New multicomponent polysaccharide microcapsules

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Introduction

Polysaccharides belong to class of polymers which due to their interesting biological properties have been recognized as class of functional materials with growing potential in many fields. Nowadays one can observe the explosion in recovery new polysaccharidic materials with specific controllable properties, including mechanical characteristic, morphology, biological activity and biodegradability. Specifically, formation and application of hydrogel microcapsules for biomedical and pharmaceutical use seem to be very promising [i]. 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 mechanical and morphological properties, especially in context of their mechanical resistance and membrane porosity.

The main aim of this study was to examine the properties of new multicomponent polysaccharide microcapsules based on polyelectrolyte complexes formed by interpolymer ionic interaction between positively and negatively charged polysaccharides. The process of hydrogel microcapsules formation described herein is based on following polysaccharides: alginate, chitosan, dextran and their anionic and cationic derivatives. In this paper the barrier and mechanical properties of proposed multicomponent immobilization systems will be presented and discussed in respect to selected process conditions. Additionally, we proposed the relaxation tests as tool for better characterization of hydrogel beads and capsules.

Materials and methods

Polysaccharides

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 [2].

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 and dextran derivatives samples 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 [2].

Quaternary ammonium derivatives of chitosan were obtained in reactionn between chitosan oligosaccharides and 3-chloro-2-hydroxypropyltrimethylammonium chloride (Sigma-Aldrich St. Louis, USA) [3], which react with the amino or hydroxyl groups of saccharide in basic pH at 60^{o}C. Such products with the same degree of substation DS=0.5 were used after precipitating in organic solvent and drying at 40^{o}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 (D5), and M_w 500,000 g/mol (D500) with sulphur content 17±1% was used.
Capsule Formation. All microbeads (MB) and microcapsules (MC) were prepared at room temperature using one - and two step procedures [2]. Hydrogel microbeads were obtained in one step method by droping 20 cm^3 of 1.5% sodium alginate solution (or different ratio ofalginate with dextran or dextran sulphate mixtures) 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 with addition of 0.01 % sodium azide to avoid the bacterial growth. In two-step microcapsule preparation method the first stage was analogical to one-step method. During second stage alginate beads were coated with a 2% solution of the different oligomer samples (0.9% NaCl, pH=7.0) for 20 min. Finally, microcapsules were washed several times with saline solution and stored similar to these obtained in one-step method.
Capsule characterization. The methods of cut-off determination and mechanical characterization of microcapsules have been described elsewhere [4].
During microcapsules relaxation tests using standard texture analyzer (Zwick/Rolle Z 2.5) beads or capsules were initially loaded/compressed until 50% of their of deformation and left for 200 seconds. The stress variation as a function of the time was recorded. Five capsules per batch were analyzed in order to obtain statistically relevant data. The following equation of correlation as exponential function after Zhao and Zhang [5] has been used:

$$F(t) = F_\infty + A \cdot e^{-kt}$$

where F_∞ – force when time t reaches infinity , A – factor related to mechanical resistance of hydrogel beads, k – decay constant related to morphological structure, t – time.
Detailed results of mechanical characterization of chitosan coated and uncoated alginate beads, including relaxation, has been recently described elsewhere [7].

Results and discussion
Four different sets of hydrogel beads and microcapsules were prepared for mechanical resistance (bursting force and relaxation) and barrier properties characterization tests.
In the first stage as anionic core material only alginate at different concentrations (Tab. 1) has been applied. As a result of 20 minutes reaction in each case we have obtained mechanically stable beads. As one could predict hydrogel beads formed at highest concentration of alginate have the lowest cut-off and highest mechanical resistance.

Table 1. Bursting force and membrane cut-off of hydrogel microbeads in function of alginate concentration.

Alginate concentration wt [%]	Membrane cut-off [g/mol]	Bursting force [N]
0.50	180 000	5.00 ±0.65
0.75	160 000	6.08 ±0.70
1.00	140 000	7.16 ±0.58
1.25	130 000	8.82 ±0.73
1.50	120 000	10.79 ±0.52

In second step 1.5% alginate beads were coated with unmodified chitosans and their quaternary ammonium derivatives (Tab. 2). Chitosan with positively charged amino groups forms complexes with anionic polymers such as alginate [2,4]. So far due to their low solubility in aqueous solutions above pH 6-6,5, especially for chitosan with M_n higher than 5,000 g/mol, the process of microcapsules formation was strictly limited to acidic aqueous solutions. Recently, to overcome this inconvenience, especially in case of bioimmobilization of mammalian cells, we have proposed the

new type of modified oligochitosan with quaternary ammonium groups [3]. The mechanical resistance of all two-step microcapsules is smaller than for corresponding 1-step beads and one can see slightly stronger reduction in case of oligochitosans with higher molar masses. Although, there is no difference of mechanical properties between modified and unmodified chitosan microcapsules.
The application of unmodified oligochitosans leads only to slightly cut-off reduction of alginate/Ca hydrogel bead outer membrane, whereas modified chitosans result in significant decrease of outer membrane porosity. Unfortunately, one could also observe that the cut-off is almost independent on chitosan molar mass [3], which makes it very difficult to control.

Table 2. Selected properties of microcapsules formed with oligochitosan and oligochitosan quaternary ammonium derivatives in 2-steps method.

Molar mass M_n of oligochitosan [g/mol]	Unmodified oligochitosan		Modified oligochitosan	
	Membrane cut-off [g/mol]	Bursting force [N]	Membrane cut-off [g/mol]	Bursting force [N]
3 500	100 000	2.40 ±0.25	12 000	2.41 ±0.14
6 500	100 000	2.84 ±0.15	12 000	2.55 ±0.12
7 800	90 000	1.77 ±0.11	18 000	1.69 ±0.17
9 100	90 000	1.76 ±0.15	16 000	1.70 ±0.18

To overcome this problem we have decided to modify such system and use some multicomponent recipes with selected commercially available dextran derivatives [6]. Firstly, the alginate beads formation has been modify by mixing the alginate with dextran sulphates (Tab. 3). Replacing of 25% and 50% wt. of alginate by both types of dextran sulfates surprisingly in most cases leads to simultaneously reduction of cut-off and mechanical resistance of formed microbeads. This reduction is more pronounced in case of higher MM of dextran derivative. Herein, one can also find some interesting correlations between properties of microcapsules and selected coefficients of relaxation curves, similarly to what has been observed recently for other alginate/Ca systems [7].

Table 3. Bursting force, membrane cut-off and relaxation coefficients for various compositions of alginate/dextran sulphate microbeads.

Microbead	Membrane cut-off [g/mol]	Bursting force [N]	Coefficients of relaxation equation		
			F_∞ [N]	A [N]	1/k [s]
MB 50A/50D5	90 000	4.56 ±0.62	0	0.02	4.27
MB 75A/25D5	80 000	11.07 ±1.52	0.012	0.11	4.99
MB 50A/50D500	60 000	1.33 ±0.06	0.02	0.18	2.94
MB 75A/25D500	40 000	8.78 ±2.19	0.02	0.13	3.13

MB 50A/50D5 – microcapsules formed with solution of 50% alginate and 50% dextran sulphate M_w 5,000 g/mol, dropped into 1% $CaCl_2$ solution, other parameters analogous for alginate microbead formation. (D500 - dextran sulphate M_w 500,000 g/mol)

In case of DEAE-dextran microcapsule the 2-step formation method has bee applied Tab. 4 (analogical to formation of oligochitosan microcapsule in Tab. 2), where 1.5% alginate/Ca beads were coated with 2% solutions of DEAE-dextrans. Based on results (Tab. 4), there is no clear evidence of any effect of DEAE dextran molar mass on capsule properties. However, there is lower reduction of cut-off values in comparison to systems based on modified chitosan (Tab. 2), what confirms that one of the most important parameter which has influence on alginate/Ca/polycation capsule properties is chemical structure of polycation.

Table 4. Bursting force, membrane cut-off of and relaxation parameters of DEAE-dextran microcapsules formed in 2-steps process.

Molar mass M_n of DEAE-dextran [g/mol]	Membrane cut-off [g/mol]	Bursting force [N]	Coefficients of relaxation equation		
			F_∞ [N]	A [N]	1/k [s]
3 200	50 000	2.8 ±0.37	0.089	0.13	3.4
7 500	20 000	3.2 ±0.28	0.066	0.09	4.95
11 000	37 000	3.4 ±0.45	0.1	0.11	4.32

Conclusions

The results of formation and properties of new multicomponent polysaccharide microcapsules based on standard polylectrolyte complex formation mechanism have been presented. They are obtained in reaction between anionic polysaccharides such as alginate and dextran sulfate and chemical modified chitosan and dextran containing cationic groups. These microcapsules based on multicomponent polylectrolytes using three components systems can be formed either in one- or two-step processes. By application of variable polymer concentrations, different molar masses and types of process formation one can obtain microcapsules with tunable in wide range properties, such as mechanical resistance and membrane cut-off. The coefficients of relaxation equations for beads and capsules are probably strictly related to its mechanical resistance and morphology, what needs to be further evaluated.

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