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Chitosan polyelectrolyte complexes for use in tissue engineering and drug delivery

S. Bubeníková[1,a], I. Lacík[2,b], D. Bakoš[1,c], L. Vodná[1,d]

[1]Department of Plastics and Rubber, Faculty of Chemical and Food Technology, Slovak Technical University, Radlinského 9, Bratislava, Slovak Republic

[2]Polymer Institute, Slovak Academy of Science, Dúbravská cesta 9, Bratislava,Slovak Republic

[a]silvia.hanzelova@stuba.sk, [b]igor.lacik@savba.sk, [c]dusan.bakos@stuba.sk, [d]lucia.vodna@stuba.sk

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Abstract. The paper presents the first part of the work focused on preparation of biodegradable chitosan microcapsules with tailored properties for potential applications in medical field as drug temporary carriers. In this paper, we aimed to prepare chitosan and chondroitin sulphate microcapsules using TPP as the second cross-linker and investigate the formation of the capsule membrane and its permeability in dependence on conditions of polyionic complexation. As a model, TPP was used to assess an influence of concentration and reaction time on the microcapsule formation. The method of inverse SEC was used for pores size and permeability limit of capsules assessment. For chitosan/CHS/TPP capsules, the distribution of pores size in the membrane is rather broad, which can be suitable for applications in tissue engineering and drug delivery systems.

Introduction

Chitosan is a biocompatible and biodegradable biopolymer, which due to its unique properties is known to have high potential for broad range of applications, especially in healthy food and biomedical areas. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been extensively examined in the pharmaceutical industry in the development of drug delivery systems [1-4].

The use of complexation between oppositely charged macromolecules to prepare chitosan beads (or microspheres) as controlled drug release formulation has attracted much attention because this process is very simple and mild [5,6]. In addition, reversible physical cross-linking by the electrostatic interaction instead of the chemical cross-linking is applied to avoid possible toxicity of reagents and other undesirable effects. For example, tripolyphosphate (TPP) cross-linked chitosan beads can be prepared simply by dropping chitosan droplets into TPP solution and this procedure was found to be useful in the pharmaceutical industry [7-9]. Furthermore, other anions (sulfate and citrate etc.) were also found to interact with chitosan by the electrostatic force [10-11]. TPP/chitosan bead usually had poor mechanical strength, which limited its usage in drug delivery. The properties of chitosan-based capsules depend on a number of parameters, the role of which one needs to understand in order to control the capsule formation process and capsule properties. For example, a novel approach was developed to improve the mechanical strength of TPP/chitosan beads by more than ten times [12]. Unfortunately, up to now, only a few ionic cross-linked chitosan beads have been reported.

Our work is focused on the preparation of biodegradable chitosan microcapsules with tailored properties for potential applications in medical field as protein and cell temporary carriers. In this paper, we aimed to prepare chitosan and chondroitin sulphate microcapsules using TPP as the second cross-linker and investigate the formation of the capsule membrane and its permeability in dependence on conditions of polyionic complexation. As a model, TPP was used to assess an influence of concentration and reaction time on the microcapsule formation.

Experimental

Chemicals. Chitosan in powder form [degree of deacetylation (DD)~85%, Mw = 780 000] was obtained from Fluka, CAS number 9012764. Chondroitin-4-sulphate (CHS) was obtained from Merck, Germany, CAS number 9082079, Mw = 50 000. Natrium tripolyphosphate ($Na_5P_{10}O_3$) (TPP) was obtained from Acros, Belgium, CAS number 7758294, Mw = 367.85.

Preparation of chitosan microspheres. Chitosan powder (1g) was dispersed in adequate amount of water containing 0.5 wt% acetic acid. Then 0.9g NaCl was added and the mixture was mechanically stirred for minimally 4h at 40°C to prepare the dissolved chitosan solution. The pH of chitosan solution was modified to pH 5 with water containing 1 wt% NaOH, so that the final concentration of chitosan solution was 1 wt%. The TPP solution is an aqueous solution composed of ionic crosslinker (TPP) (1, 1.5, 2 wt%) and 0.9 wt% NaCl. The chitosan solution was directly dropped trough a syringe needle into the solution of TPP (collection time 5s), and the chitosan droplets stood in the solution for 30, 60, 120s to form chitosan microspheres. After reaction, the spheres were separated and washed thoroughly with buffer (0.9 wt% NaCl, pH=7) and collected in a buffer with the same properties.

Chitosan/TPP/CHS capsules were prepared from the polycation solution containing 1 wt% chitosan and 0.9 wt% NaCl adjusted to pH=5, and the polyanion solution with 0.5 wt % CHS, 0.5 wt % TPP and 0.9 wt% NaCl adjusted to pH=7. Reaction time of complexation 30 s was selected for this type of capsules. The multi-loop reactor (Fig. 1) was used to control the reaction time of complexation.

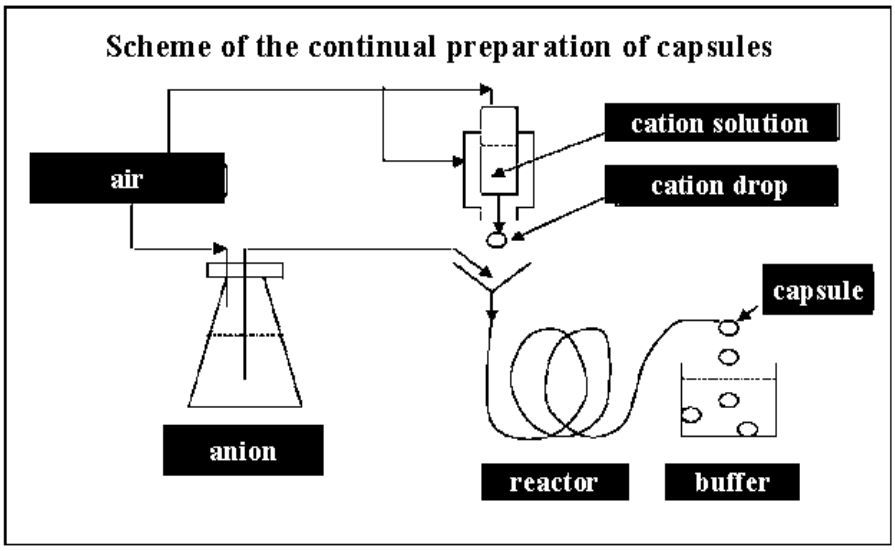

Fig.1. Scheme of the continual preparation of capsules [13] used in the experiment

Capsule size and morphology. The capsule diameter, membrane thickness and size distribution of capsules were determined using microscope Kappa 2000, Kvant s.r.o., Slovakia fitted with a color CCD camera Mintron CC-63KWIP, Malaysia interfaced to a PC operating with Prover Image Forge v 1.1, Prover s.r.o., Slovakia image analysis software. The sample of 15 capsules was examined, and the mean standard deviation was determined.

Inverse SEC. The method was used for pores size and permeability limit of capsules assessment. Specifications: Glass column 10 x 250 mm (adjusted pluger), the setup - pump Waters 515, Rheodyne injector 7725i, DRI Detector Waters 2410, PSS WinGPC 7 ALLTECH, USA; eluent - 0.9 M NaCl with 200 ppm NaN_3; flow rate: 0.2 ml/min. The polysaccharide standards were used for the calibration curve for capsules, which served as the column bed. The basic step was the determination of the distribution coefficient K_d for the standards $K_d = (Vi - Vo)/(Vt - Vo)$ with values <0 ; 1>. Coefficients K_d of the standards were used to construct the calibration curve log Mw = f(1 - K_d) and for the calculation of the distribution curve.

Results and discussion

Chitosan dropped into the polyanionic solution produced immediately capsules with a spherical shape, formed through the ionic complexation at the interface of chitosan droplets. We experienced that the degree of deacetylation and molar mass of chitosan are dominant parameters determining the capsule formation process, which led to the selection to use a relatively high molecular weight polymer. Other parameters, such as pH and ionic strength during the complex formation, concentration, gelation time and ratio between TPP and CHS4, need to be optimized to form non-sticky, spherical and chemically and mechanically stable core-shell type microcapsules shown in Fig. 2. It is clear from the pictures that the reaction time is a very sensitive factor influencing markedly the capsule membrane. In this case, TPP itself was used as a polyanion for the complexation and for the study of uniformity of microcapsules. To control the reaction time in the range of tens of seconds, it is absolutely crucial to use the multi-loop reactor [14-15] for this capsule type. This type of the reactor facilitates a high control of the process, which is connected with adjusting the size, shape and topology of the capsules. Regarding the applications of capsules, this continual process enables to control the permeability of the capsule membrane.

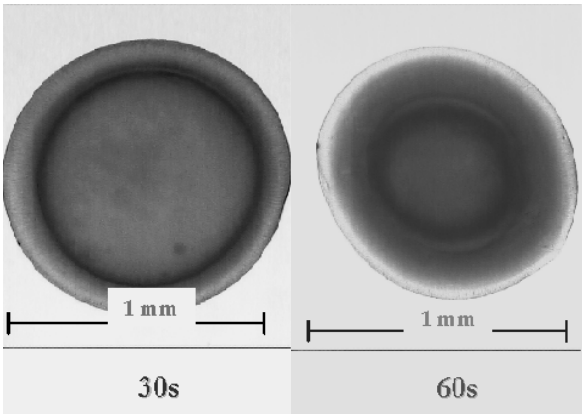

Fig. 2. The form and shape of microcapsules prepared using different reaction times

The microcapsules prepared continually in the multi-loop reactor with the reaction time 30 s were uniform with a regular spherical shape. The effects of various concentrations of TPP and various reaction times of the complexation on the capsule size and the membrane thickness can be seen from Table 1. Regarding the measured size of capsule, here it is clear that the process of

Table 1. Membrane thickness of the uniform capsules in dependence on TTP concentration and reaction time

TPP concentration [%].	Reaction Time [s]	Capsule size [mm]	Standard deviation	Membrane thickness [mm].	Standard deviation
1	30	1.281	0.067	0.129	0.047
	60	1.170	0.054	0.231	0.012
	120	1.102	0.039	0.479	0.015
1.5	30	1.027	0.007	0.182	0.012
	60	0.982	0.007	0.258	0.011
	120	1.058	0.017	0.479	0.014
2	30	1.095	0.010	0.200	0.011
	60	1.050	0.024	0.312	0.012
	120	1.113	0.039	0.471	0.037

capsules preparation is standard and the size of the capsules is uniform. Great differences are shown in the membrane thickness, which is markedly influenced by the reaction time. The standard deviation has the lowest value for the TPP concentration 1.5 % for both capsule size and membrane thickness.

Besides of the membrane composition, it is necessary to know the local concentration and distribution of polymers in the membrane in the direction from the surface to the core of the microcapsule. Information on this parameter is rarely emphasized despite of its importance for the membrane stability, permeability, pores size distribution, as well as on the influence of chemical and physical environment on a active compound encapsulated.

The results from SEC using the polysaccharide standards to characterize the chitosan/CHS/TPP capsules are given in Table 1. The scale of Mw of standards corresponded to the required pores size to describe their distribution in the capsule membrane. The calculated values of the dependence of 1 – Kd on log Mw together with the theoretical curve of this dependence is shown in Fig. 3.

Table 2. SEC results for the standards and the calculated values of parameters for capsules characterisation

Standard	Mw	Elution volume [ml]	Kd	Pore size [nm]
Pullulan	788000	7.47	0.000	26.3
	212000	7.54	0.009	12.8
	112000	8.32	0.120	9.0
	43700	9.51	0.289	5.6
	22800	9.08	0.228	3.7
	11800	9.75	0.323	2.6
	5900	11.86	0.622	1.8
	738	14.46	0.990	0.6
Saccharose	342	14.53	1.000	0.4

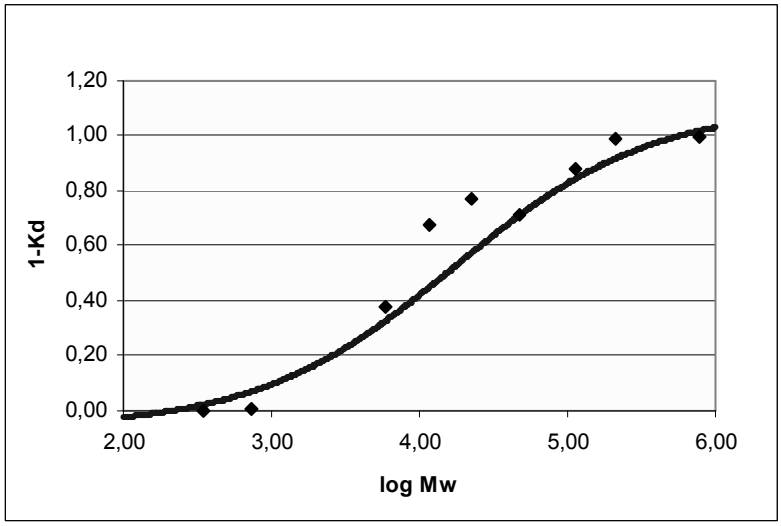

Fig. 3. The experimental (spots) and theoretical (curve) dependence of 1 – Kd on Mw

The derivative curve represents the distribution curve of pores according to size in the membrane. For chitosan/CHS/TPP capsules, the distribution of pores size in the membrane is rather broad as it is seen from Fig. 4. This is different from the alginate beads, which can be prepared with a very narrow distribution of pores size [16]. In the case of alginate beads, this can be an advantage

since they are developed for the encapsulation of live cells, particularly for encapsulation of pancreatic islands. As the membrane has a function to protect cells against antibody molecules, the narrow distribution of pores of a required size is acceptable. On the other hand, if applications of chitosan/CHS/TPP capsules are focused on delivery of bioactive compounds, a broad distribution of pores size does not play such a role. In this case the parameters of capsules, which can be influenced by environment in a given application, are more important (pH, swelling, enzymes, degradation, erosion, etc.)

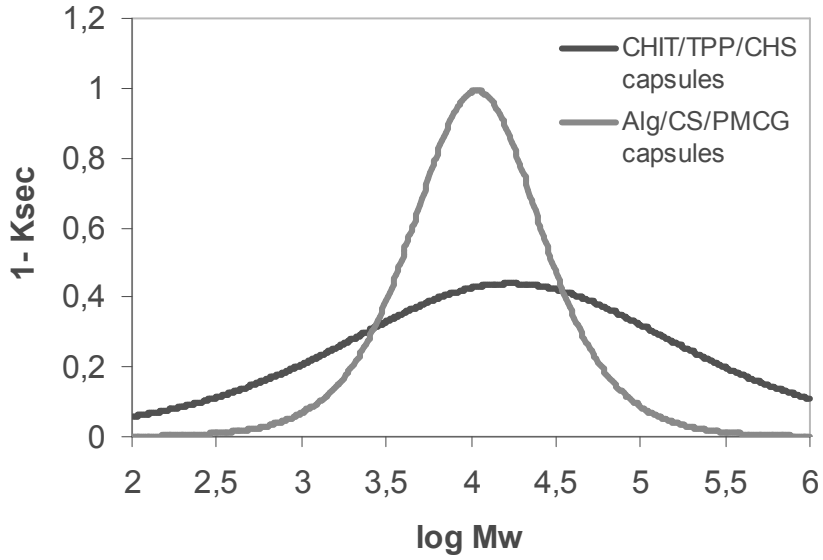

Fig. 3. Distribution curves of pores size for chitosan/CHS/TPP and alginate capsules

Summary

Spherical microcapsules with chitosan core and CHS/TPP shell with an average diameter around 1.0 mm were prepared. The optical microscopy shows that the microcapsules have a promising membrane permeability potentially allowing to control the diffusion properties of the membrane. Based on our knowledge from capsule formation process as well as from the polymer chemistry, this capsule will be further optimized for offering to medical and pharmaceutical applications, where the features of this biodegradable microcapsule can be utilized.

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