알지네이트내에 리보좀을 이용한 천연산화제의 캡슐화

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Encapsulation of natural antioxidants using liposome-in-alginate

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Nowadays natural antioxidants are desirable in different food products since they preserve flavour and colour of the food and prevent vitamin destruction. It is also well known that antioxidants have strong impact on health; hence, food with addition of natural antioxidants is often demanded by the market. Still, natural antioxidants are very sensitive; therefore, there is a need for their protection [1-2]. Encapsulation is proposed as one of convenient method for that. It was already explained that, both, liposomes and polymers can protect antioxidants from the light and oxygen and preserve their stability and activity to some extent. In order to achieve prolonged release of the antioxidants, in this study we developed a complex systems based on liposomes incorporated within a polymer matrix [3]. Non-toxicity, biocompatibility, thermal and chemical stability are the main features, on account of which the alginate is selected for a polymer. Resveratrol was used as a model antioxidant substance [4-5]. It is phytoalexin found in several plants and it is also well known compound of red wines. According to literature, proliposome method appeared to be a convenient way to produce liposomes with encapsulated RSV. The aim of this paper is to determine mass transfer resistance provided by the membranes (liposomal and calcium alginate).

Natural antioxidants are usually very sensitive compounds and it is necessary to protect them from the light and oxygen in order to preserve their stability and activity. Convenient way to achieve this is to encapsulate antioxidants in proper matrix system. In this work resveratrol is used as a model antioxidant substance. The aim of this study was to encapsulate resveratrol in a complex system liposome-in-alginate in order to achieve its prolonged release, particularly in water based medium.

Experimental

Preparation of liposome with encapsulated RSV

Phospholipon 90G (P90G) (Natterman Phospholipids Germany) was the major component used in liposome formulation and it is more than 90% phosphatidilcholine (PC). In brief 2g P90G and 0,1g RSV was added to ethanol (96%) and stirred until the P90G was dissolved. In the

mixture heated to 60 0C for a few minutes after the addition of a small portion of water. Then the mixture was cooled down to ambient temperature and 100 ml distilled water was added into system as aqueous phase. The obtained suspension was stirred for 1 h at 800 rpm.

Preparation of liposome-in-alginate systems

Low viscosity Na-alginate was dissolved in distilled water in three different concentrations and after the addition of liposome suspensions, the final alginate concentrations of the solutions were 1%, 1.5% and 2.5% w/v (LV 1%, LV 1.5% and LV 2.5%). Three more samples were prepared by dissolving medium viscosity Na-alginate in water and after mixing with liposome suspensions, the final alginate concentrations were 1% and 1.5% w/v (MV 1% and MV 1.5%). The extrusion was done under an applied electric field between the positively charged needle and grounded collecting solution (distance 3.0 cm). The potential difference was controlled by a high voltage unit (Model 30R; Bertan Associates, Inc., New York, USA) and kept at a constant voltage of 6.3 kV. Collecting solution contained 50 mL of 2% w/v Ca-chloride. Solution droplets formed spherical insoluble hydrogel liposome-in-alginate systems (microbeads), after ions exchange. The liposome-in-alginate systems were left in the cross-linking solution for 30 min and then used for further analysis.

Results and Discussion

Encapsulation efficiency and average diameter

The results of encapsulation efficiency are shown in Table 1. As seen, all liposome-in-alginate microbeads were able to incorporate liposomes loading RSV in high yields (83-95%). As more dense the alginate network is the more of RSV it encapsulates. Thus, the highest EE was achieved in microbeads made of 2.5% medium viscosity alginate (EE% 95.06%) and the lowest one was determined for microbeads produced from 1% of low viscosity alginate solution (EE% 83.90%). According to previous studies as the alginate network is less concentrated, larger pores are within and the surrounding membrane is less tick (Shapiro & Cohen, 1997). Large pore structure of a network enables easy leakage of the encapsulated compound to an external solution. The size of microbeads is also influenced by the concentration and type of alginate (Table 1). Thus, the average diameter increased from ~360 to ~670 μm with the increase in concentration from 1 to 2.5% w/v for low viscosity alginate. The result is in agreement with previous studies on electrostatic extrusion in which the size of droplets forming microbeads is related to viscosity of the polymer solution. Manojlovic and co-workers have shown that the increase in viscosity of the polymer solution resulted in larger microbeads with wider size distribution.

Table 1. The influence of alginate viscosity and concentration on encapsulation efficiency of RSV and average diameter of microbeads.

Sample (w/v)	EE%	Average diameter (μm)
LV 1.0%	83.90	360.98
LV 1.5%	90.42	446.49
LV 2.5%	94.36	671.36
MV 1.0%	94.27	381.66
MV 1.5%	95.06	583.26

The release studies

The release studies were performed using Frantz diffusion cell and the release kinetics is presented in Fig. 1. The release started after approximately 30 minutes and diffusion equilibrium was reached after about 300 minutes. Obviously, alginate matrix of liposome-in-alginate microbeads provided extended release of RSV compared to liposomes. Fig. 1 also shows that diffusion rate is higher as the alginate is less concentrated. Here, it should be emphasized that the rate of RSV release is also influenced by the membrane thickness (membrane is composed of acetate-cellulose membrane plus layer of microbeads) presented in Table 2; thus, microbeads made from medium viscosity alginate was not able to retain RSV better than microbeads with low viscosity alginate of the same concentration (since values of membrane thickness are higher for samples with MV than LV alginate). Since RSV has low solubility in aqueous surroundings such as the one in hydrogel, it was released only up to 1.5 % (expressed on the base of the initial concentration of RSV in the donor compartment of the Frantz cell).

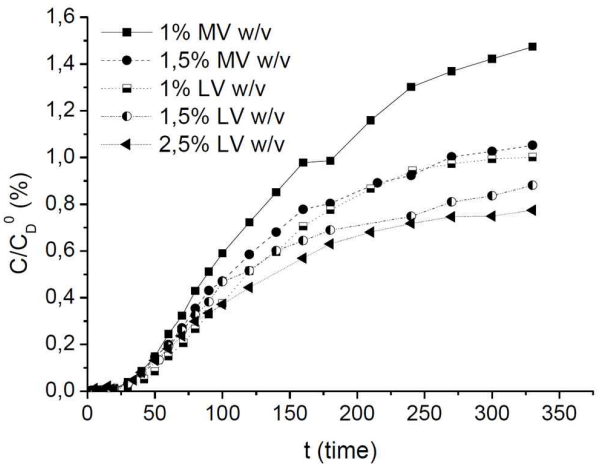

Fig. 1 RSV release curve for liposome-in-alginate microbeads

Table 2. Diffusion coefficients for different liposome-in-alginate systems and membrane thickness of the Frantz cell.

Sample (w/v)	δ, mm	D, m²/s
LV 1.0%	2.60	$9.37 \cdot 10^{-10}$
LV 1.5%	2.93	$9.04 \cdot 10^{-10}$
LV 2.5%	2.26	$6.69 \cdot 10^{-10}$
MV 1.0%	2.54	$8.03 \cdot 10^{-10}$
MV 1.5%	2.42	$7.36 \cdot 10^{-10}$

Diffusion coefficients and mas transfer resistances

Fick's second law was used for determination of diffusion coefficients of RSV from liposome-in-alginate microbeads and the values are given in Table 2. As shown the diffusion coefficients decrease with the increase in alginate concentration for both types of alginate (LV and MV). As expected, the values are lower for samples made of low viscosity alginate when compared with the microbeads produced with medium viscosity alginate of the same concentration. Diffusion coefficients of the same order were reported by Pjanović and co-workers who investigated encapsulation of lidocaine hydrochloride and dihydroquercetin in liposomes

which were incorporated within sodium polyacrylate and carbopol resin matrixes. For sake of comparison, the values are one order of magnitude smaller than those which have been obtained in our recent report for diffusion coefficient of RSV from free liposomes. The release of active compound from any system is directly affected by the mass transfer resistance provided by the membrane. Comparison of mass transfer resistance provided by both, lipid membrane and alginate matrix cumulatively, for liposome-in-alginate microbeads are given in Fig. 2. As it was predictable, the mass transfer resistance increased with alginate viscosity and alginate concentration increasing. Among all samples, the highest value was obtained for liposome-in-alginate microbeads with 2.5% w/v low viscosity alginate (2.80·106 s/m).

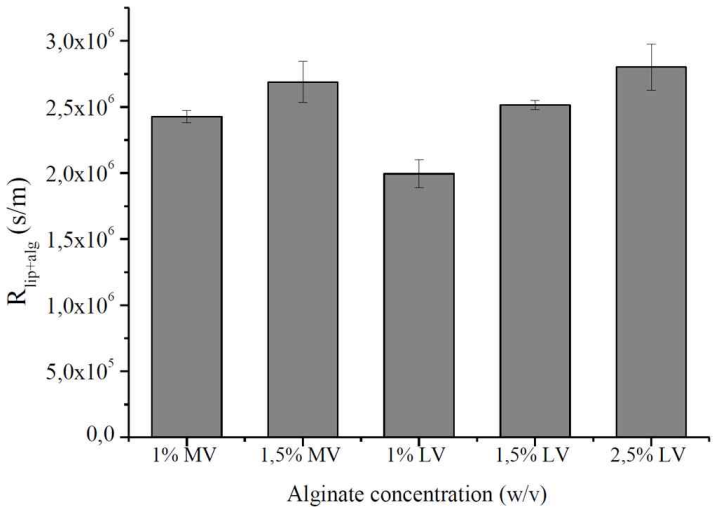

Fig. 2 Mass transfer resistances of liposome-in-alginate systems

Conclusion

In this study RSV was efficiently encapsulated in liposomes-in-alginate microbeads. The increase in alginate concentration and/or alginate viscosity led to an increase in size of microbeads and efficiency of encapsulation.

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