Mechanical stability of multicomponent polysaccharide microcapsules

A. Bartkowiak[1*] and **W. Brylak**[2]
[1] Chair of Food Packaging and Biopolymers, University of Agriculture in Szczecin, Kazimierza Królewicza 4, Szczecin, Poland
e-mail: artur.bartkowiak@tz.ar.szczecin.pl
[2] Polymer Institute, Szczecin University of Technology, Pulaskiego 10, Szczecin, Poland

Introduction

Nowadays one can observe the explosion in recovery of new polysaccharidic materials with specific controllable properties, including mechanical characteristic, morphology, biological activity and biodegradability. Specifically, formation and application of hydrogel microcapsules by biomedical and pharmaceutical industry seem to be very promising (Wang, et al., 2006). Despite many successful applications of various systems described already in literature, including the most popular alginate/Ca one, it is still very challenging to obtain materials with fully tunable morphological properties (membrane cut-off) and which are mechanically stable during storage and application at various conditions This is particularly critical in physiological solutions and for in vivo applications (for example stability during some weeks stirring in bioreactor or some months after transplantation etc.). Therefore, numerous groups all over the world have been looking for the best way to improve the mechanical stability of classical alginate/polyvalent metal capsules.

Recently the new multicomponent polysaccharide microcapsules based on standard polylectrolyte complex formation mechanism have been proposed (Brylak, Bartkowiak, 2006). Their stable outer membranes are formed during the PEC reaction between anionic polysaccharides such as alginate and dextran sulfate and chemical modified chitosan containing quaternary ammonium groups (Brylak, Bartkowiak, 2004). By application of variable polymer concentrations, different molar masses and selection of process formation one can obtain microcapsules with tuneable properties in wide range (Brylak, Bartkowiak, 2006), such as mechanical resistance and membrane cut-off.

The main aim of this study was to examine the mechanical stability of above mentioned multicomponent polysaccharide microcapsules during long term storage either in saline or PBS solutions.

Material and methods

Sodium Alginate - Keltone HV (Kelco/NutraSweet, San Diego, CA, USA) with intrinsic viscosity [η] of 840 mL/g in 0.1 M NaCl at 20°C, what corresponds to a molar mass (MM) of 420,000 g/mol, was used for capsule preparations.
Chitosan with a Mη of 1.200 kDa, degree of deacetylation 85% (Yuhuan Ocean Biochemical CO. Ltd. CH-K05011512, China) and dextran T2000 of MM 2 000 000 g/mol (Pharmacia Fine Chemicals AB, Uppsala Sweden) were used in all chemical modifications.
Chitosan with varying molar masses were prepared by controlled radical degradation via continuous addition of hydrogen peroxide to 2.5% high MM respective polysaccharide solution at different time and temperature. All samples, after degradation had similar polydispersities in MM (1.5-2.2). The detailed procedure of degradation and purification of chitosan is described elsewhere (Bartkowiak, Hunkeler, 2000).
Quaternary ammonium derivatives of chitosan were obtained in reactionn between chitosan oligosaccharides and 3-chloro-2-hydroxypropyltrimethylammonium chloride (Sigma-Aldrich St.

Louis, USA) (Brylak, Bartkowiak, 2004), which react with the amino or hydroxyl groups of saccharide in basic pH at 60°C. Such products with the same degree of substation DS=0.5 were used after precipitating in organic solvent and drying at 40°C without any further purification.
Commercial dextran derivatives. Diethylaminoethyl-Dextran DEAE-Dextran (pK Chemicals A/S, Koge, Denmark) with M_w 500 kDa and degree of substitution DS=0.5 was used as the starting material, DEAE-dextran of M_n 4,300 – 11,000 g/mol, were prepared by controlled radical degradation described above, where no change of DS has been observed.
Dextran sulphate (pK Chemicals A/S, Koge, Denmark) with molar masses M_w 5,000 g/mol (DS 5kDa), and M_w 500,000 g/mol (DS 500kDa) with sulphur content 17±1% was used.

Capsule formation. All microcapsules (MC) were prepared at room temperature using two step procedure [2]. Hydrogel microbeads were obtained in one step method by droping 20 cm^3 of 1.5% alginate/dextran sulfate solution in (80/20 ratio of alginate to dextran sulphate) into 200 mL of 1% calcium chloride. After 20 min reaction time hydrogel beads of around 2.0-3.0 mm in diameter were collected, three times washed and stored at 4°C in 0,9% NaCl solution with addition of 0.01 % sodium azide to avoid the bacterial growth. During second-step hydrogel beads were coated with a 2% solution of the different oligomer samples (0.9% NaCl, pH=6.5) for 20 min. For preparation of all solutions as solvent 0,9% NaCl has been applied. Finally, microcapsules were washed several times with saline solution and divided into two parts, where one has been stored in 0,9% NaCl (pH = 6-6.5) or PBS solution (pH 7.2).

Capsule characterization. The methods of cut-off and mechanical resistance determination of microcapsules have been described elsewhere (Brylak, Bartkowiak, 2004). Capsule where characterized mechanically after one day, following 1, 2 and 3 weeks of storage.

Results and discussion

On of the most important problems related to capsule stability is a possible change of capsule properties after specific bioapplication, such as transplantation or bioreactor. Generally, the mechanical resistance after formation should remain constant, however in the case of the polyelectrolyte complexes there will be always some decrease of the mechanical resistance due to the structure reorganization and egress of some weak bonded oligocations or even polyanion molecules. Due to the relatively "weak" type of the chemical bonds between charge groups within the PECs, resulting microcapsules are sensitive to environmental conditions and, therefore, tend to change their properties during the change of composition of the storage medium.

Eigth different sets of microcapsules were prepared for mechanical resistance (bursting force) and barrier properties (cut-off) characterization tests during storage at various conditions (Table 1). In the first stage of hydrogel bead formation as main variable only molar mass (5,000 vs. 500,000 g/mol of DS (Tab. 1) has been applied.

In second step two types of beads were coated with oligochitosans (3,000 or 7,000 g/mol) either as unmodified or their quaternary ammonium derivatives (Tab. 1). The effect of additional components on the properties of multicomponent microcapsules can be visualized using the alginate/oligochitosan microcapsules, prepared in saline which after the formation have been stored in to different solutions (Fig. 1 – saline, 2 - PBS). Already after one day there is a significant reduction in case of mechanical resistance for capsules stored in PBS solution (4-10 times). For capsules after 3 weeks of seasoning, either in 0,9% NaCl or PBS solutions, one can observe a significant reduction of mechanical resistance from 0 to 50% of the initial value (Fig. 1-2, Tab. 1). The obtained results confirm that mechanical stability of capsules coated with unmodified oligochitosan is related to the molar mass of dextran sulfate. For DS of MM 5,000 g/mol they are more mechanically stable (clearly after one week of stabilization) after seasoning in

pure saline solution, where for DS 500kDa in PBS solution. In most case application of modified chitosans led to mechanically more stable microcapsules.

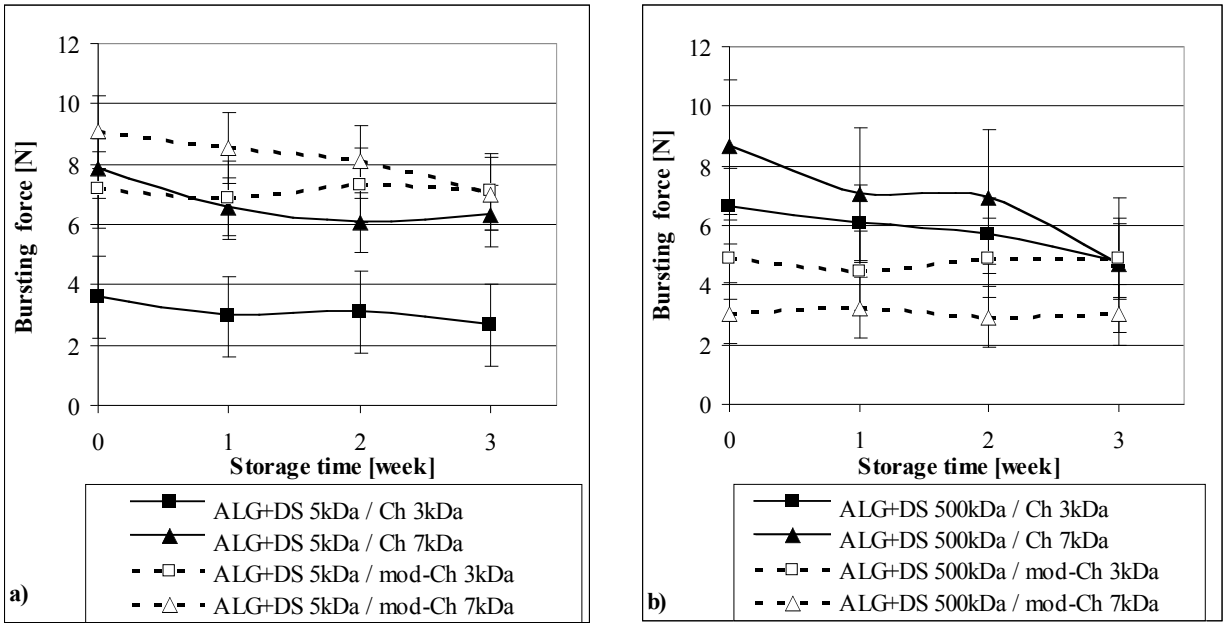

Figure 1: Changes of the alginate+DS/oligochitosan microcapsule mechanical resistance stored in 0.9% NaCl with dextran sulfates of molar mass: a) M_w = 5,000 g/mol; b) M_w = 500,000 g/mol

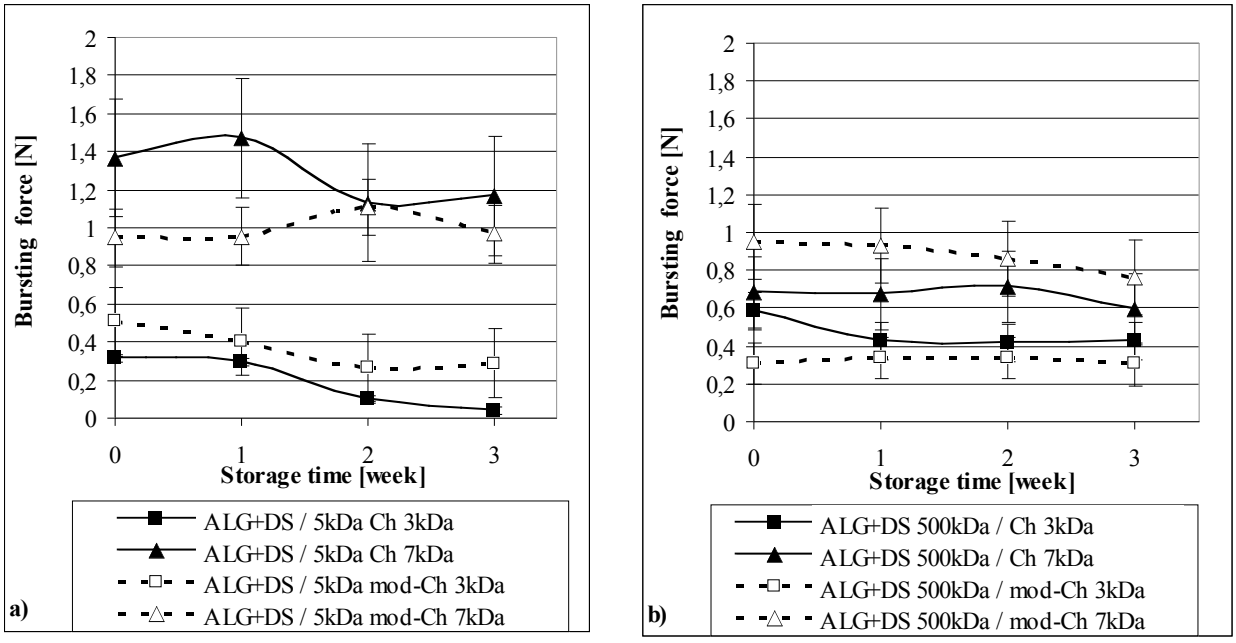

Figure 2: Changes of the alginate+DS/oligochitosan microcapsule mechanical resistance stored in PBS (molarity of the PBS solution corresponds to 0.9% NaCl) with dextran sulfates of molar mass: a) M_w = 5,000 g/mol; b) M_w = 500,000 g/mol

All microcapsules have very similar and relatively dense membranes with cut-off below 40,000 g/mol. However, one can observe that higher reduction of cut-off values are for DS and modified oligochitosans of higher molar masses (Tab. 1). This confirms that molar mass and type of cationic groups along the oligomer chain are the most important parameters which have influence on properties of these multicomponent capsules.

Table 1: Bursting force, membrane cut-off of and relaxation parameters of microcapsules formed in 2-steps process

Type of microcpsules	Membrane cut-off [g/mol]	Bursting force – first day [N]		Bursting force – after 3 weeks [N]		Change of bursting force* in respect to initial properties [%]	
		0,9% NaCl	PBS	0,9% NaCl	PBS	0,9% NaCl	PBS
ALG+DS 5kDa Ch 3kDA	39 000	3,616 ±1,36	0,314 ±0,23	2,640 ±0,49	0,035 ±0,01	-26,9	-88,9
ALG+DS 5kDa Ch 7kDa	30 000	7,871 ±0,690	1,364 ±0,31	6,285 ±1,00	1,166 ±0,29	-20,1	-14,5
ALG+DS 5kDa mod-Ch 3kDa	26 000	7,147 ±1,26	0,505 ±0,18	7,088 ±0,87	0,286 ±0,16	-0,8	-43,4
ALG+DS 5kDa mod-Ch 7kDa	18 000	9,072 ±1,20	0,949 ±0,06	7,010 ±1,89	0,966 ±0,15	-22,7	+1,8
ALG+DS 500kDa Ch 3kDa	35 000	6,641 ±1,27	0,588 ±0,11	4,786 ±0,74	0,425 ±0,05	-27,9	-27,7
ALG+DS 500kDa Ch 7kDa	34 000	8,638 ±2,26	0,684 ±0,13	4,675 ±1,43	0,593 ±0,02	-45,9	-13,3
ALG+DS 500kDa mod-Ch 3kDa	22 000	4,866 ±0,85	0,304 ±0,12	4,913 ±1,33	0,303 ±0,05	+1,0	-0,3
ALG+DS 500kDa mod-Ch 7kDa	16 000	3,042 ±0,75	0,95 ±0,14	3,016 ±0,41	0,763 ±0,20	-0,85	-19,7

* - (+) - increase and (-) - decrease of mechanical resistance

The most stable microcapsules during storage either in saline or PBS (Tab. 1) are those, where high molar mass dextran sulfate and low molar mass modified oligochitosan has been applied.

Conclusions

The results of mechanical stability of new multicomponent polysaccharide microcapsules based on standard polylectrolyte complex formation mechanism during storage in two different salt solutions have been presented. These microcapsules based on multicomponent polylectrolytes using three polymeric systems (alginate, dextran sulfate and chitosann or their chemical derivative containing quaternary ammonium groups) can be formed either in one- or two-step processes. All presented here systems can not be considered as fully thermodynamically stable, as they are changing their properties during the storage. Although, by proper selection and application of polymer with specific molar masses and chemical structure one can obtain microcapsules with tunable properties, which are mechanically stable during storage in typical buffer solutions.

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