One-step microfluidic production of β-carotene-loaded ultrathin shell double emulsions aiming food applications

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Abstract: We demonstrate a microfluidic production of β-carotene-loaded W/O/W double emulsion using a three-dimensional glass-capillary device by combining co-flow and flow-focusing in coaxial glass-capillaries. The diameter of W/O/W emulsion droplets decreased with increasing flow rate of continuous phase, which ranged between approximately 100 and 180 μm. The microfluidic process developed is potentially useful for a broad range of applications in protection and delivery of food active compounds.

Key-words*: capillary;β-carotene; droplet.*

Introduction: The double water-in-oil-in-water (W/O/W) emulsions are three-phase dispersions composed of inner aqueous droplets dispersed in larger oil droplets, which are themselves dispersed in another aqueous phase. A variety of applications has been demonstrated due to their unique and highly hierarchized structure, such as encapsulation and controlled release of active compounds. However, preparation of W/O/W emulsions by conventional methods is not trivial and, in general, two emulsification steps are needed. Firstly, W/O single emulsion is produced from high shear mixing of immiscible liquids while in the second step, a W/O/W double emulsion is formed using milder shear conditions to avoid the disruption of the inner water droplet. Generally, this two-step process shows poor reproducibility with a broad size distribution of W/O/W double emulsion droplets being formed. To overcome these drawbacks, the microfluidic emulsification devices consisting of networks of flow microchannels have received increased attention as versatile and powerful tools for preparing in a single-step highly monodisperse W/O/W double emulsions. Here, we demonstrate a microfluidic production of β-carotene-loaded W/O/W double emulsion using a three-dimensional glass-capillary device by combining co-flow and flow-focusing in coaxial glass-capillaries.

Experimental procedure: The capillary devices were built on a glass slide, and consisted of two glass cylindrical capillaries inserted into the opposite ends of a square capillary, according to shown in Fig. 1(a). The cylindrical tube with smaller inner diameter was treated to render its surface hydrophobic, and the larger diameter tube to render its surface hydrophilic. For injection of aqueous innermost phase, a third cylindrical capillary was stretched, and inserted into the injection capillary. The W/O/W double emulsions were obtained using an innermost aqueous phase containing 1% (w/v) PVA and 9% (w/v) dextran. The phospholipid middle phase consisted of a mixture of 0.5% (w/v) soybean lecithin and 0.125% (w/v) β-carotene dissolved in the following organic solvent mixtures (1:1.8 v/v): chloroform/hexane; ethyl acetate/hexane or ethyl acetate/pentane. Besides, the continuous phase used in this study was an aqueous solution 10% (w/v) PVA. The innermost (q1) and middle oil (q2) phases were injected in stretched tube and cylindrical tube with smaller inner diameter, respectively, at a flow rate 1000 μl/h, according to Fig. 1(a). On the other hand, the continuous phase (q3) flowed through the interstices between the cylindrical tapered capillary and the square capillary, at a flow rate ranging between 3000 and 12000 μl/h. Bright field and fluorescence images were obtained with an inverted fluorescence confocal microscope (DMIRBE, Leica Microsystem, Germany).

Results and discussion: This process configuration forces the water droplets to become re-emulsified leading to formation of monodisperse W/O/W emulsion droplets with an ultra-thin middle oil phase at the orifice of the capillary collection tube, as shown in Fig. 1(a,b). In this dripping regime, the breakup of droplets is governed by the balance between the interfacial tension that constrains the droplet to the tip of the tapered tube and the drag force exerted by the continuous phase that pulls the droplet downstream. Therefore, droplets detachment is proportional to the viscosity of the continuous phase, but mainly to the velocity difference between the continuous and oil phases. Thus, an accurate control of W/O/W droplet diameter generation was observed by finely tuning the flow rate of continuous phase, as shown in Fig. 1(b) and Fig. 2(a). The diameter of W/O/W emulsion droplets decreased with increasing flow rate of continuous phase, which ranged between approximately 100 and 180 μm for all solvent mixtures (data not shown). Besides, the W/O/W emulsion exhibited high uniformity with coefficients of variation in the range of 3.0-6.0%. The thickness of ultrathin shell shows

generally a few micrometers, as shown in Fig. 1(d), much smaller than in typical double emulsion obtained by conventional methods. According to Fig. 2(a) the W/O/W double emulsion size obtained using ethyl acetate and pentane mixture showed a linear behavior as function of continuous flow rate, and thus a better capacity to fine-tuning of droplet diameter at higher continuous flow rates (9000 and 12000 µl/h). This result can be related with its lower viscosity in comparison to other mixtures, which can facilitate the droplets detachment and improve size control. Thus, it was possible to confirm that single-step microfluidic production of W/O/W emulsion droplet was efficient using solvents with low toxicity potential to human health, such as ethyl acetate and pentane, replacing solvents with higher toxicity potential, such as chloroform and hexane, commonly used in these processes. Fig. 2(b) shows that β-carotene was successfully incorporated inside phospholipid ultrathin shell due to its intrinsic fluorescence for all solvent mixtures. Because of the absence of polar groups in β-carotene structure, its localization was restricted only to the lipid membrane that was regulated by van der Waals interactions with the fatty acid chains. The confocal micrographs and particle size distributions indicated that W/O/W double emulsion droplets were highly monodisperse.

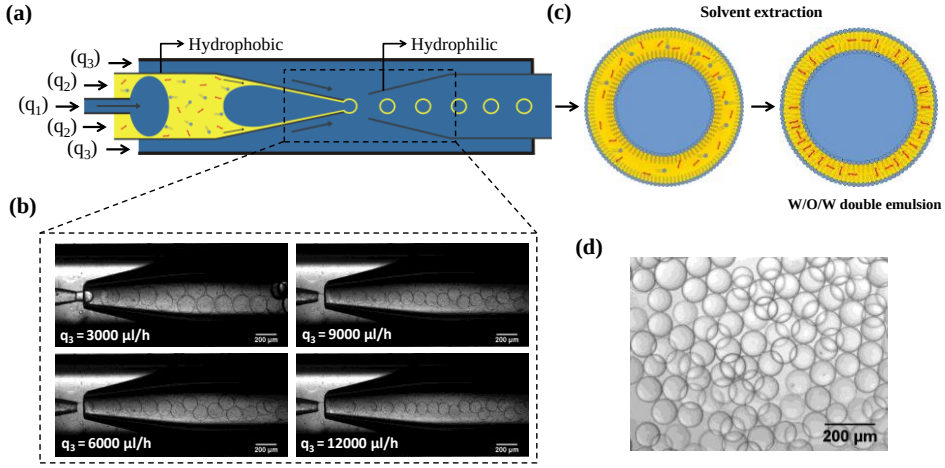

Fig 1. (a) Microfluidic production of W/O/W double emulsion with ultrathin shells containing β-carotene; **(b)** Optical microscope images of microfluidic process at continuous flow rates (q_3) ranged from 3000 µl/h to 12000 µl/h at flow rate of innermost (q_1) and middle lipid (q_2) phases equal 1000 µl/h; **(c)** Diagram of organic solvent extraction process; **(d)** Inverted optical microscope images of monodisperse W/O/W double emulsion droplet templates in the bottom of collection solution.

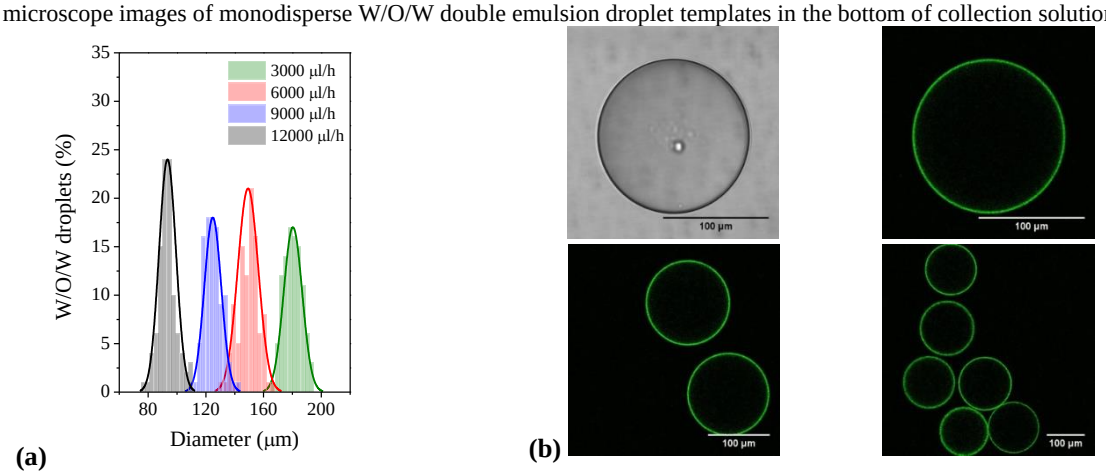

Fig. 2. (a) Influence of the continuous flow rate (q_3) on W/O/W droplet diameter distribution, at flow rate of innermost (q_1) and middle lipid (q_2) phases equal 1000 µl/h) using organic solvent mixture of ethyl acetate/pentane (1:1.8 v/v); **(b)** Confocal micrographs of β-carotene-loaded W/O/W droplet using an organic solvent mixture of ethyl acetate/pentane (1:1.8 v/v).

Conclusions: It was demonstrated that W/O/W double emulsion for food applications can be produced using glass-capillary microfluidic devices. Besides, the solvent mixtures replacement containing chloroform and/or hexane to green solvents with low toxic potential, such as ethyl acetate and pentane, allowed successfully the formation of stable W/O/W double emulsion. It was possible to demonstrate β-carotene incorporation inside lipid shell. Thus, this microfluidic process proposed is potentially useful for a broad range of applications in protection and delivery of food and pharmaceutical active compounds.

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