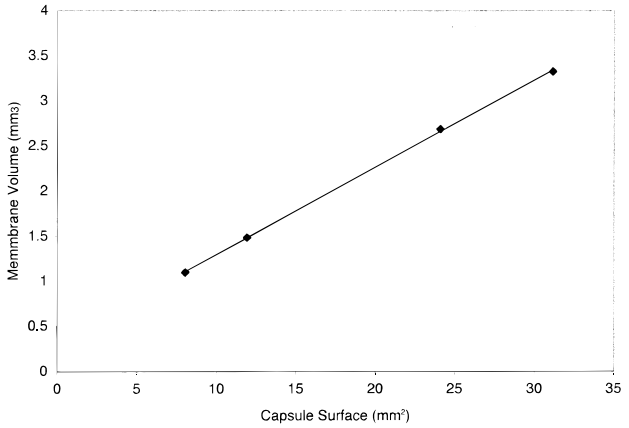

Figure 7. Membrane volume of capsules as a function of external surface area. Reaction between a 1.2% alginate (Keltone HV) solution and a 1% chitosan solution (M_n = 2.9 kDa) at pH 6.8. The correlation fit to a curve $y = 0.1x + 0.33$.

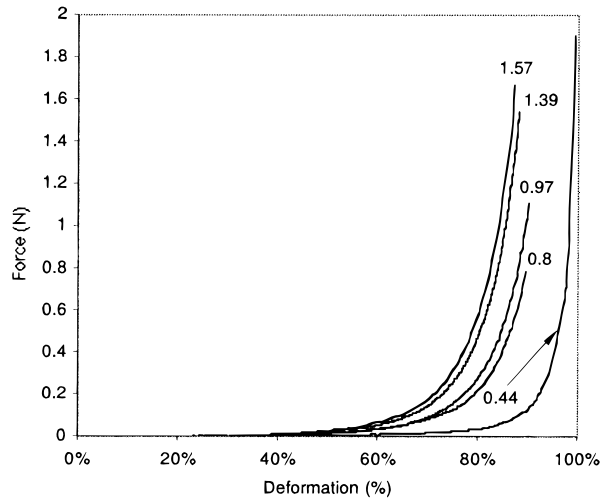

Figure 8. Force imposed on the capsules as a function of deformation. The capsule radius was varied between 0.44 and 1.57 mm.

capsules of radius 0.44 mm deformed between rigid plates of the texture analyzer is completely filled with the complexed membrane. Therefore, they are infinitely deformable (Figure 8), as might be expected based on the improved mechanical properties generally observed at higher surface curvatures. The direct comparison of capsule mechanical properties based on bursting force values is impossible due to their differences in diameter, which influence the size of the capsule contact surface (S) during the deformation, and as a result, the recorded force does not reflect to the capsule internal pressure during compression. On the basis of optical observations and recorded data during the loading process, all microcapsules start to burst at deformation higher than 80% (Table 2). At such high deformation, we can assume that the shape of deformed capsule is similar to the shape of the flat cylinder with surface S (Figure 9). Following the assumption that during compression there is no change in total volume of the capsule, one can calculate the contact surface S expressed as relationship between initial radius of capsule r and strain at bursting d_f at the bursting point:

$$S = \frac{2}{3}\Pi \frac{r^2}{(1 - d_f/100)}$$

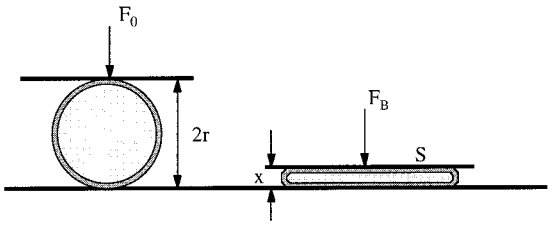

Figure 9. Geometry of a single spherical capsule, filled with an incompressible fluid, during compression between two rigid plates. Left, before deformation, and right, at bursting point above 80% of deformation, where r is the initial radius of capsule, F_B is the bursting force, x is the distance between rigid plates, and S is the contact surface between plate and capsule at bursting point.

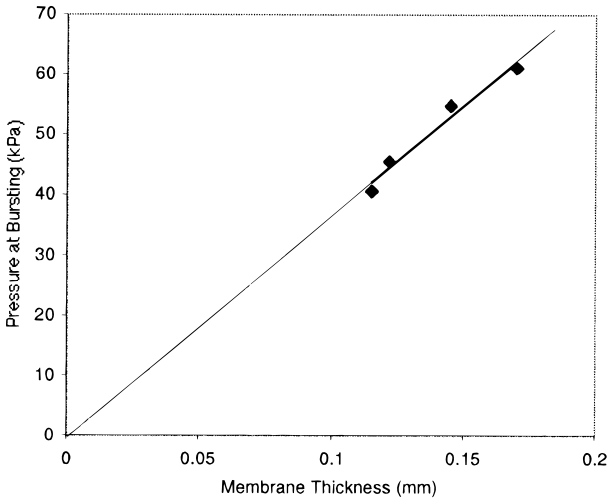

Figure 10. Internal pressure at bursting as a function of membrane thickness. The correlation fit to a curve $y = 363x$.

where $x = (1 - d_f/100)2r$.

On the basis of the preceding equation, one can calculate the internal pressure for capsule with different diameters (Table 2), where the force applied by the plates at the bursting point (F_B) divided by the area of contact between the capsule and the plate (S) is the internal pressure (P) within the capsule. As was aforementioned, the internal pressure is an absolute value indicating the mechanical resistance of capsules. On the basis of the calculations summarized in Table 2, one can conclude that the mechanical resistance of the capsule increases with reduced capsule diameter as a result of thicker membrane formation. Moreover, the internal pressure is directly proportional to membrane thickness (Figure 10), where from the slope of the trendline one can estimate that mechanical resistance of the alginate−chitosan membrane is approximately 360 MPa/m. This proves the postulate of Ma et al. that membrane strength is directly proportional to the membrane thickness when the same materials and methods of capsule preparation are used.[25]

Capsule Permeability. Similar to the case of mechanical resistance, a direct analysis of the effect of diameter on capsule permeability is impossible due to the difference in surface-to-volume ratio. Therefore, all the results were recalculated and normalized to diffusion through 1 m^2 of capsule (Table 4, Figure 11). One can observe

(25) Ma, X.; Vacek, I.; Sun, A. *Art. Cells, Blood Subs. Immob. Biotech.* **1994**, *22* (1), 43.

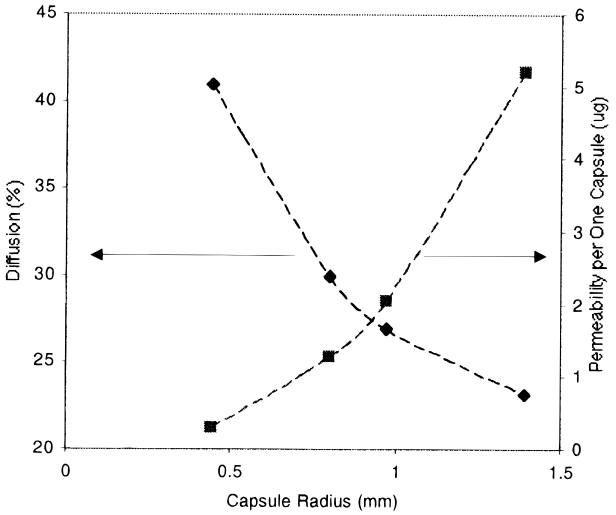

Figure 11. Permeability percentage and diffusion of dextran 110 kDa through capsules differing in size.

that the capsules have a cutoff above 110 kDa (in all cases, diffusion above 2%; see Table 4), and the diffusion of the dextran standards increases with capsule size, which is likely caused by the thicker membrane (Figure 11). Moreover, the virtually identical values of the diffusion, correlated to the membrane thickness (last column in Table 4), indicate that the differences in permeability of capsules differing in size are likely only related to changes in the membrane size. These results likely confirm that there are no changes in microstructure of the membrane during formation of capsules with different diameters. Furthermore, capsule size control could be used as a process tool in modulating the diffusion and mechanical properties of the capsules.

3. Coating of Capsules with Alginates of Different Molar Mass. Recently, we have postulated that the permeability or molar mass cutoff of alginate−oligochitosan membranes could be controlled with the alginate concentration.[18] In the present paper, we showed that by increasing the polyanion concentration one could reduced capsule permeability. However, unless the alginate MM is also changed, a variation in polyanion concentration results in a change in the microcapsule's mechanical characteristics. For some biomedical applications, the toxicity is very sensitive to alginate MM, and therefore it may not be used as a capsule variable. Under such circumstances, simultaneous changes in mechanical properties and permeability can be obtained by varying the alginate concentration during the reaction and adding a polyanionic coating following encapsulation. Such strategy could be advantageous for bioartificial organs that require the usage of biocompatible materials. For example, a significant increase in host reaction was observed for microcapsules containing an outer layer of a polycation, such a polylysine, as compared with alginate beads without coating.[26]

To prevent implant rejection as well as cell overgrowth, the surface of the capsule was coated with an outer layer of polyanion. In our coating test, the capsules within the size 2.4−2.6 mm prepared in standard conditions were kept for 10 or 20 min in 0.05% alginate

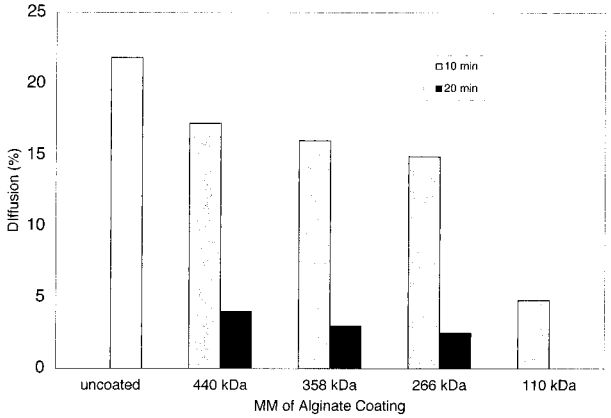

Figure 12. Influence of 10 and 20 min coating with alginates of different MM on the permeability of dextran 110 kDa through alginate−oligochitosan capsule membranes.

of different MM solutions (Table 1). All capsules prepared had similar mechanical resistances with slight shifting toward higher values (5−10%) for lower alginate MMs and longer coating times. Both alginate MM and coating time influence permeability (Figure 12). Generally, one reduces the capsule permeability by coating it with alginate, where the prolonging coating time strongly diminishes final capsule permeability. The reduction is higher for lower MM and longer reaction times where capsules coated with alginate solutions for 20 min have cutoff lower than 110 kDa. This could be explain by formation of a thicker and denser oligochitosan−alginate complex outerlayer. Similarly, it was observed that the selective binding of alginate to alginate−poly-L-lysine capsule is more effective and is enhanced by reducing the molar mass of the polyanion.[27]

Conclusions

The properties of the alginate−oligochitosan capsules can be controlled by polyanion molar mass and concentration. The changes of alginate concentration simultaneously influence the capsule permeability and the mechanical resistance. However, the variation of the alginate molar mass influences only capsule durability. Therefore, by using polyanions of different MM it is possible to decouple these two properties.

The postencapsulation coating with alginate was found to be an effective way to reduce membrane permeability and capsule cutoff in applications when the need for biocompatibility or sterilizability prohibit a change in alginate MM.

Capsules of different sizes prepared under the same conditions differ in their macroscopic characteristic (membrane thickness, mechanical resistance, and permeability) due to the changes in surface/volume ratio. However, no changes in morphological structure and characteristic of the membrane during formation of capsules were observed. Moreover, the postulate that capsule mechanical resistance is linearly proportional to their membrane thickness was proven, and thereby, alginate-oligochitosan membranes were designed to resist up to 360 MPa/m of membrane.

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(26) Vandenbossche, G. M. R.; et al. *J. Pharm. Pharmacol.* **1993**, *43*, 115.

(27) Thu, B. et al. *Biomaterials* **1996**, *17*, 1031.