Alginate−Oligochitosan Microcapsules: A Mechanistic Study Relating Membrane and Capsule Properties to Reaction Conditions

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A new generation of microcapsules was prepared by utilizing a polyelectrolyte complexation reaction between two oppositely charged polysaccharides, one of which is oligomeric. This capsule combines extremely high deformability (>80%) and elasticity with permeability control and can be applied in various bioencapsulation technologies. The relative number of interchain ionic bonds, which determine the cross-linking density and membrane properties, can be controlled by either pH or ionic strength. Specific binding with respect to the molar mass of chitosan occurs during capsule formation. With increasing ionic strength and pH, a shift toward higher molar masses involved in membrane formation was observed. The preparation of capsules under physiological conditions (pH 7.0, 0.9% NaCl) was demonstrated, overcoming the limitation of all other microcapsules based on chitosan which must be prepared at pH below 6.5. It has been shown that the parameters such as reaction time, chitosan molar mass, and concentration primarily influence the mechanical properties, whereas alginate concentration effects both mechanical and porosity characteristic of the capsule membrane.

Introduction

Polyelectrolyte complexes (PECs) formed through the mixing of oppositely charged polymers, have recently attracted considerable attention due to their potential application as microcapsules for medical implants. A variety of approaches, based on various polymer chemistries, processes for membrane formation, and encapsulation technologies, have been evaluated.[1,2] However, over the past two decades the overwhelming majority of scientists have restricted their studies on the alginate/poly-L-lysine polyelectrolyte complex system, where solid alginate/calcium beads are coated with a solution of oppositely charged poly-L-lysine (PLL) and subsequently converted into a permeable capsule by liquefying the ionotropically gelled anionic polysaccharide.[3]

Chitosan-Based Capsules. Chitosan, as one of the few abundantly available and naturally derived biocompatible cationic polysaccharides, has been a subject of many studies and is an alternative to PLL.[4] The polyelectrolyte complex formed between alginate and chitosan can be considered as essentially irreversible and stronger than binding of poly-L-lysine to an alginate gel.[5] This high stability is likely caused by cooperative ionic bounds between $-NH_3^+$ and $-COO^-$ groups of chitosan and alginate, respectively. McKnight et al. produced chitosan/alginate/Ca^{2+} capsules with a molecular weight cutoff of 200 kD for use in cell engineering as well as in controlled release technology.[6] The quantitative binding of chitosan has been shown to depend strongly on the capsule formation procedure. Microcapsules produced from calcium alginate beads, with calcium chloride present in the chitosan solution, bind ∼100 times more chitosan than capsules produced by dropping the alginate solution into 20 kD a chitosan solution in the absence of salt.[7]

Reversed chitosan−alginate complex coacervate capsules, formed by the dropwise addition of chitosan solution into alginate solution, were reported to be fragile even following hardening for 3 h.[8] The addition of calcium and a reduction of the pH during the reaction significantly improved structural stability; however, this approach requiring acidic conditions cannot be generally adopted for the majority of mammalian cells. Other attempts have focused on the replacement of alginate by either natural or modified polysaccharides. For example a one-step capsule formation between carboxymethyl cellulose (CMC) and chitosan has been applied for the encapsulation of hybridoma cells.[9] The reaction was limited to 3 min probably due to the relatively low pH, which is needed to solubilize chitosan although it negatively effects immobilized cell viability.

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Thermodynamic Behavior of Capsules. Due to the presence of a relatively thin (3 μm) membrane CMC−chitosan capsules have the tendency to swell during the first 2 days of incubation and increase the volume by up to 300%. Different characteristic surface structures including hemispheres are created by interaction between chitosan and gellan through polyion complex formation.[10] The swelling degree and strength of the capsule depend on both chitosan concentration and reaction time. Moreover, it was demonstrated that very strong polyelectrolyte complexes can be formed between fully deacetylated chitosan and other negatively charged polysaccharides (chondroitin sulfate, hyaluronic acid, and carrageenans).[11,12] The reaction has stoichiometric character and depends on degree of ionization of the ionizable sites on the polyanionic and polycationic chains. Several of these preparation methods have been patented.[13−15] However, all techniques employ high molar mass chitosan (>10 kD) with reactions carried out below a pH of 6.6 to ensure its solubility.

Recently, we 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 modulus. Specifically, oligocations with a MM between 2 and 20 kD are favored.[16] The novel chemistry forms a membrane directly between two oppositely charged polyelectrolyte solutions in the absence of simple electrolyte and involves a single-step process to generate the microcapsule.[17] The method does neither require the high temperature or polyvalent metal ions to promote gelling of the polymer solution nor the use of organic counterions in the precipitation bath. Furthermore, the selection of an optimum molar mass provides an additional degree of freedom, permitting the simultaneous regulation of mechanical properties and permeability. The effects of the MM of chitosan and the concentration of chitosan, as well as the presence of sodium chloride on the preparation, physical properties, and release characteristics of the capsules at pH 6.5 have been studied.[17] The use of low molar mass chitosan (<3 kD) permits the formation of capsules having good mechanical properties at a physiological pH, which represents a strong advantage over existing chitosan-based microcapsules.[16]

The present investigation involved the elucidation of the mechanism of alginate/chitosan binary microcapsule formation at variable pHs and ionic strengths. Specifically, the effect of reaction parameters on mechanical resistance and membrane permeability of capsules formed under physiological conditions has been studied.

Experimental Section

Materials. *Alginate.* Keltone HV−sodium alginate (lot. 54650A) was obtained from Kelco/NutraSweet (San Diego, CA). An intrinsic viscosity [η] of 880 mL/g was measured in 0.1 M NaCl at 20 °C in a capillary viscosimeter (Viscologic TI 1, SEMA Tech, France). This reflects to MM of 440 kD.[18]

Chitosan. Samples with varying molar masses (1−4 kD) were obtained in controlled radical degradation via continuous addition of hydrogen peroxide (0.8−6.4 mMol/g of polysaccharide) to 2.5% chitosan solution of pH 3.5−4.0 at 80 °C.[19,20] Chitosan with a molar mass of 50 kD and a degree of deacetylation >97% was used as the starting material (Hutchinson/McNeil Int., Philadelphia, PA, product E-055). All samples after degradation as a chloride salts had similar polydispersities in MM (1.5−1.8) and high degrees of deacetylation (>95%).

Molar masses of chitosan samples were estimated by GPC at a flow rate of 0.5 mL/min (LaChrom L-7110 isocratic pump, Merck, Darmstadt, Germany) equipped with a refractometric detection (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.[21] Poly(ethylene glycol) standards (PSS, Mainz, Germany) were used for column calibration and as a relative reference for MM calculation. All other reagents were of analytical grade.

The degree of chitosan amino group ionization (α) at different pHs were determined from potentiometric titration curves, where 20 mL of 0.1% polymer solutions (pH 2.5) were titrated with 0.02 M NaOH. The degree of protonation is defined as $\alpha = \alpha' + [H_3O^+]/c$, where α' is the degree of neutralization, $[H_3O^+]$ is the proton concentration (deducted from pH), and c is equivalent concentration of chitosan.

Measurements of pH were made at ambient temperature with a pH meter 744 (Metrohm, Herisau, Switzerland), calibrated at pH 4.0 and 7.0.

Microcapsule Preparation. Capsules were produced from a pair of oppositely charged polysaccharides. A 0.5−2% aqueous sodium alginate was prepared in deionized water or 0.9% NaCl. Approximately 4 mL of this solution was introduced into a 5-mL disposable syringe with a 0.4-mm flat-cut needle (Becton Dickinson AG, Basel, Switzerland). The droplets were sheared off for 60 s at a flow rate 1 mL/min (kdScientific syringe pump−Bioblock Scientific, Frenkendorf, Switzerland) into 20 mL of solution of 1% chitosan (M_n varied between 2 and 3 kD) at pH 6, 6.5, and 7.0 previously adjusted with 1 M NaOH, respectively. The resulting microcapsules (2.5−3 mm in diameter) were allowed to harden for 20 min under gentle stirring (200 rpm) with small magnetic bar, filtered, and rinsed with the solvent used for preparation of the polysaccharide solutions. Collected microcapsules (~2 mL in volume) were stored at 4 °C in 0.9% NaCl/0.01% sodium azide. The entire capsule formation procedure described herein was performed at ambient temperature.

The effect of chitosan molar mass on the relative mechanical strength of the prepared chitosan/alginate capsules was discussed previously.[17] In brief, oligochitosan requires an "lower critical oligomer chain length" (M_n > 1−2 kD) for stable capsule formation with a maximum in mechanical capsule strength observed at a MM of 2−3 kD. All oligochitosan samples used in this study were prepared within this range (2.2−2.8 kD).

Chitosan Conversion Determination. Samples of 400 μL of chitosan solutions were withdrawn from th reaction bath every 5 min during capsule formation and characterized by using the aforementioned chromatographic method. By assuming that the total concentration of the solute is proportional to the GPC elution curve area (Beers' Law), the conversion and MM of chitosan were calculated from the respective chromatogram derivatives.

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Permeability Measurements. Two milliliters of a 0.1% polymer standard solution (dextran 40, 70, and 110 kD in 0.9% NaCl), were added under 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 SG-G and SB-803 HQ 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, calculated from decrease in dextran concentration, was maximal at 33.3% of polymer diffusion. 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 mechanical deformation tests were performed at 0.1 mm/s mobile probe speed until bursting was observed. The force (milliNewtons) exerted by the probe on the capsule was recorded as a function of the compression distance leading to a force vs strain relation. Twenty capsules per batch were analyzed to obtain statistically relevant data.

Microscopic Observation. Capsules size and membrane thickness were visually examined under a fluorescence confocal microscope and a standard inverted light microscope (LSM 410 Invert Laser Scan Microscope and Axiovert 100, respectively; Carl Zeiss Jena GmbH, Jena, Germany). The capsules were stained in 0.01% fluorescence dye solution (rhodamine or eosine, both from Fluka, Buchs, Switzerland) for 30 min and then washed with 0.9% NaCl. The eosine dye is more suitable due to it anionic character and ability of specific binding to amino groups of chitosan. Images were acquired by normal light microscopy followed by a confocal scan. The thickness of the optical section was on the order of several micrometers. Changing the plane of focus vertically up or down by 10 μm had no effect on the apparent membrane thickness. All fluorescence pictures were analyzed using the Scion Image software (Scion Corporation, Frederick, MD).[22]

Results and Discussion

I. Membrane Size and Shape. Examination of the microcapsules under the optical microscope revealed an approximately spherical shape with a diameter in the range 2−2.5 mm. In most cases capsules possessed transparent membranes. A comparison of the capsules after reaction times of 10 and 20 min is presented in Figure 1. With the use of both imaging methods, confocal fluorescence (Figures 1a and 2a) and light microscope (Figures 1b and 2b), it is possible to distinguish the capsule wall thickness. Moreover, from fluorescence intensity one can observe that capsules have asymmetric membrane structures with higher concentrations of chitosan close to the surface, gradually decreasing toward capsule center. This agrees with a former description of asymmetric capsule membrane formation between two oppositely charged polyelectrolytes as a two-step process.[23] According to this model, membrane formation begins with the spontaneous creation of a semisolid complex "skin" at the droplet surface as a result of the phase separation process. After this initial step, a macroporous "trabecular" membrane is built up, a process which is controlled by diffusion of the cationic polymer.[24]

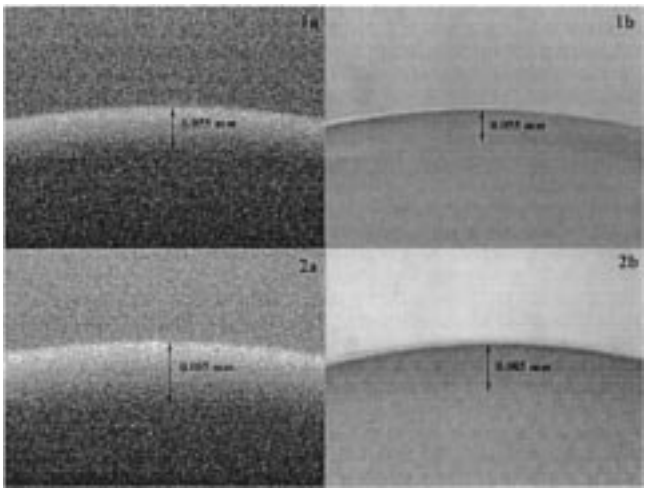

Figure 1. Photomicrographs of capsule membranes prepared in 0.01% Eosin under fluorescence confocal microscope (a) and standard light microscope (b). The microcapsules were obtained through a reaction between a 1% alginate (Keltone HV) solution at a 1% chitosan solution, $M_n = 2.7$ kD (pH 7.0, 0.9% NaCl) after 10 (1) and 20 min (2).

II. Thermodynamic Aspects Related to Capsule and Membrane Formation. Capsule permeability is controlled by the cross-linking density of the polymer network. As the membrane formation is an electrostatic process, pH and ionic strength influence complexation, with the protonation degree of chitosan being the key variable.

Effect of Chitosan Protonation. Chitosan is weak base and has therefore a limited solubility in the higher pH region, i.e., precipitation occurs when the pH exceeds 6.0−6.5. However, it has been reported that for chitosans with MMs lower than 8 kD, precipitation occurs at higher pH values.[17] The solution properties of this natural polycation are control by the degrees of acetylation and amino group protonation.[25] Figure 2 shows the ionization degrees of 50kD chitosan and of two oligochitsan samples as a function of pH. At a pH 4.0, all samples are in their fully protonated form. However with increasing pH protonation becomes highly dependent on the MM. For high MM chitosan (50 kD) ionization of 50% of the cationic groups is observed at pH 6.1, whereas half of the oligochitosan (2.8 kD) chains become ionized at pH 6.5. This is in agreement with earlier work where a gradual decrease of the dissociation constant for chito-oligosaccharides with increasing monomeric units in the chain (1−7) was observed.[26] Therefore, a likely explanation for the enhanced solubility of lower MM chitosans in neutral, or even slightly basic solutions is a shift of their pK_a's toward higher values.

The capsules obtained at different pHs (6, 6.5, and 7) in water or saline varied in their surface roughness, membrane thickness, and morphology (Figure 3). Capsules prepared in water had smooth surfaces and their membrane thickness increased as a function of pH.

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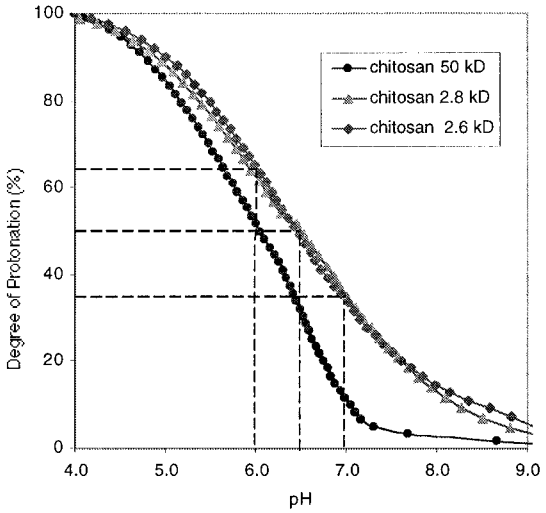

Figure 2. Degree of protonation of chitosans of various MMs as a function of pH.

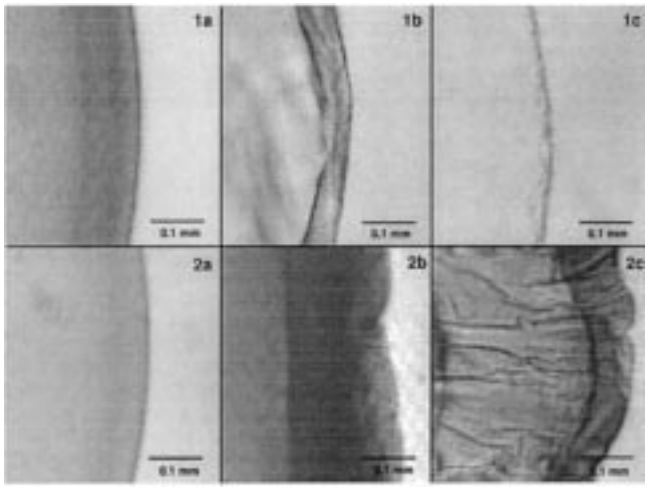

Figure 3. Photomicrographs of capsules prepared in solution of various ionic strengths (1,water; 2, 0.9% NaCl) and at various pH's (a, 7.0; b, 6.5; and c, 6.0). For conditions, see Table 1. Clearly, only the capsules in photos 1a and 2a have well-defined membrane morphologies and would be suitable in biomedical applications.

These differences can be explained by changes in the protonation degree of chitosan (35%, 50%, and 65% respectively; see Figure 2), which influences the cross-linking density of the polyelectrolyte complexes produced. Interestingly, by introducing a monovalent metal salt during capsule formation (in 0.9% NaCl), this trend changed and the membrane thickness decreased with increasing pH. Furthermore, the capsules were less homogeneous and rougher in their structure in comparison to those prepared in pure water (Figure 3). This may indicate that the salt shielded the polyanion, decreasing the extent of intermolecular interaction.

Control of Cross-Linking Density. Taking into account the difference in charge density (potentiometric titration), the conversion of chitosan (from GPC measurements), and the membrane thickness (microscopic observation) of capsules prepared with 20 min reaction time, one can calculate the relative cross-linking density (CD) given by the $-NH_3^+/-COO^-$ ratio (Table 1). Generally, in pure water, the CD increased with the lowering of pH, resulting in denser and less permeable

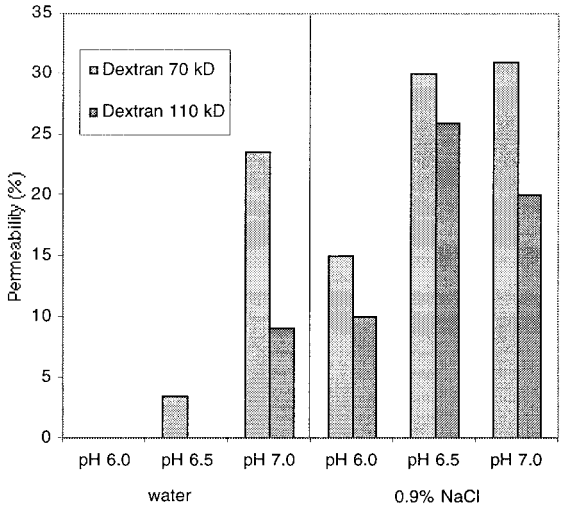

Figure 4. Permeability of capsules synthesized in various solutions. For conditions, see Table 1. Solute diffusion of dextran of different molar masses.

membranes. From dextran permeability measurements one can observe a significant difference in membrane porosity. At pH 6.0, the MM cutoff was below 40 kD,[27] at pH 6.5, it was between 70 and 110 kD, and at pH 7.0, it exceeded 110 kD (Figure 4). Lee et al.[28] observed that alginate/chitosan/Ca^{2+} microcapsules prepared in one-step reaction at a relatively low sodium chloride concentration (0.05% NaCl) and at pH 4.80 showed a minimum release rate of guaifensin, as a result of dense membrane formation between the polymeric chains with similar charge density. The release rate increased at higher pH due to more porous membrane structure, which can be explained by a loop formation of polyelectrolyte backbone chains with different charge densities during complex formation. This agrees with the increase in permeability observed in this study.

The presence of low MM salt (0.9% NaCl) during the binary capsule formation accelerated the diffusion of the oligocations and led to thicker capsule walls with lower CD (Figure 3 and Table 1). As a consequence, these capsules had similar porosities with a higher cutoff (>110 kD). This implies that the differences in diffusion kinetics are associated with a variation in membrane thickness.

Mechanism. The structure of the chitosan/alginate microcapsule membrane may be represented as alternative sequences of ionic interchain bonds and looplike regions incorporating the uncoupled units of both chains.[29] More compact membranes, with smaller "loops" and lower cutoffs, are formed at lower pHs, although only in the simple aqueous system (capsule prepared in water). The introduction of low MM ions changes the polyelectrolyte solution conformation. In particular longer chains, as in alginate, transform from elongated into more compact coil structures. As a result, the short oligochitosan chains can more easily penetrate the alginate chain network and form thicker and less dense membranes. This apparent difference in the mechanism

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Table 1. Properties of Capsules Prepared at Different pH and Ionic Strength[a]

solvent	pH	degree of chitosan protonation α (−)	membrane thickness (μm)	V_{mem}/V_{cap}[b] (%)	chitosan conversion (%)	membrane CHIT/ALG[c] (g/g)	cross-linking density $-NH_3^+/-COO^-$ (mol/mol)
water	6.0	0.65	13	3.1	3.08	19.9	14.8
	6.5	0.50	72	16.3	5.19	6.37	3.94
	7.0	0.35	105	23.1	6.59	5.70	2.43
0.9% NaCl	6.0	0.65	300	56.1	7.25	2.59	1.93
	6.5	0.50	190	39.0	7.08	3.63	2.28
	7.0	0.35	75	16.9	6.74	7.98	3.40

[a] Capsules (2.5 mm in diameter) were obtained through a reaction between a 1% alginate (Keltone HV) solution and a 1% chitosan, M_n = 2.6 kD (20-min reaction time, ambient temperature). [b] Membrane/capsule volume ratio. [c] Chitosan/alginate ratio in the capsule membrane.

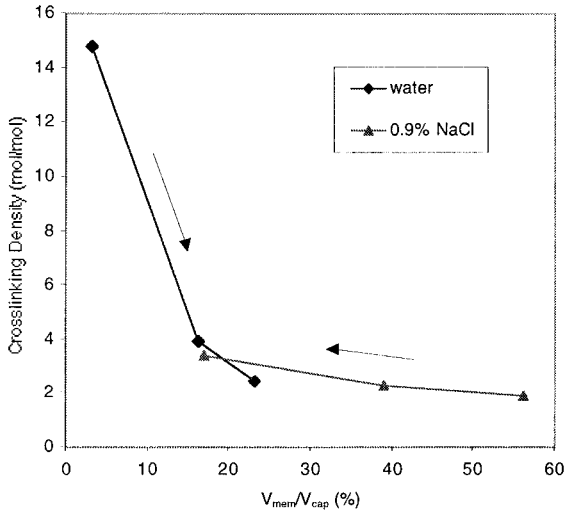

Figure 5. Relative cross-linking density of capsule membranes in function of membrane/capsule volume ratio. For conditions, see Table 1. The arrows indicate the direction of the pH increase (from 6.0 to 7.0).

of membrane formation is well-visualized in a plot of CD in function of membrane/capsule volume ratio (Figure 5). While for the pure water system we observed a strong decrease (high slope) of the CD with increasing pH, for the saline system the changes went into the opposite direction with a significantly lower variation in CD values (lower slope). This implies that for all but nonneutral conditions, the charge suppression on the macroions due to increased salt reduces the extent of complexation. However, both curves cross over at approximately neutral pH, where capsules have comparable membrane thickness and porosity (Table 1, Figure 3).

III. Kinetics of Alginate−Oligochitosan Complexation. *Effect of Molar Mass Distribution.* Table 2 summarizes the chitosan MM bound within the capsule membrane after 5 and 20 min of reaction. A very selective binding with respect to the MM of chitosan was observed during capsule formation in solutions which differ in pH and ionic strength. This effect is most prominent between capsules prepared in solutions with varying ionic strengths, such as water and 0.9% NaCl. Generally, the lower MM portion of chitosan is involved in membrane formation in salt-free system. This specific complexation is caused by the so-called polyelectrolyte effect. It has been shown for a number of interpolymer reactions between mixtures of oligomers that preferential binding to the longest chains takes place.[30] However, this conclusion was primarily based on hydrogen bond complexation systems. In the case of polyelectrolytes in

Table 2. Molar Masses of Chitosan Bonded during Capsule Formation[a]

reaction conditions					
	pH	time (min)	M_n (kD)	M_w (kD)	M_w/M_n
chitosan−starting solution			2.60	4.70	1.8
water	6.0	5	1.80	4.20	2.3
		20	1.80	4.20	2.3
	6.5	5	1.95	4.20	2.2
		20	1.85	3.00	1.6
	7.0	5	2.80	4.50	1.6
		20	2.85	4.55	1.6
0.9% NaCl	6.0	5	2.60	5.50	2.1
		20	2.80	5.20	1.9
	6.5	5	2.80	4.40	1.6
		20	3.40	5.20	1.5
	7.0	5	4.20	10.00	2.4
		20	4.10	8.60	2.1

[a] The preparation is identical to that described in Table 1.

salt-free solutions, inter- and intramolecular electrostatic interactions occur which strongly influence the solution properties as well as the mechanism of complex formation. Recently, this observation was experimentally confirmed for polyelectrolyte complexes between poly(diallyldimethylamonium chloride) PDADMAC and poly(styrene sulfonate) PSS of different molecular weights.[31] In the salt-free solution and at extremely low salt concentrations preferential binding of PSS (8 kD) was found. At higher ionic strength a pronounced complexation of higher MM PSS (356 kD) was observed due to the shielding effect of sodium cations during polyelectrolyte complex formation.

Influence of the Kinetics on Capsule Properties. During capsule formation in pure water the lower MM part of chitosan is selectively incorporated into the membrane. With increasing polycation protonation degree, through lowering the pH, a clear shift toward shorter oligomeric chains is observed. This leads to more dense and thinner membranes and consequently to mechanically less stable capsules (Table 3). When the cutoff of the primary membrane is too low, polycation molecules may not be able to diffuse, and membrane growth is slowed or even stopped. This was the case when chitosan of MM higher than 30 kD was used or when capsules were prepared at lower pH in salt-free solutions. Therefore, at neutral pH in water, the conversion of chitosan increased proportionally with the reaction time and the reaction rate was significantly reduced at lower pHs (Figure 6).

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Table 3. Mechanical Properties of Capsules Prepared at Different pH and Ionic Strength[a]

solvent	pH	membrane thickness (μm)	CD $-NH_3^+/-COO^-$ (mol/mol)	bursting force (mN)	strain at bursting (%)
water	6.0	13	14.8	30 ± 15	64
	6.5	72	3.94	180 ± 60	77
	7.0	105	2.43	1850 ± 500	85
0.9%	6.0	300	1.93	100 ± 30	72
NaCl	6.5	190	2.28	220 ± 90	72
	7.0	75	3.40	190 ± 30	72

[a] The preparation is identical to that described in Table 1.

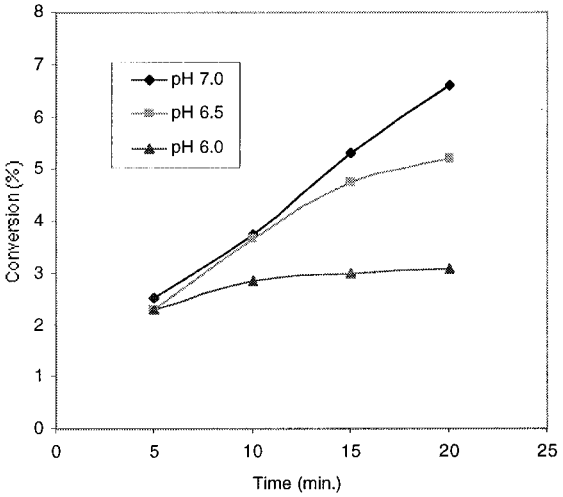

Figure 6. Conversion of chitosan during capsule membrane formation in water. Reaction between a 1% alginate (Keltone HV) solution and a 1% chitosan solution (M_n = 2.6 kD).

By introducing sodium chloride into the reaction media the mechanism of the membrane formation was changed. In this case, the MM of the complexed oligocation was higher than the starting material and with increasing of pH shifted into the direction of larger MM values. One also observes that the conversion rate is independent of the pH and is proportional to the reaction time, similar to the conversion in water at neutral pH (Figure 6). As a result of this complex phenomenon, a formation of capsules with similar mechanical and structural properties was observed, which was independent of the protonation degree of the chitosan.

For capsules prepared in water at pH 7.0, Tables 2 and 3 show that capsules with the best mechanical strength (1850 mN) and flexibility (85% bursting strain) were obtained when chitosan reacted without any preferential reaction early in the process and hence uniformly with time (Table 2). Therefore, uniformity in kinetics leads to improved mechanical properties. Moreover, although capsules prepared at pH 7.0 in water or saline had similar relative cross-linking densities, membrane thicknesses, and porosities, they showed about 10 times higher mechanical resistance and very high (~85%) deformability in water alone. This is evidence that by simply varying the ionic strength of the solvent at neutral pH we can significantly influence the mechanism of capsule formation and decouple the mechanical properties from permeability. The uniformities of the capsules prepared in water and saline are different due to the shift of MM of chitosan built into the membrane toward higher values with introducing the salt during the membrane formation.

Table 4. Properties of Capsules in Function of Reaction Time[a]

reaction time (min)	membrane thickness (μm)	permeability (after 3 h) (%)		bursting force (mN)
		40 kD	110 kD	
5	40	33	24	50 ± 30
10	60	33	23	110 ± 60
15	80	32	23	200 ± 70
20	95	33	22	370 ± 150

[a] Capsules (2.5 mm in diameter) were obtained through a reaction between a 1% alginate (Keltone HV) solution and a 1% chitosan, M_n = 2.8 kD (pH 7.0, 0.9% NaCl, ambient temperature).

Table 5. Properties of Capsules Obtained with Chitosan of Different MM and Concentration[a]

chitosan conc (%)	M_n of chitosan (kD)	membrane thickness (μm)	permeability (after 3h) (%)		bursting force (mN)
			40 kD	110 kD	
1	2.8	95	33	22	370 ± 100
1	2.6	75	33	21	190 ± 30
0.5	2.6	125	33	25	50 ± 20

[a] Capsules (2.5 mm in diameter) were obtained through a reaction between a 1% alginate (Keltone HV) solution and a chitosan solutions (pH 7.0, 0.9% NaCl, 20 min reaction time, ambient temperature).

IV. Capsule Preparation under Physiological Conditions. Bioencapsulation in general, and applications in the biomedical field in particular require physiological conditions for immobilization and immunoisolation of living cells. Therefore, an additional set of capsules was prepared at pH 7.0 in 0.9% NaCl in order to demonstrate the versatility of the new capsule chemistry.

Effect of Reaction Time. The properties of the capsules vary remarkably as a function of the reaction conditions with the reaction time having the most profound influence on the process of binary capsule formation. Generally, the mechanical strength of the capsules increases with reaction time due to the increase of the capsule wall thickness (Table 4). However, no significant differences in capsule permeability and cutoff were measured. This indicates that the process of "skin" formation during the first minutes of reaction controls the cutoff of the membrane. However the subsequent diffusion-controlled building up of the inner membrane is responsible for the capsule's mechanical resistance.

Effect of Chitosan MM and Concentration. Table 5 illustrates that lower MM chitosans (2.6 kD) form slightly denser membrane skins which limit oligocation diffusion and membrane build-up and, as a consequence, lead to mechanically less resistant capsules. In addition, decreasing the oligochitosan concentration changes the kinetics of membrane formation, engendering a significant reduction in mechanical stability. However, in both cases no significant difference in membrane permeability was observed.

Effect of Alginate Concentration. The capsule membrane grows more slowly at higher alginate concentrations (Table 6). Oligocation diffusion seems to be retarded by the higher concentration of the polyanion chain network, which leads to a thinner, but due to the

Table 6. Properties of Capsules Prepared with Different Concentration of Alginate[a]

alginate conc (%)	membrane thickness (μm)	permeability (after 3h) (%)		bursting force (mN)
		40 kD	110 kD	
1.0	95	33	22	370 ± 100
1.2	80	33	18	450 ± 110
1.5	70	24	10	570 ± 150

[a] Capsules (2.5 mm in diameter) were obtained through a reaction between alginate (Keltone HV) solutions and 1% chitosan, M_n = 2.8 kD (pH 7.0, 0.9% NaCl, 20 min reaction time, ambient temperature).

higher charge density, denser membrane with lower cutoff and higher mechanical resistance.

Conclusions

The ionic strength and the pH of the solution utilized during the capsule formation process strongly influence the structure of the alginate/oligochitosan membrane. 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. The permeability of the new alginate−oligochitosan microcapsules, therefore, depends mainly of two factors: (1) the density of the membrane which 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 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 effects both mechanical and porosity characteristic of the capsule membrane. We believe that the control of the membrane cutoff will require either the use of alginates at different concentrations and MMs, or other polyanions of various charge densities. The alginate−oligochitosan capsule described herein is currently being tested as an immunoisolation device in vitro and in vivo.

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