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Note

Permeability and stability of chitosan-based capsules: effect of preparation

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Abstract

Capsules were obtained by interpolymer complexation between chitosan (polycation) and sodium hexametaphosphate (oligoanion). The effect of preparation variables such as the pH, ionic strength as well as the reagent and porogen concentration on the capsule characteristics was evaluated. By decreasing the chitosan/SMP ratio, adding mannitol up to 1% or maintaining the salt concentrations below 0.15 w/v%, the diffusion characteristics can be modulated without disturbing the capsule mechanical stability. Higher concentrations of the cross-linking agent (2.25 w/v%) produced stable capsules only in the absence of electrolyte and low polyol amounts. Furthermore, by increasing the ionic strength, or the pH of the initial chitosan solution, the membrane exclusion limit shifted to higher values concomitant with a significant loss in the membrane compression resistance. The results obtained showed that the capsule characteristics could be independently controlled by manipulating the coacervation conditions. © 2002 Published by Elsevier Science B.V.

Keywords: Chitosan; Oligophosphate; Capsules; Diffusion; Stability; Gene delivery; Encapsulation

Microcapsules based on the electrostatic interactions between oppositely charged polyelectrolytes have been prepared using a variety of materials and for many applications (Benita, 1996; Uludag et al., 2000). Clearly, capsule mass-transfer and mechanical properties must be adapted to the requirements of the final uses which range from highly specific biomedical formulations (Li, 1998) to large scale bioreactors (Park and Chang, 2000). While many systems are based on naturally occurring polyanions (such as alginate), there are few natural polycations which have been exploited.

Chitosan (CS), a natural polyamine, has been used to prepare microspheres by complex coacervation or ionotropic gelation with numerous counterions (Kas, 1997; Gåserod et al., 1998). Despite being extensively investigated, as either coating material or membrane forming cross-linker, there are few systems utilizing CS as a capsule core base (Mi et al., 1999). The most well-studied, CS–tripolyphosphate, produces beads with poor mechanical stability (Aral and Akbuga, 1998).

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The influence of a variety of parameters such as the reagent concentration, pH, ionic strength, additives and the size on the properties of CS–sodium hexametaphosphate (SMP) capsules has been systematically studied (Angelova and Hunkeler, 2001a,b). A protocol was developed to produce capsules with tailored mass-transfer characteristics wherein the stability is not compromised. The results, published in two recent publications (Angelova and Hunkeler, 2001a,b) and summarized herein, are important to understand how the formation process can be used as a means to control capsule performance.

Capsules were prepared as previously described (Angelova and Hunkeler, 2001a). Briefly, CS solution was dropped into SMP solution at a given concentration, pH, ionic strength and low-molar mass additive amount. After washing with saline the resulting capsules were characterized in term of their size, membrane thickness, sphericity, and swelling capacity as well as permeability and stability. The membrane permeation was evaluated by measuring the dextran standards diffusion when capsules were incubated for different time periods with a dextran solution. The concentration changes were determined by size-exclusion chromatography as previously reported (Angelova and Hunkeler, 2001a). The membrane mechanical stability test was performed applying uniaxial compression until the capsule bursting (Angelova and Hunkeler, 2001b).

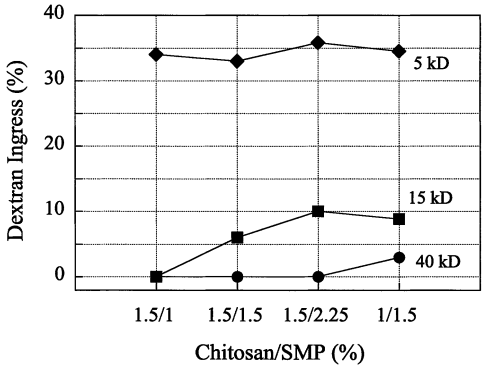

Fig. 1. Dextran ingress as a function of the ratio CS/SMP; diffusion time 6 h; 20 °C.

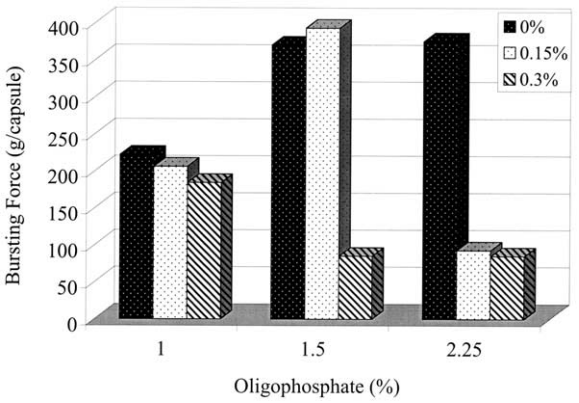

Fig. 2. The effect of SMP amount on the capsule compression bursting resistance. Capsules were made with 1.5% CS, pH 4; SMP solutions pH 7; reaction time 2 min. The legend indicates the NaCl concentration.

Fig. 1 presents the effect of CS/SMP ratio on the ingress of dextran with different molar masses. At the same concentration of the network base material, CS, the increased cross-linker (SMP), in the absence of salt, favored the formation of greater voids in the membrane and allows dextran with higher molar mass (15 kD) to diffuse in. At the same ratio, a more porous membrane was formed using 1% CS compared with 1.5%, indicated by the slight ingress of 40 kD dextran.

The compression test showed that the influence of the SMP concentration on the capsule stability strongly depends on the salt amount present in the preparation solution (Fig. 2). Specifically, the data demonstrate that, despite an increase in the permeability, elevated SMP levels do not affect the stability of capsules prepared in the absence of NaCl. Furthermore, low salt levels ($< 0.15\%$) resulted in a significant decrease of membrane bursting forces when high concentrations (2.25%) of cross-linker were used for capsule formation. Due to the salt-induced retardation in the membrane formation kinetics, the membrane thickness gradually increased with increasing ionic strength (Fig. 3). The thicker membranes were observed to result in a low capsule compression stability, due to the decreased free internal deformation volume (Rehor et al., 2001). Moreover, the thicker membrane appears also to be more porous and the ingress of the both standards (15 and 40 kD)

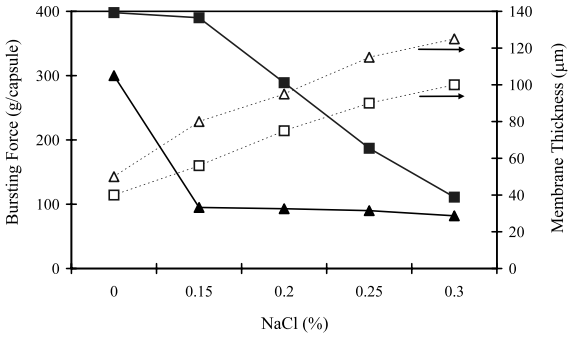

Fig. 3. Relationship between the compressive strength (left scale) and membrane thickness (dashed line, right scale) as a function of ionic strength. Capsules were made with 1.5% CS, pH 4 and (■) 1.5% SMP; (▲) 2.25% SMP; pH 7; reaction time 2 min.

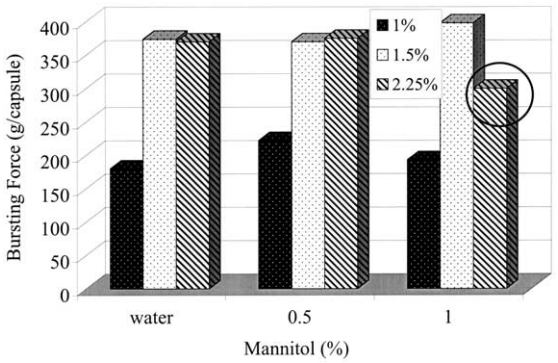

Fig. 5. The compression breaking force values versus concentration of mannitol. Capsules were made from CS 1.5% and pH 4; SMP 1.5% and pH 7; reaction time 2 min. The legend indicates the SMP concentration.

doubled when the NaCl concentration increased from 0.15 to 0.2% and showed no remarkable differences afterwards up to 0.9% NaCl (data not shown).

An important finding of the study is that polyols (e.g. mannitol) could be applied as porogens without affecting the stability of the membrane. The exclusion limit moved from 15 to 40 kD when 1% mannitol was used compared with 0.5% (Fig. 4) with no changes in membrane bursting values (Fig. 5). This reveals that the increased membrane cut-off in presence of polyols is induced by a different mechanism to the salt induced effect. In the presence of mannitol the enhanced hydrophobic interactions between the CS chains produced a more "structured" membrane. The reorganization of the network segments opens larger voids but at high degree of cross-linking (2.25% SMP) formed a membrane with increased rigidity and slightly low compression resistance (Fig. 5, circled).

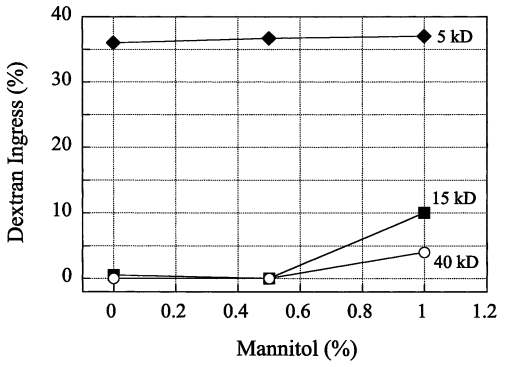

Fig. 4. Dextran ingress through capsule membranes prepared at different concentrations of mannitol in the oligophosphate solution; diffusion time 6 h; 20 °C.

In conclusion, it could be shown that appropriately selecting the preparation parameters, both the diffusion and stability properties of the capsules might be modulated over a broad range. The mannitol amount and the CS/SMP ratio affect the membrane porosity without disturbing the polyelectrolyte interactions and thus the membrane stability. The salt concentration directly influences the molecular charge density and could be varied within the limits given herein. As the reported capsules have a relatively low permeability and very good stability, they are promising candidates for various biomedical applications such as gene delivery and enzyme immobilization.

References

Angelova, N., Hunkeler, D., 2001a. Effect of preparation on properties and permeability of chitosan–sodium hexametaphosphate capsules. J. Biomater. Sci., Polym. Ed. 12, 1317–1337.

Angelova, N., Hunkeler, D., 2001b. Stability assessment of chitosan–sodium hexametaphosphate capsules. J. Biomater. Sci., Polym. Ed. 12, 1207–1225.

Aral, C., Akbuga, J., 1998. Alternative approach to the preparation of chitosan beads. Int. J. Pharm. 168, 9–15.

Benita, S. (Ed.), 1996. Microencapsulation: Methods and Industrial Applications. Marcel Dekker, New York.

Gåserod, O., Smidsrod, O., Skjak-Bræk, G., 1998. Microcapsules of alginate–chitosan. I. A quantitative study of the interactions between alginate and chitosan. Biomaterials 19, 1815–1825.

Kas, S.H., 1997. Chitosan: properties, preparation and application to microparticulate systems. J. Microencapsulation 14, 689–711.

Li, R.H., 1998. Materials for immunoisolated cell transplantation. Adv. Drug Deliv. Rev. 33, 87–109.

Mi, F.L., Shyu, S.S., Lee, S.T., Wong, T.B., 1999. Kinetic study of chitosan–tripolyphosphate complex reaction and acid-resistive properties of the chitosan–tripolyphosphate gel beads prepared by in-liquid curing method. J. Polym. Sci. B: Polym. Phys. 37, 1551–1564.

Park, J.K., Chang, H.N., 2000. Microencapsulation of microbial cells. Biotechnol. Adv. 18, 303–319.

Rehor, A., Canaple, L., Zhang, Z., Hunkeler, D., 2001. The compressive deformation of multicomponent microcapsules: influence of size, membrane thickness, and compression speed. J. Biomater. Res., Polym. Ed. 12, 157–170.

Uludag, H., De Vos, P., Tresco, P.A., 2000. Technology of mammalian cell encapsulation. Adv. Drug Del. Rev. 42, 29–64.