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Novel resveratrol delivery systems based on alginate-sucrose and alginate-chitosan microbeads containing liposomes

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ABSTRACT

We reported the design of liposome-loaded Ca-alginate microspheres as a drug delivery system for controlled release of resveratrol. The effect of admixed sucrose and chitosan coating was assessed in terms of physicochemical, thermal and release properties of liposome-in alginate systems with encapsulated resveratrol. The diameter of liposomes produced by proliposome method increased from 412 to 471 nm with addition of sucrose as a cryoprotectant. DSC analysis revealed that phospolipids interact with each other while forming the lipid bilayer and that resveratrol was incorporated within the lipid bilayer, causing destabilizing effect in the two temperature regions (137–202 °C and 240–270 °C). Liposomes were entrapped within polymer network and remained intact as determined by SEM cross-sectional observation of the microbeads. Liposomes interfered with the thermal behavior of alginate in the temperature region above 220 °C. The presence of liposomes decreased the strength of the beads in comparison to placebo beads, according to mechanical tests on compression. Release studies performed in Franz diffusion cell showed the overall resistance to mass transfer one order of magnitude higher (10^6 s/m) than the resistance ascribed solely to the liposomal membrane. The chitosan coating, visible as a dense surface layer (~7 μm thick) in dry state, caused decrease in encapsulation efficiency of resveratrol (85% *vs.* 91%) and in size of the particles (d_{50} of 387 *vs.* 440 μm); the chitosan also caused weakening of the polymer matrix, but increased resistance to drug diffusion (11.4×10^5 s/m) in comparison to the uncoated alginate-liposome formulation (9.1×10^5 s/m).

1. Introduction

Resveratrol (RES) is a natural phenolic compound with remarkable antioxidant activity, upon some conditions even stronger that those of vitamin C and E (Stojanović, Sprinz, & Berde, 2001). Resveratrol has been shown to modulate the metabolism of lipids and to inhibit the oxidation of low-density lipoproteins and aggregation of platelets (Dalaklioglu, Genc, Aksoy, Akcit, & Gumuslu, 2012; Lim et al., 2014; Toliopoulos, Simos, Oikonomidis, & Spyros, 2013). Moreover, as phytoestrogen, resveratrol provide cardiovascular protection (Das & Das, 2010). This compound also possesses anti-inflammatory and anticancer properties (Amri, Chaunmeik, Sfar, & Charrueau, 2012). Nevertheless, the utilization of resveratrol in the food industry is limited due to its high sensitivity and instability. Earlier researches highlighted several obstacles regarding the resveratrol application: (1) low stability against oxidation (Pineiro, Palma, & Barroso, 2006); (2) high photosensitivity; (3) insolubility in water and (4) short biological half-life (Lopez-Nicolas, Nunez-Delicado, & Perez-Lopez, 2006).

Encapsulation technology has emerged as a major strategy to improve stability and bioavailability of resveratrol and to achieve targeted and/or prolonged release. The contemporary researches on this topic propose a number of different systems for resveratrol encapsulation. Among them, liposomal resveratrol formulations have been proved to have high entrapment efficiency, improve resveratrol stability and biological activity against UV-B-induced oxidative damage, decrease the cytotoxicity of resveratrol at high

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concentrations and improve the efficiency of resveratrol on the cell-stress response (Caddeo, Teskač, Sinico, & Kristl, 2008; Isailović et al., 2013; Kristl, Teskac, Caddeo, Abramovič, & Šentjurc, 2009). However, the lipid-based systems, such are liposomes, often suffer from the problem of instability, which constrains their application especially in food industry. A possible solution is drug loaded liposomes in hydrogel matrices; this kind of delivery systems has been proposed by several authors in the last decade, but mainly in the form of wound dressings, injectable depot systems and formulations for topical treatment of diseases (Gao et al., 2014; Jøraholmen, Škalko-Basnet, Acharya, & Basnet, 2015; Lee, Oh, Baxa, Raghavan, & Blumenthal, 2012; Pjanović et al., 2010), while very little, if any, work was done on resveratrol formulations aimed at oral applications. The combination of the two (polymer-based and the lipid-based) systems integrate their advantages and avoid disadvantages. Besides that, more designable drug release kinetics could be achieved by hydrogel-microencapsulated liposomes compared with that by microcapsules or liposomes alone. Inspired by this knowledge we aimed to design novel resveratrol delivery systems based on hydrogel microbeads containing liposomes with incorporated resveratrol.

However, the fabrication methods of liposomes-in-gel systems are generally time-and cost-consuming. To deal with this problem, the systems developed in this study were prepared with some modifications. Firstly, resveratrol loaded liposomes were produced with a commercial lipid mixture by proliposome method which is considered as easy to scale-up and proven to be effective in encapsulation of resveratrol (Isailović et al., 2013). Secondly, sucrose was used for liposome preparation as a cryo- and lypoprotectant; namely, the sugar molecules interact with the phospholipid headgroups, counteracting fusion or membrane disruption (Stark, Pabst, & Prassl, 2010). Thirdly, thus obtained MLVs were encapsulated into alginate together with the drug remained nontrapped in MLVs. In this way, drug losses were reduced to minimum, and separation step (for separation of liposomes from free drug) has been avoided. The encapsulation of liposomes in alginate was performed by applying electrostatic extrusion to form micro scale particles containing resveratrol loaded liposomes.

Alginate is biodegradable, biocompatible and non-toxic natural polysaccharide, generally regarded as safe (GRAS) by FDA (George & Abraham, 2006). Alginate gels are particularly suitable for oral applications due to their properties of bioadhesiveness and pH sensitivity. In our particular case, shrinking of the alginate gels at low pH (gastric conditions) and dissolution of alginate in intestinal conditions would allow the release of liposome containing resveratrol, and subsequently resveratrol at the targeted place of human gastrointestinal tract – small intestine. In addition, it is likely to expect that alginate matrix around liposomes, acting as a mass transport barrier would decrease lipase activity (and thus the rate of lipid digestion and resveratrol release) by decreasing the rate of lipase diffusion. Despite all aforementioned, the alginate gels have a limitations reflected in two main problems: (1) bioactive compounds loss during gels formation (Won, Kim, Kim, Park, & Moon, 2005) and (2) burst release of bioactives (George & Abraham, 2006). These undesirable tendencies derive from large pore size of alginate gels (Gombotz & Wee, 2012). As a solution to this problem, in this study an attempt was made to formulate the optimal dosage forms, either by using sucrose as a filler of the alginate matrix or by coating of the microbeads with chitosan. The mixtures of alginate and other materials (that act as a pores filler and/or form additional layer surrounding alginate gels) have been utilized before to control shrinkage upon drying, to improve mechanical properties and retard the drug release (Belščak-Cvitanović et al., 2011; Chan et al., 2011; Stojanović et al., 2012).

In this study are developed hybrid delivery systems based on alginate-sucrose and alginate-chitosan particles with entrapped resveratrol-bearing liposomes. These are characterized in terms of the rheology, particle size, morphology, mechanical, thermal and release properties with an aim of determining the contribution of each compound of such complex systems and interactions between them. This study provides valuable information for administering the complex system liposome-in-hydrogel with encapsulated bioactive compound(s) into functional food products.

Liposomes are biocompatible carriers that can be produced by different techniques. They can stabilize the encapsulated materials and can be used for entrapment, delivery, and release of poorly soluble compounds, such as resveratrol. Liposomes are commonly formed from naturally occurring components, and thus their application to food systems as a new formulation should be easily implemented.

2. Materials and methods

2.1. Materials

Phospholipon 90 NG was supplied by Natterman Phospholipids (Germany). It is the major component used in liposome formulation composed of more than 90% phosphatidilcholine (PC). *Trans*-resveratrol standard (RES, >99% pure) was obtained from ChromaDex (Irvine, CA, USA) and it was used as a model antioxidant substance. Na-alginate (medium viscosity) was supplied by Sigma-Aldrich (St. Louis, MO, USA). Calcium-chloride dehydrate, 99+%, as well as chitosan (molecular weight 100.000–300.000 Da) were obtained from Acros organics, USA, while sucrose was obtained from VWR, Prolabo, Belgium. All other chemical used were of analytical grade.

2.2. Liposome preparation

Multilamelar lipid vesicles (MLV) were prepared by proliposome method (PRO) from commercial lipid mixture Phospolipon 90NG (P90NG) as described by Isailović et al. (2013). In brief, P90NG, ethanol, resveratrol and small quantity of water was stirred with magnetic stirrer with heating at 60 °C for a few minutes. When the mixture was cooled to room temperature, 50 ml of water was added in small portions. The suspension was stirred for one more hour at 800 rpm. The final concentration of the lipids in the liposomes was 20 mg/ml, while the ratio between resveratrol and P90G was 1:20 w/w. When the liposomes were prepared with addition of sucrose the procedure was the same except that in the last step the 50 ml of sucrose solution (30% w/v) was used as aqueous phase.

2.3. Microbeads preparation

Sodium-alginate was dissolved in distilled water and then mixed with liposome suspension in volume ratio 1:1 to obtain final concentration of sodium alginate 1.5% w/v (Dai, Wang, Zhao, Li, & Wang, 2006; Strasdat & Bunjes, 2013). The solution was extruded through a blunt stainless steel needle (23 G) and a constant flow rate of 39.3 ml h^{-1} was achieved by a syringe pump (Razel Scientific Instruments, Stamford, CT, USA). The extrusion was done under an applied electric field between the positively charged needle and grounded collecting solution (distance 2.5 cm). The potential difference was kept at a constant voltage (6.3 kV) using a high voltage unit (Model 30R; Bertan Associates, Inc., New York, USA). The collecting solution was based on 2% w/v calcium-chloride. When the liposomes with sucrose were used for microbeads preparation, 15% w/v of sucrose was added in the collecting solution. The third kind of microbeads was prepared when the collecting solution was consisted of 0.5% w/v chitosan, 2% w/v ascorbic acid and 2% w/v