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Influence of Changes in Composition of the Membrane-forming Solution on the Structure of Alginate-polyethersulfone Microcapsules

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The aim of this study was to evaluate the effects of changes in composition of the membrane--forming solution on the structure of alginate-polyethersulfone microcapsules as determined by optical microscopy and scanning electron microscopy. The microcapsules were produced from 4 solutions of different concentrations and molecular weights of synthetic polymer (polyethersulfone, PES) and porophore (polyvinylpyrrolidone, PVP). The composition of the membrane-forming solution strongly affected the structure of microcapsules. An increase in PES concentration caused a decrease in the membrane thickness. The inner and outer layers of the membrane became thinner and denser, while the pores of the middle finger-like zone turned into more regular, channel-like structures. The size of the pores was not directly affected by the molecular weight of porophore, however, an increase in its concentration resulted in formation of the larger inner surface pores, but the smaller outer surface pores.

K e y w o r d s: alginate-polyethersulfone microcapsules, scanning electron microscopy, optical microscopy, microcapsule structure, membrane porosity

1. Introduction

Microcapsules intended for medical applications such as hybrid organs, advanced drug delivery systems or extracorporeal life support systems are commonly produced by double-step method developed by Lim [1]. In this method an alginate

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core of microcapsule is coated with one or several layers of polyaminoacids such as poly-L-lysine, polyornitine [2, 3] or poly-co-guanidine [4]. The microcapsules produced according to Lim's method are highly biocompatible [5], but the thin alginate-polyaminoacid membrane cannot provide sufficient mechanical resistance [6]. In addition the control over porosity of such membranes is very limited. These difficulties have been overcome by a development of membranes produced from synthetic polymers such as polyethersulfone or polypropylene. This type of membranes has been well characterized and extensively used in medicine for separation, dialysis or tissue engineering [7–10]. So far, only one method of production of synthetic polymer microcapsules, intended for encapsulation of living cells, was proposed (Sefton, 1982) [11]. It is a single-step method, in which cells suspended in Matrigel of Ficoll-400 are encapsulated by submerged nozzle-liquid jet extrusion in a thin membrane composed of HEMA-MMA copolymer [12]. These microcapsules have been tested on HepG2 [12] and Chinese hamster ovary fibroblast cell lines [13] and shown to create a favorable environment for cells. The membrane permeability was improved by the addition of porophore to the membrane-forming solution [14].

Our method for manufacturing microcapsules is a single-step process using a triple nozzle [15, 16] and an electrostatic droplet generator. It enables production of mixed polysaccharide polymer microcapsules. A spherical hydrogel core (alginate gel) is coated with a semi-permeable membrane composed of synthetic polymer polyethersulfone (PES). The membrane-forming polymer solution is enriched with porophore polyvinylpyrrolidone (PVP) in order to enhance porosity of the membrane. Because of the opacity of microcapsules, in order to analyse their structure, microscopic examination is required. This paper presents a study of structural parameters of the alginate-polyethersulfone microcapsules produced from solutions of different concentrations and molecular weights of PVP and PES by two microscopic methods: optical microscopy and scanning electron microscopy (SEM).

2. Materials and Methods

The structural analysis was performed on 18 samples of the alginate-polyethersulfone microcapsules. The microcapsules were produced by coextrusion of 3 solutions: alginate (Sigma), glycerin (POCH) and membrane-forming solution containing: polyethersulfone (BASF), polyvinylpyrrolidone (Sigma) and N-methylpyrrolidone (Fluka). Two types of polyethersulfone were alternatively used: PES2020 (Ultrason E2020P, Mw 42 000 g/mol) or PES6020 (Ultrason E6020P, Mw 58 000 g/mol). The membrane-forming solution contained either porophore of molecular weight 10 000 g/mol (PVP10) or 40 000 g/mol (PVP40). The production of microcapsules was based on the phase-inversion method, in which PES and alginate jellify when

dropped into water bath containing calcium ions ($CaCl_2$, Ubichem) and surfactant Tween-80 (Serva) or methanol (POCH). The obtained microcapsules were assigned to 4 different groups depending on composition of the membrane-forming polymer solution and type of the gellifying bath used, as given in Table 1.

Table 1. Compositions of membrane-forming polymer solutions and gellyfying baths used for production of polyethersulfone-alginate microcapsules

Group number	Composition of membrane-forming polymer solution [%]	Viscosity at 20ºC [mPas]	Gellyfying bath composition
I	PES6020 – 7.0 PVP10 – 5.3	72	1.1% $CaCl_2$aq + methanol (2:1)
II	PES2020 – 11.0 PVP10 – 9.0	199	1.1% $CaCl_2$aq + 1% Tween-80
III	PES2020 – 11.3 PVP40 – 9.4	407	1.1% $CaCl_2$aq + 1% Tween-80
IV	PES2020 – 15.5 PVP40 – 8.0	1040	1.1% $CaCl_2$aq + 0.5% Tween-80

Samples were analysed using the scanning electron microscopy (SEM) and optical microscopy. Prior to observation the microcapsules were rinsed with deionized water and dehydrated with ethanol. Samples intended for observation under scanning electron microscope (Hitachi, TM-1000) were freeze-fractured under cryogenic conditions using liquid nitrogen. Sections of the microcapsules were dried for 15 minutes at 80ºC and observed. The microcapsules examined by optical microscopy were prepared for observation by embedding in polyacrylic resin (Leica Historesin Embedding Kit 7022 31731) and sectioning through their geometric center with microtome (Leica RM 2265). The 20 μm thick sections were placed on microscopic slides and examined. The images of microcapsules were captured and analysed with regard to the size of microcapsules, thickness of the membrane, the membrane internal arrangement and porosity of the inner and outer surfaces.

3. Results and Discussion

Tables 2 and 3 summarize results of the structural analysis of the microcapsules. The images of one microcapsule from each of 4 groups were selected as examples. The first column of Table 2 contains the optical microscope images of cross-sections through geometric center of the microcapsules. The second column presents the images of the microcapsule interior from the scanning electron microscope.

Table 2. Images of cross-sections through alginate-polyethersulfone microcapsules from optical microscope (column 1) and SEM (column 2, magnifications used are indicated below the images)

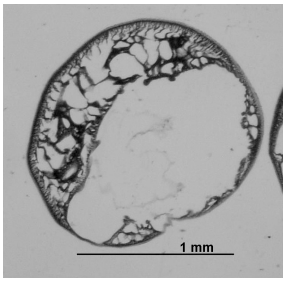
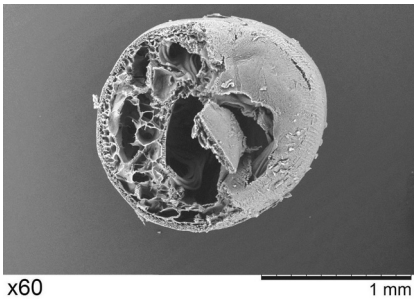
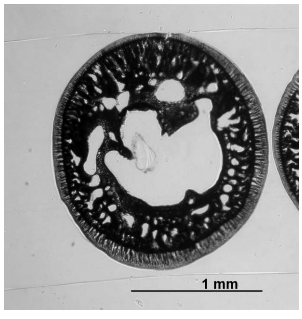
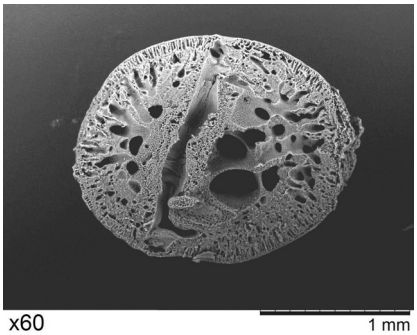
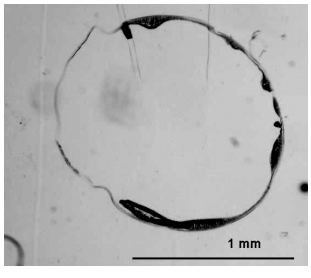
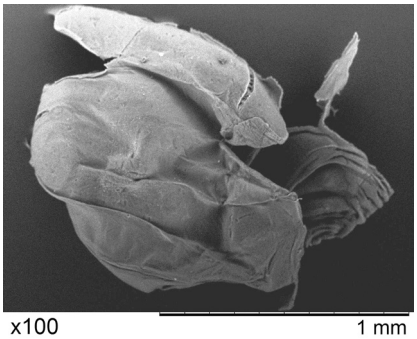
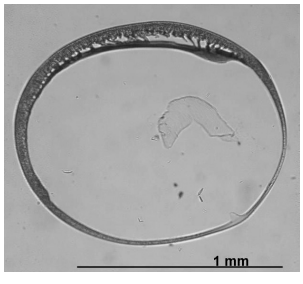
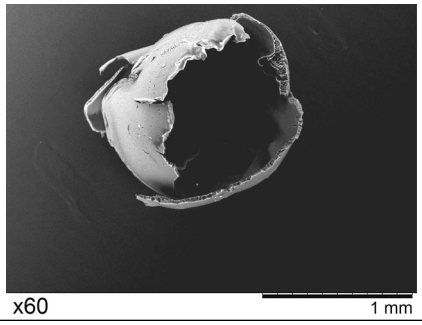
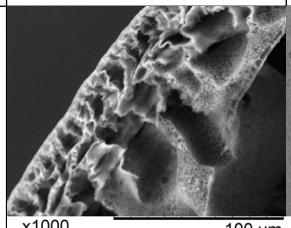
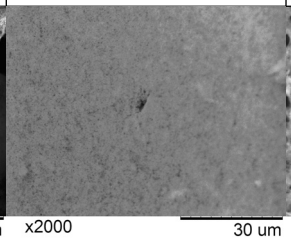
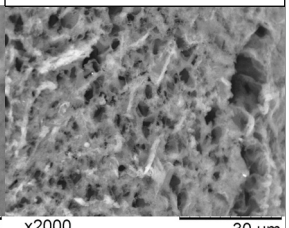
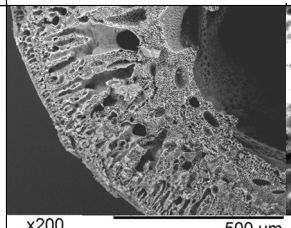
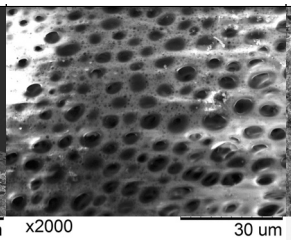
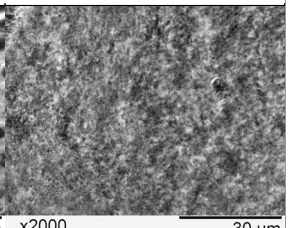
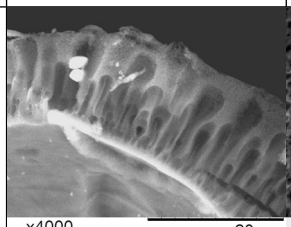
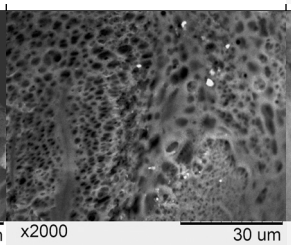
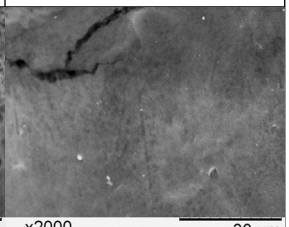
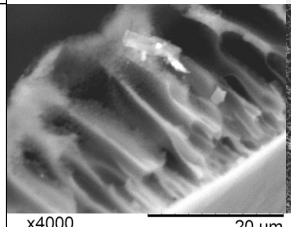
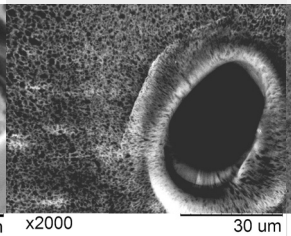
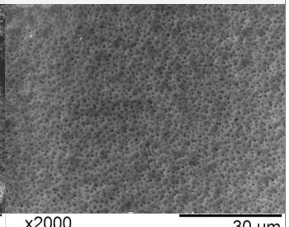
Group	Optical microscopy image	SEM image
I		
II		
III		
IV		

Table 3 contains SEM images depicting the internal organization of the membrane and the structure of inner and outer surface of the microcapsule.

Table 3. SEM images of polyethersulfone-alginate microcapsules structure: cross-section through the membrane (column 1), inner surface of the membrane (column 2) and outer surface of the membrane (column 3). Magnifications used are indicated below the images

Group	Cross-section through the membrane	Inner surface of the membrane	Outer surface of the membrane
I	 x1000 100 um	 x2000 30 um	 x2000 30 um
II	 x200 500 um	 x2000 30 um	 x2000 30 um
III	 x4000 20 um	 x2000 30 um	 x2000 30 um
IV	 x4000 20 um	 x2000 30 um	 x2000 30 um

All groups I–IV consisted of round to oval microcapsules of regular shape. In groups I and II the size of microcapsules ranged from 1.5 to 2.1 mm and the capsules' inner space was partially filled with a porous mass. The microcapsules from groups III and IV, despite almost the same size as in groups I and II (group III: 1.2–1.5 mm; group

IV: 1.3–2 mm), were mechanically fragile and got deformed during freeze-fracturing. The fragility of the microcapsules was caused by a low thickness of the membranes (group III: 20–150 μm; group IV: 30–98 μm) compared to groups I (127–235 μm) and II (478–566 μm), as well as lack of any submembrane material, which could serve as a support as observed in groups I and II. The variation in the membrane thickness between groups resulted from differences in the membrane-forming solution composition. At high concentrations of polymer (groups III and IV) the membranes obtained were thinner than at low PES concentrations (groups I and II). Figure 1 shows that an increase in PES2020 concentration from 11.0 to 15.5% causes 70% decrease in the average membrane thickness (from 517 μm to 146 μm).

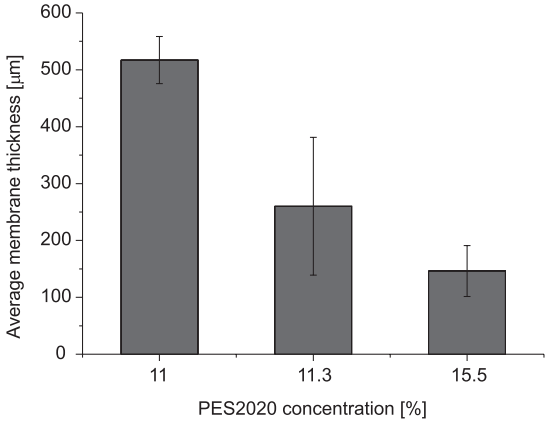


Fig. 1. Average membrane thickness in microcapsules produced from membrane-forming solutions of various concentrations of PES2020

Polymer concentration had also a large effect on the membrane internal structure (Table 3). The membranes in all groups were composed of three distinct layers: external skin layer, middle finger-like layer and internal skin layer. However, thickness and porosity of particular layers differed significantly between the groups. Within group I the middle zone of the membrane was loose and composed of irregular pores. Outer skin layer was thick and porous. In group II the membranes had much denser structure with more compact external skin layer. The microcapsules of groups III and IV shared a similar structure of the membrane. The inner and outer skin layers were thin and dense, while the middle layer consisted of regular long and narrow channel-like pores.

Porosity of the membrane was also affected by the composition of the membrane-forming solution (Table 3, Fig. 2). In groups I and IV the pores of the inner membrane surface were very small (0.5–2.5 μm), whereas in groups II and III much larger (3–10 μm). The size of the pores on the outer surface of the microcapsules did not correspond to the inner surface pore diameter. In groups II, III and IV the pore size was extremely small (0.5–1 μm), while in group I it ranged from 1 to 5 μm.

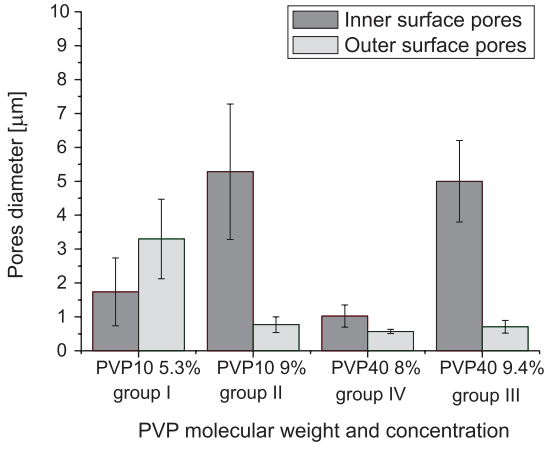


Fig. 2. Correlation between PVP molecular weight/concentration and the size of inner surface pores and outer surface pores

This data show that the size of the pores is not directly related to the molecular weight of polyvinylpyrrolidone. However, it was observed that an increase in PVP concentration caused a rise in size of the pores on the inner surface. The opposite effect was observed for the outer surface pores – their size decreased as the content of PVP in the solution was elevated (Fig. 2).

The third factor influencing the structure of the membranes was a type of gellyfing bath used for production of the microcapsules. The microcapsules which jellified in a bath containing methanol formed loose membranes with thick and porous outer skin layer (Table 3). The presence of a surfactant (Tween-80) in a gellifying bath resulted in formation of much more dense membranes with thin, compact outer skin layer.

Preparation for observation under SEM caused a shrinkage of the microcapsules. In all 4 groups 7–23% decrease in diameter was reported (Table 4). These values exceeded the allowable diameter variation defined by coefficient of variation (VC, Table 4). This reduction in size of the microcapsules results probably from removal of ethanol from the inner space of the microcapsule and pores of the membrane during drying at 80ºC. Drying prior to the observation removes ethanol from the microcapsule which prevents its sudden boiling and rupture of the membrane under vacuum in the specimen chamber of the microscope. Apart from the microcapsule diameter, also the thicknesses of the membrane and its layers determined by SEM were lower compared to corresponding results from optical microscopy analysis (data not shown). Therefore SEM analysis, despite its high resolution, may produce slightly underestimated results due to changes in dimensions of the microcapsules during preparation for the observation. This should be taken into account when drawing conclusions on the basis of obtained results.

Table 4. Percentage decrease in diameter of microcapsules after preparation for SEM observation

Group number	D_0 [mm]	D_{SEM} [mm]	$D_0 - D_{SEM}$ [mm]	$\Delta D = \frac{D_0 - D_{SEM}}{D_0} \times 100\%$ [%]	VC D_0 [%]
I	1.75	1.45	0.30	17	11
II	2.25	1.74	0.51	23	2
III	1.73	1.47	0.26	15	5
IV	1.37	1.27	0.10	7	5

Abbreviations: D_0 – initial diameter of microcapsules, D_{SEM} – diameter of microcapsules after preparation for SEM observation, ΔD – percentage decrease in diameter of microcapsules after preparation for SEM observation, VC D_0 – coefficient of D_0 variation.

4. Conclusions

Both optical and scanning electron microscopy were shown to be good methods for observation of the alginate-polyethersulfone microcapsules. The images of cross-sections obtained by both the techniques exhibit high similarity (Table 2). However, optical microscopy can be applied only to examination of general structure of microcapsules. The porosity and the detailed internal structure of the membranes cannot be determined this way. For these purposes scanning electron microscopy is preferable. The main disadvantage of SEM is the risk of deformation of the mechanically fragile microcapsules during freeze-fracturing. In addition, shrinkage of the microcapsules during preparation for observation under SEM may lead to underestimation of their size and other structural parameters such as membrane thickness or diameter of pores. We conclude that combination of both techniques is necessary for proper analysis.

All microcapsules examined had three-layered membranes of structure typical for flat and capillary membranes produced by the phase-inversion method [17, 18]. The membranes were composed of three clearly distinguishable layers: external skin layer, middle finger-like layer and internal skin layer. However, the microcapsules produced from the membrane-forming solutions of various concentrations of polymer and porophore were shown to vary with regard to membrane thickness and porosity. As concentration of the polymer increased the average thickness of the membrane decreased. The inner and outer skin layers became thinner and denser, while the pores of the middle zone turned into more regular, channel-like structures. In addition, the microcapsules formed in gellifying bath containing a surfactant (Tween-80) had more dense membranes with thin and compact outer skin layer.

The size of the pores on the membrane surface was not related to the molecular weight of porophore, but an increase in its concentration resulted in formation of larger inner surface pores. At the same time the pores of outer surface were reported to get smaller. These findings clearly indicate that alteration in composition of the membrane-forming solution allows to produce microcapsules of desired membrane thickness, structure and permeability.

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