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Microencapsulation of oil droplets using cold water fish gelatine/gum arabic complex coacervation by membrane emulsification

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ABSTRACT

Food grade sunflower oil was microencapsulated using cold water fish gelatine (FG)–gum arabic (GA) complex coacervation in combination with a batch stirred cell or continuous pulsed flow membrane emulsification system. Oil droplets with a controllable median size of 40–240 μm and a particle span as low as 0.46 were generated using a microengineered membrane with a pore size of 10 μm and a pore spacing of 200 μm at the shear stress of 1.3–24 Pa. A biopolymer shell around the oil droplets was formed under room temperature conditions at pH 2.7–4.5 and a total biopolymer concentration lower than 4% w/w using weight ratios of FG to GA from 40:60 to 80:20. The maximum coacervate yield was achieved at pH 3.5 and a weight ratio of FG to GA of 50:50. The liquid biopolymer coating around the droplets was crosslinked with glutaraldehyde (GTA) to form a solid shell. A minimum concentration of GTA of 1.4 M was necessary to promote the crosslinking reaction between FG and GTA and the optimal GTA concentration was 24 M. The developed method allows a continuous production of complex coacervate microcapsules of controlled size, under mild shear stress conditions, using considerably less energy when compared to alternative gelatine types and production methods.

1. Introduction

Microencapsulation is a technique by which material is coated or entrapped within another one which forms a protective shell or wall (Burgess & Ponsart, 1998; Madene, Jacquot, Scher, & Desobry, 2006). Usually it is performed in order to isolate and protect the material from the environment or to promote controlled release (Benita, 2005). Complex coacervation is a spontaneous phenomenon that occurs between two oppositely charged polymers and the neutralization of these charges induces a phase separation (polymer rich phase vs. aqueous phase), which has been applied extensively in microencapsulation (Bungenberg de Jong, 1949; de Kruif, Weinbreck, & de Vries, 2004; Schmitt et al., 1998; Turgeon, Schmitt, & Sanchez, 2007). Although spontaneous, this phenomenon occurs only under very specific conditions which will depend on the charge of the polymers, their charge density, the surface tension in the system, the temperature at which the system is maintained and the dynamics of the system (stirring and cooling) (Leclercq, Harlander, & Reineccius, 2009).

Typical steps in microencapsulation of hydrophobic material by complex coacervation process involve: i) emulsification of hydrophobic material (oil) in an aqueous solution containing two different polymers (most commonly a protein and a polysaccharide), usually at a temperature above the gelling point of protein and pH that is above the isoelectric point of protein; ii) separation into two liquid phases (an insoluble polymer rich phase and an aqueous phase that is depleted in both polymers) as a result of attractive electrostatic interactions between oppositely charged polymers caused by lowering the solution pH below the isoelectric point of protein; iii) wall formation due to deposition of the polymer rich phase around the droplets of the hydrophobic material — induced by controlled cooling below the gelling temperature; and iv) wall hardening by addition of a crosslinking agent in order to obtain hard microcapsules. The most classical example of complex coacervation is the gelatine/gum arabic (GE/GA) system in which GE and GA are used as positive and negative polyelectrolytes, respectively (Lemetter, Meeuse, & Zuidam, 2009). The system has been successfully used for different applications such as the production of carbonless paper (Green & Schleicher, 1957), the encapsulation of flavour compounds (Leclercq et al., 2009; Soper, 1997; Wampler, Soper, & Pearl, 1998; Yeo, Bellas, Firestone, Langer, & Kohane, 2005) and lipophilic drugs (Jizomoto, Kanaoka, Sugita, & Hirano, 1993; Junyaprasert, Mitrevej, Sinchaipanid, Boonme, & Wurster, 2001). Gelatine can be obtained from pig skin, bovine hide, pork and cattle bones, and marine sources (warm- and cold-water fish skins, bones and fins). Worldwide religious sentiments, vegetarian nutritional requirements, the risk

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associated with the transmission of pathogenic vectors such as Bovine Spongiform Encephalopathy prions by tissue-derived collagens and gelatines have led to alternatives to mammalian gelatine. In this regard, gelatine obtained from marine sources has received increased interest in recent years (Karim & Bhat, 2009; Surh, Decker, & McClements, 2006).

The use of fish gelatine (FG), as an alternative to mammalian gelatine, can bring various advantages in complex coacervation processes. The gelling point of FG (8–25 °C and 11–28 °C for cold- and warm-water fish gelatines, respectively) enables a process at about ambient or slightly above-ambient temperatures that allows i) to reduce the duration of the heating step shortening the overall processing time, ii) to prevent the loss of volatile encapsulantes such as flavours and fragrances, and iii) to preserve the bioactivity of heat sensitive components such as drugs or biomolecules. Warm water FG having a gelling temperature of 27 °C and gum arabic (GA) were used to prepare microcapsules by complex coacervation (Dardelle & Normand, 2012; Soper, 1997). The process was carried out at 27 °C and the mixture was cooled down to 20 °C. The interaction occurring between cold water FG and GA molecules in aqueous solutions has been investigated (Yang, Anvari, Pan, & Chung, 2012). Quan, Kim, Pan, and Chung (2012) reported the use of FG for the production of microcapsules with a solid lipid core. The melted lipid droplets were initially produced by sonication, but the resulting microcapsules had a wide size distribution.

The design of microcapsules with tailor-made properties in terms of composition, size and size distribution and the development of mild (minimal shear) encapsulation techniques is still an important challenge in microencapsulation technology. In recent years membrane emulsification has been explored as an advanced encapsulation technique that allows the production of uniform particles of controlled size. It is a dispersion process to produce droplets of one liquid phase (e.g. oil) in a second immiscible liquid phase (e.g. water) by injecting the dispersed phase through the membrane using low energy per unit volume. The droplet size is mainly influenced by the pore size and the shear stress applied on the membrane surface (Vladisavljević & Williams, 2005). Conventional emulsification devices (high pressure homogenisers, ultrasonic processors, rotor–stator systems) are not suitable for use when dealing with shear sensitive substances, because they apply more energy than needed for the disruption of droplets and the energy input within the device is not uniform, leading to the droplets with a wide size distribution. Membrane emulsification has been used to produce polymeric microspheres and microcapsules (Chu, Park, Yamaguchi, & Nakao, 2002; Gasparini, Kosvintsev, Stillwell, & Holdich, 2008; Wie et al., 2008), solid lipid microcapsules (Kukizaki & Goto, 2007), and agarose microbeads (Zhou, Wang, Ma, & Su, 2007) but microencapsulation of oil droplets produced by membrane emulsification has not yet been demonstrated using complex coacervation. A microchannel emulsification system has been used to prepare gelatine/acacia coacervate microcapsules (Nakagawa, Iwamoto, Nakajima, Shono, & Satoh, 2004). However microchannel emulsification, due to the low volume flow rate of the dispersed phase, is mainly applied at the small batch scale for niche production and laboratory research.

The paper reports the use of membrane emulsification for production of uniform microcapsules with diameters between 40 and 270 μm having a single liquid oil core using cold water fish gelatine and gum arabic. The influence of operating parameters, pH, biopolymer ratio, total biopolymer concentration, presence of surfactant, and dispersed phase concentration on the production of microcapsules was investigated in a Dispersion Cell (Stillwell, Holdich, Kosvintsev, Gasparini, & Cumming, 2007). For scaling up of the process the conventional cross-flow membrane emulsification, where the shear is induced by recycled flow of the continuous phase, is not convenient for the production of droplets larger than 20 μm, due to break up in the pump. Therefore the continuous membrane emulsification with pulsed (oscillatory) flow (Holdich, Dragosavac, Vladisavljević, & Piacentini, 2013) was investigated.

2. Methods and materials

2.1. Experimental

Commercially available food grade sunflower oil was used without further purification. Fish gelatine from cold water fish skin, gum arabic from Acacia tree, lactic acid and 50% w/w glutaraldehyde were purchased from Sigma-Aldrich, UK.

In all the experiments mixtures of FG and GA were used as a continuous phase. Separate stock solutions of FG (10% w/w) and GA (10% w/w) were prepared by dispersing the weighed amounts of FG and GA in distilled water. The solutions were gently stirred and incubated at 40 °C for 1 h and were left to cool down to room temperature. Aqueous biopolymer mixtures were prepared by mixing and diluting the stock solutions of FG and GA with distilled water.

During the formulation stage, which included finding the optimum pH, biopolymer ratio and concentration, emulsions were produced using a Dispersion Cell (Dragosavac, Sovilj, Kosvintsev, Holdich, & Vladisavljević, 2008; Gasparini et al., 2008; Stillwell et al., 2007) fitted with a flat disc hydrophilic nickel membrane placed under the paddle stirrer (Fig. 1a). The paddle stirrer was driven by a 24 V DC motor (Instek Model PR 3060) and the rotation speed, controlled by the applied voltage, was varied from 170 to 1700 rpm corresponding to shears between 0.7 and 24 Pa (Eq. (2)). The dispersed phase was injected using a peristaltic pump (Watson-Marlow 101 U/R). Most of the experiments were carried out at room temperature (20 °C). The dispersed phase (sunflower oil) was injected through the membrane into the continuous phase at a constant flow rate of 1 mL min^{-1} corresponding to a dispersed phase flux of 75 L m^{-2} h^{-1}. The volume of the aqueous biopolymer mixture in the cell (continuous phase) was 90 cm^3.

Once the best formulation was determined, continuous membrane emulsification with pulsed (oscillatory) flow and tubular nickel hydrophilic membrane was used (Fig. 1b). The dispersed and continuous phases were injected using peristaltic pumps (Watson-Marlow Sci-Q 400 Pump, Cornwall, UK). An accelerometer (PCB Piezotronics model M352C65) was connected to a National Instruments Analogue to Digital converter (NI eDAQ-9172) which was interfaced to a LabView executable programme running on a PC. The information provided by the programme from the accelerometer was the frequency and amplitude of the oscillation: the frequency being determined from the direction of travel, and the amplitude was deduced from the acceleration measurement. The oscillation signal to the amplifier used to drive the oscillator was provided by a National Instrument frequency generator. The power amplifier drove an electro-mechanical oscillator which was connected to a bellow designed for use in a diaphragm pump, which was submerged in the continuous phase, and its oscillation provided pulsation of the liquid. This electro-mechanic means to generate pulsed flow provided separate control of frequency and amplitude. The continuous phase oscillations with a frequency of 20 to 50 Hz and amplitude between 0.6 and 1.6 mm within the membrane tube were used, corresponding to the shears between 1.3 and 12.3 Pa (Eq. (6)) and the influence of the dispersed phase flow rate was also investigated. The emulsion droplets produced in the membrane module were collected in a stirred tank and slowly agitated.

In both systems 10 μm membranes with 200 μm pore spacing and porosity of 0.2% were used. The flat disc membrane had a diameter of 33 mm corresponding to the effective membrane area of 8.4 cm^2, whilst the tubular membrane had an internal diameter of 14 mm and working length of 60 mm and had three times bigger effective membrane area (26 cm^2). Membranes, Dispersion Cell as well as continuous membrane emulsification system with pulsed (oscillatory) flow were supplied by Micropore Technologies Ltd. (Hatton, UK). After each experiment, the membranes were cleaned with commercially available soap in an ultrasonic bath for 5 min followed by treatment in 4 M NaOH solution for 5 min and finally rinsed with distilled water.