Journal of Colloid and Interface Science 498 (2017) 387–394

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

Facile microfluidic production of composite polymer core-shell microcapsules and crescent-shaped microparticles

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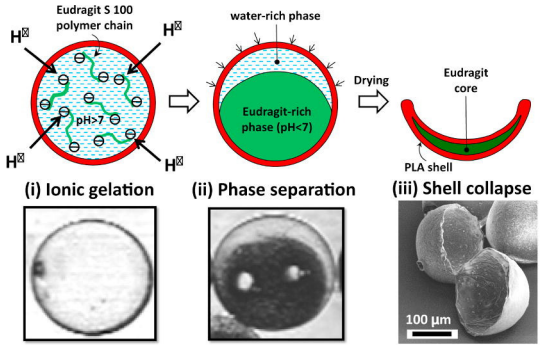
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 January 2017
Revised 14 March 2017
Accepted 15 March 2017
Available online 18 March 2017

Keywords:
Biodegradable polymers
Core-shell microcapsules
Eudragit
Cell encapsulation
Crescent particles
Ionic gelation
Microfluidics

ABSTRACT

Hypothesis: Core-shell microcapsules and crescent-shaped microparticles can be used as picolitre bioreactors for cell culture and microwells for cell trapping/immobilisation, respectively.
Results: Monodisperse polylactic acid (PLA) core-shell microcapsules with a diameter above 200 µm, a shell thickness of 10 µm, and 96% water entrapment efficiency were produced by solvent evaporation from microfluidically generated W/O/W emulsion drops with core-shell structure, and used to encapsulate *Saccharomyces cerevisiae* yeast cells in their aqueous cores. The morphological changes of the capsules stained with Nile red were studied over 14 days under different osmotic pressure and pH gradients.
Findings: The shell retained its integrity under isotonic conditions, but buckling and particle crumbling occurred in a hypertonic solution. When the capsules containing 5 wt% aqueous Eudragit® S 100 solution in the core were incubated in 10^{-4} M HCl solution, H^+ diffused through the PLA film into the core causing an ionic gelation of the inner phase and its phase separation into polymer-rich and water-rich regions, due to the transition of Eudragit from a hydrophilic to hydrophobic state. Crescent-shaped composite microparticles with Eudragit cores and PLA shells were fabricated by drying core-shell microcapsules with gelled cores, due to the collapse of PLA shells encompassing water-rich crescent regions.

1. Introduction

Microfluidic emulsification combined with solvent evaporation is a facile method for continuous production of monodispersed microparticles of versatile morphology and internal structure

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http://dx.doi.org/10.1016/j.jcis.2017.03.067
0021-9797/

including non-spherical particles, e.g. crescent-moon-shaped [1], dendritic [2], and toroidal [3], surface-patterned particles with surface patches [4] and dimples [5], microcapsules composed of controlled number of inner compartments such as multi-compartment colloidosomes [6], polymersomes [7], and liposomes [8], and asymmetric particles with spatially segregated sections, such as Janus [9] and ternary [10] particles. Alternative fabrication methods, such as masking/unmasking techniques [11], microcontact printing, layer-by-layer deposition [12], internal phase separation, and direct polymerisation [13], are typically batch-wise, involve complex and multi-step processing, specific formulations and lead to poor particle size uniformity. The particles produced by microfluidic routes are monodispersed and their size and morphology can be controlled by changing the size and morphology of the parent drops. Glass capillary microfluidic devices are increasingly used for manufacturing microparticles [14–16], due to their 3D geometry, cheap fabrication process, ability to generate complex droplets in single emulsification step, and excellent optical properties and chemical resistance of borosilicate glass. Furthermore, glass surface can be readily functionalized to tailor its surface wettability.

Hydrogel microbeads are difficult to produce in microfluidic devices because the drop pinch-off must be decoupled from the gelation process to avoid clogging of the channels. However, typical ionic reactions that occur during ionotropic gelation are much faster than the drop generation. To overcome this problem, gel beads were produced in microfluidic systems by coalescence-induced gelation [17], chaotic mixing [18], competitive ligand exchange crosslinking [19], internal gelation using calcium or barium carbonate nanoparticles dispersed in the aqueous phase and acidified oil phase [20], and external gelation using the oil phase containing $CaCl_2$ [21–23]. These fabrication routes usually result in the formation of coherent gel microcapsules with uniform internal structure.

Composite microcapsules with gelled shells and oily cores were fabricated in microfluidic systems using O/W/O emulsions as templates [24,25]. Microcapsules with gelled shells and aqueous cores were fabricated via W/W/O emulsions and used for encapsulation of carcinoma cells [22]. To the best of our knowledge microcapsules with gelled cores and biodegradable polymer shells have not yet been generated by microfluidic methods.

Crescent-moon-shaped polymer particles were obtained by polymerisation of microfluidically emulsified Janus droplets composed of non-curable phase and photocurable phase [4,26] selective polymer leaching from acorn-shaped Janus particles [27], and non-uniform solvent evaporation from Janus droplets [28].

In this study, novel core-shell microcapsules with gelled or non-gelled aqueous cores and biodegradable polymer shells were fabricated and used as templates for composite crescent microparticles. The fabrication method is based on single-step microfluidic generation of core-shell W/O/W emulsion droplets, followed by solvent evaporation and an off-chip ionic gelation of a pH-sensitive, biocompatible polymer in the core. The fabricated microcapsules with aqueous cores were loaded with yeast cells to demonstrate high encapsulation efficiency of the fabrication method. In addition, morphological changes of the fabricated microcapsules over prolonged storage were investigated under different incubation conditions.

2. Materials and methods

2.1. Droplet generation and microparticle production

Core-shell drops were generated using glass capillary device with a combined co-flow/counter-current flow focusing geometry shown in Fig. 1. The device was fabricated and operated using the procedures described elsewhere [29,30]. The middle fluid was a mixture of 7 wt% PLA (poly(dl-lactic acid), M_w = 89,000 g/mol, IngeoTM 2060D) and 2 wt% PGPR (polyglycerol polyricinoleate, E476, Abitec Ltd., New Milton, UK) in DCM (dichloromethane, HPLC grade, Fisher Scientific, UK). Nile red (9-diethylamino-5-benzo[α] phenoxazinone, Sigma-Aldrich, UK) was added in the trace amounts as a lipophilic stain to visualize PLA shells by fluorescence microscopy after DCM evaporation. The inner fluid was pure water prepared using a Millipore 185 Milli-Q Plus water purification system or 5 wt% aqueous solution of Eudragit® S 100 (methacrylic acid-methyl methacrylate copolymer 1:2, Evonik, Germany) dissolved in 0.1 M NaOH. Eudragit® S 100 is soluble at pH ≥ 7 and precipitates at pH < 7. The continuous fluid was 5 wt% aqueous solution of PVA (poly(vinyl alcohol), M_w = 13,000–23,000 g/mol, 87–89% hydrolysed, Sigma-Aldrich).

At optimum fluid flow rates, monodispersed single core or dual-core drops were generated, as shown in Fig. 1 and Videos 1 and 2 in the supplementary material. Monodispersed core-shell particles with thin PLA shells and aqueous cores were produced upon DCM evaporation from the middle phase. A pH-triggered gelation of an aqueous Eudragit® S 100 solution from the cores was achieved by incubating the core-shell particles in the acidic environment. Finally, the capsules with gelled cores were collapsed by drying-induced buckling under ambient conditions to produce crescent-shaped microparticles with Eudragit core and PLA shell.

2.2. Confocal fluorescence microscopy

A drop of the particle suspension was placed on a microscope slide and allowed to dry. As a result of the inclusion of Nile red dye in the middle phase formulation, hydrophobic regions of the produced particles were fluorescent and visualized using a Nikon Eclipse TE300 confocal inverted microscope connected to a computer running Zeiss LaserSharp 2000 software. Nile red was excited with argon laser at a wavelength of 488 nm and helium-neon laser at 543 nm. The total emission was divided into two wavelength regions, detected by two photomultiplier tubes (PMTs): PMT1 captured fluorescence at 515 ± 30 nm (the green region) and PMT2 captured fluorescence above 570 nm (the red region). Only PMT2 images are presented in this work. Corresponding optical images of microparticles were simultaneously acquired using the same microscope.

2.3. Focused ion beam (FIB) imaging

To avoid particle distortion and disruption due to high beam energy, 2 kV was used throughout. External imaging of the particle was performed at 30 pA and a tilt angle of 52° before a protective platinum layer was deposited at 0.3 nA. If needed, the particle cross section was milled at 20 nA and cleaned at 7 nA, followed by the final cleaning at 3 nA. Finally, the current was reduced to 30 pA to preserve the exposed cross section and prevent its modification due to exposure to ion beam during imaging, which was carried out at 2 min/image for noise reduction.

2.4. Prediction of particle size

Fig. 2a shows the two extreme morphological transformations of a core-shell drop during solvent evaporation from the shell, Path A and B, where 0% and 100% core-water entrapment scenarios are depicted respectively.

The diameter of the particle formed via path A depends on the density of the dispersed phase, ρ_d prior to solvent evaporation, the density of the formed particle, ρ_p, the mass fraction x_p of the