A novel approach to the polymer microcontainer fabrication

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INTRODUCTION

Nowadays many different methods apply to encapsulate various bioactive substances, which possess hydrophilic or lipophilic properties. These methods involve emulsion or microemulsion polymerization, evaporation, diffusion, precipitation polymerization, entrapping in colloidosomes, liposomes etc. (Kaur 2007).

In most cases water-immiscible component is encapsulated by emulsification method, which requires additional stabilization of a system by different types of surfactants, surface-active polymers, proteins, polysaccharides or mixtures of them. Today to produce emulsion, different techniques could be used, such as high pressure homogenization, microfluidization, membrane contractor technique, and ultrasonication (Grigoriev 2009).

The present study demonstrates a new system for microcontainer fabrication, which can be obtained from direct and invert emulsions by applying a low-frequency ultrasound. The mixture of two biopolymers (Chitosan and Xanthan Gum) was used to form a shell of the microcontainers. Various types of hydrophobic materials were encapsulated by ultrasound treatment using the proposed approach.

MATERIALS AND METHODS

Chitosans (800-2000 cP, 20-300 cP), oligochitosan (average MW 5,000), Xanthan gum (from Xanthomonas campestris), poly-L-lysine (PLL) (MW 15000-30000), hylauronic acid (HA) (MW 1000), soybean oil, hexane, cyclohexane, toluene, fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich. Miglyol 840 was supplied from Sasol Germany GmbH.

***Microcontainer formations*:** Firstly, in order to eliminate an electrostatic interaction between Chitosan (Chit) and Xanthan Gum (XG), pH of their solutions (0.25% in all cases) was adjusted to 2. In the next step, the solutions of Chit and XG were mixed at a ratio 1:1. In the next stage, the direct O/W emulsion was formed by stirring 20 ml of the polymer mixture and 0.5 ml of oil (Miglyol, soybean oil, hexane, cyclohexane or toluene). Then the system was exposed to high-intensity ultrasonication (intensity of 25 $W \cdot cm^{-2}$ and frequency of 20 kHz). The obtained microcontainers were removed by centrifugation (6.5 × g, 10 min) and washed three times with water. Figure 1 shows a scheme of the microcontainer fabrication. The microcontainer surface was modified with polymers (PLL and HA) by consecutive incubation of the microcontainers in the polymer solutions (2 ml, 2 $mg \cdot ml^{-1}$, 0.15M NaCl) for 20 min under shaking (Vortex-genie 2, Scientific Industries, Inc., USA).

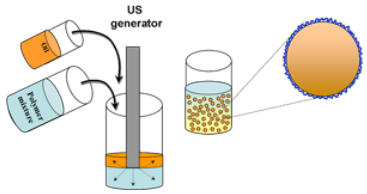

Fig. 1. The schematic representation of the microcontainer formation.

Microcontainer characterization: Fourier transform infrared (FTIR) measurements were carried out with a Bruker Hyperion 2000 IR. Confocal images were obtained using a Leica TCS SP confocal scanning system (Leica, Germany). Scanning electron microscopy (SEM) measurements were performed using a Gemini Leo 1550 instrument. Z-potential and size of the microcontainers were measured by a Zeta Sizer Nano (Malvern Instruments, UK).

RESULTS AND DISCUSSION

Since the polycation Chit can electrostatically interact with the polyanion XG, pH of their solutions was decreased to 2. Prevention of a complex formation is an important factor for the microcontainer fabrication. A low-frequency ultrasound was used to produce the emulsion of Miglyol in the mixture of Chit and XG. Miglyol was chosen as pure neutral oil, which shouldn't impact the ultrasonication procedure. Influence of several parameters on the container yield was examined, including duration of ultrasonic irradiation, polymer concentrations, and a volume of the sonicated solutions. The optimal conditions were found to produce the stable containers of Miglyol.

Chit and XG are known to form complex under neutral conditions, but they remain stable in the mixture at acidic pH. Since both polymers possess functional groups such as amino, hydroxyl and carboxyl, we suggest that they could be cross-linked by direct interaction between their polymer chains. Well known, that ulrasonication results in the formation of the cavitation bubble, which induces the production of highly reactive free radicals, especially $OH^{\cdot}$ and $H^{\cdot}$ (Canselier 2002). The hydroxyl radicals

undergo different reactions in the present of oxygen, which include superoxide ($HO_2^{\cdot}$) formation. Superoxides can crosslink polymer moieties located at the interface between a dispersed phase and a dispersion medium, which results in the polymer interaction. It leads to the creation of a stable structure at the surface of the emulsion droplets and subsequently the permanent shell of the microcontainers formation.

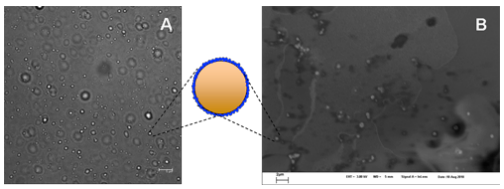

Fig. 2. A. CLSM image of the microcontainers. B. SEM micrograph of the microcontainers.

The influence of ultrasound on the polymer mixture was investigated by FTIR spectroscopy. The observations showed that the ultrasonic treatment leads to the changes in a chemical structure of the polymers that could be due to result of their interaction.

The microcontainers were assayed by confocal scanning microscopy (Figure 2A) and scanning electronic microscopy (Figure 2B). The observations reveal that the containers are not aggregated and remain stable after 2 month of storage at 3°C.

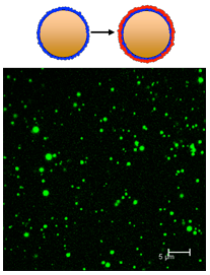

Fig. 3. The CLSM observation of the system modified by PLL-FITC.

In the next step, the containers were coated with an additional layer of PLL and HA. To demonstrate a deposition of PLL onto the nanocontainer surface, polypeptide was labeled with fluorescein isothiocyanate (FITC). The investigation by CLSM microscopy demonstrates a presence of fluorescence due to the adsorption of PLL-FITC (Figure 3). Additionally, the measurements of electrophoretic mobility showed the reversal of the surface charge from -30 mV (outer layer is XG) to +25 mV (outer layer is PLL-FITC). Both the facts proved the polymer adsorption on the nanocontainer surface.

The proposed procedure was applied to fabricate the containers loaded with another oils such as soybean oil, hexane, cyclohexane and toluene. The stability of the containers was not changed, which evidences that the encapsulated material (Miglyol) doesn't influence a generation of free radicals and consequently the cross-linking process.

Finally, we investigated one possible way to use the obtained microcontainers. They were incorporated into polymer film composed of HA. Figure 4 reveals the confocal observations of the coating that proves a relatively homogeneous and non-aggregated container distribution within the polymer film.

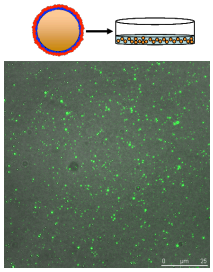

Fig. 4. The HA coating with the embedded microcontainers.

CONCLUSIONS

We proposed new type of the microcontainers fabricated by the ultrasound treatment. The microcontainer surface was successfully modified with positively and negatively charged polyelectrolytes followed by embedding into the polymer coating, which shows perspectives for their future applications. Although the conditions for the container preparation could be found not perfect for biological utilization, the technique is perspective and allows encapsulating variety of bioactive molecules.

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