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A step forward towards the design of a continuous process to produce hybrid liposome/protein microcapsules

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ABSTRACT

Microfluidics and electrospraying, two revolutionary technologies with industrial potential for the microencapsulation of lipophilic bioactive ingredients, have been combined to produce hybrid liposome/protein microencapsulation structures in a semi-continuous process, reducing the number of steps required for their manufacture. Three different microfluidic mixing devices, one of them consisting of a simple straight microchannel (cross junction design) and the other two exhibiting patterned microchannels with different geometries (Tesla and 'splitting and recombination' designs), were used to mix a liposome suspension with a whey protein concentrate dispersion. The Tesla design showed the best mixing performance, as observed by fluorescence microscopy, so it was selected to be assembled to an electrospraying apparatus. The proposed in-line setup was successfully used to produce the micron-sized encapsulation structures, as observed by scanning electron microscopy.

1. Introduction

Liposomes can be used as delivery vehicles for the incorporation of lipophilic bioactive compounds into food products, increasing their stability and bioavailability (Frenzel et al., 2015). However, the high semi-permeability of their membranes and low physical stability (Frenzel and Steffen-Heins, 2015) limit their practical application. Microencapsulation of the liposomes within dry biopolymeric matrices, obtaining convenient powdery ingredients, has been proposed as a strategy to overcome these limitations (Gültekin-Özgüven et al., 2016; Tan et al., 2016; Van Den Hoven et al., 2012; Wang et al., 2015).

Specifically, the electrospraying technique, a drying technology based on the electrohydrodynamic processing or atomization of polymeric fluids, has been very recently used to produce dry liposome/protein microencapsulation structures, which proved to successfully stabilize and improve the bioaccessibility of curcumin (Gómez-Mascaraque et al., 2017). The main advantage of electrospraying over other more commonly used drying techniques such as spray-drying is its operation under mild temperature conditions avoiding the thermal degradation of thermosensitive bioactive ingredients (Gómez-Mascaraque and López-Rubio, 2016).

On the other hand, conventional technologies used for the manufacturing of liposomes usually require numerous pre- and post-processing steps (Tien Sing Young and Tabrizian, 2015). Microfluidics, a technology which allows manipulation of tiny volumes of fluids inside micron-sized channels, has been proposed as an alternative for the continuous production of liposomes by flow focusing (Balbino et al., 2013a).

The combination of microfluidics and electrospraying would allow the design of an industrially attractive continuous process for the production of liposome/protein microencapsulation structures similar to those previously developed in batch (Gómez-Mascaraque et al., 2017). For this purpose, an intermediate mixing step would be necessary in order to blend the liposomes with the polymer prior to electrospraying the mixture. Due to the low flow rates used in both techniques, this mixing step should be also accomplished by means of microfluidics.

Several designs for microfluidic mixing devices ('micromixers') have been proposed. The most simple design is based on straight channels (Bothe et al., 2006). In these devices, mixing occurs through a diffusion mechanism due to the low Reynolds numbers

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(Re) obtained for the fluids, which flow in the laminar regime as a consequence of the micro scale (Yang et al., 2015). However, diffusion-driven mixing is time-consuming and inefficient, and thus passive micromixers based on the modification of the flow channel geometry by adding obstacles in the flow path have been also designed in order to enhance fluid mixing by increasing contact between the two fluids. Several patterned microchannels with different geometries have already been developed (Afzal and Kim, 2012; Bhagat and Papautsky, 2008; Hong et al., 2004; Tran-Minh et al., 2014).

In this work, we report on the study of three different micromixer designs based on the cross flow junction, Tesla and SAR ('splitting and recombination') designs (Bothe et al., 2006; Hong et al., 2004; Tran-Minh et al., 2014), to achieve an effective mixing of a liposome suspension with a whey protein concentrate (WPC) dispersion. The selected micromixer was then assembled to an electrospraying apparatus to produce liposome/protein microencapsulation structures in a one-step process.

2. Materials and methods

2.1. Materials

A whey protein concentrate (WPC), under the commercial name of Lacprodan® DI-8090 was kindly donated by ARLA (ARLA Food Ingredients, Denmark). Pure phosphatidylcholine (98% ± 4%) stabilized with 0.1% ascorbyl palmitate, was obtained from Lipoid GmbH (Germany). Rhodamine B was obtained from Sigma-Aldrich (Germany). Absolute ethanol (>99.5%) was purchased from Synth (Brasil). Deionized water (>18 MΩ cm^{-1} resistivity) was purified using Milli-Q® SP Reagent water system plus from Millipore Corp. (USA). Sylgard 184 Silicone Elastomer Kit (Dow Corning, Midland, MI, USA) was used as material precursor of PDMS layers for the microfluidic devices, and NANO™ SU-8 photoresist formulation (MicroChem Corp., USA) was used to prepare the masks.

2.2. Production of the microfluidic mixing devices (micromixers)

Three different designs for the micromixers were projected using AutoCAD (Autodesk). The geometry of their channels is depicted in Fig. 1. The polydimethylsiloxane (PDMS)/glass microfluidic devices were then produced following the procedure described in (Balbino et al., 2013b), which is based on the conventional UV photolithographic and soft-lithography methods. The cross-section of the microchannels was rectangular in all cases, with a depth of 50 μm.

2.3. Preparation of protein and liposome dispersions

WPC (25% w/v) was dispersed in milliQ water at room temperature under vigorous magnetic stirring. Liposome dispersions (80 g/L) were prepared using the ethanol injection method followed by ultrasonication as described previously (Gómez-Mascaraque et al., 2017). Both dispersions were filtered prior to their pumping into the micromixers, to avoid clotting. The liposome dispersions were filtered using 0.45 μm, nylon, Whatman® syringe filters, while the WPC dispersions were filtered using filter paper under vacuum due to its high viscosity.

2.4. Mixing of protein and liposome dispersions using micromixers

The dispersions were introduced in glass syringes and were pumped through the selected microfluidic mixer devices with digitally controlled syringe pumps model KDS-100 (KDScientific, Massachusetts, USA). The protein dispersion was infused through the lateral inlets of the micromixers, each stream flowing at 0.95 μL/min. The liposomes were introduced through the central inlet at 0.6 μL/min. These rates were selected by carrying out a mass balance taking into account the prerequisites of the subsequent electrospraying process, which was optimized in a previous work (Gómez-Mascaraque et al., 2017) and required a total flow rate of 2.5 μL/min and a final WPC concentration in the mixture of 20% (w/w).

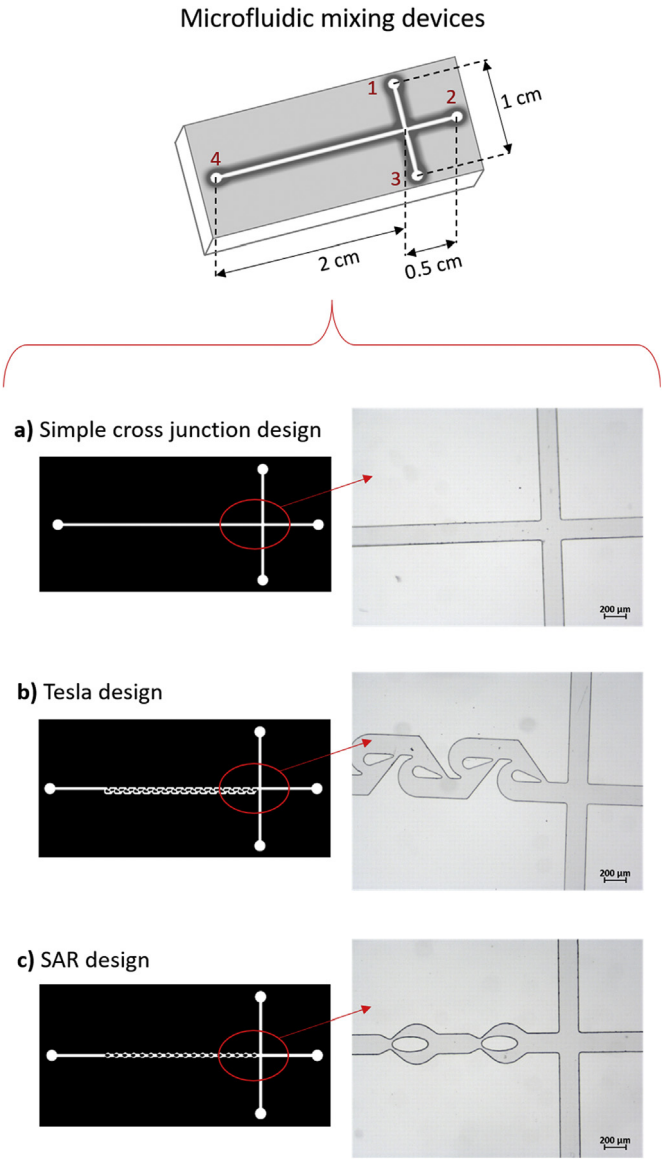

Fig. 1. Schemes and corresponding micrographs of the three different designs of microfluidic mixing devices used in this work. The microchannels are 200 μm wide and 50 μm deep.

2.5. Assessment of the quality of mixing

To assess whether the different microfluidic devices were effective in mixing both suspensions, 0.1 mM rhodamine B was added to the liposome stream as a fluorescent probe and the flow of the fluids through the microchannels was observed under an inverted research microscope Eclipse Ti-U, (Nikon, Tokyo, Japan) equipped with a digital imaging head which integrates an epi-fluorescence illuminator. A digital camera head (Nikon 1.5 MP DS-Qi1Mc, Japan) was attached to the microscope. Nis Elements

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