Colloids and Surfaces A: Physicochem. Eng. Aspects 384 (2011) 53–57

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Formation of monodisperse cross-linked nanospherial condensates based on flow-focusing and droplet diffusion techniques

Qianjun Zhang[a], Guangyao Lin[a], Yong Wang[a], Fujun Yang[b], Long Ba[a,*], Degang Fu[a]

[a] State Key Laboratory of Bioelectronics, School of Bioengineering and Department of Physics, Southeast University, Nanjing 210096, China
[b] Department of Engineering Mechanics, Southeast University, Nanjing 210096, China

ARTICLE INFO

Article history:
Received 13 January 2011
Received in revised form 1 March 2011
Accepted 3 March 2011
Available online 10 March 2011

Keywords:
Microfluid
Nanosphere
Monodisperse
Polylysine

ABSTRACT

By generating the monodisperse droplets of disperse phase in the continuous phase and keeping them stable during condensing process, it is possible to fabricate monodisperse spherical condensates using emulsification–diffusion technique. In this work we fabricated the monodispersive polylysine micro- and nano-spheres by using "flow-focusing" and droplets diffusion techniques. By keeping the flow rates of dispersed phase and continuous phase constant, the size of condensed and glutaraldehyde cross-linked polylysine spherical particles was tuned from tens micrometers to tens nanometers by varying the concentration of the polylysine solution. Near monodisperse spheres can form from tens micrometers to hundreds nanometers with similar size distribution. The luminescence experiment shows that the stability of small molecules trapped by cross-linked polymer can be stable over tens hours when sufficient aldehydization keeps the condensates stable.

1. Introduction

The fabrication of microspheres of various biomaterials was promoted by various applications during recent years, e.g. controlled drug release [1,2], bio-labeling [3,4]. Among various fabrication methods, precipitating or coacervating solutes from solutions of different concentrations provides a robust routine for the controllable formation of the microscale or nanoscale spherical particles. Using emulsification–evaporation [5], emulsification–diffusion [6,7] or spray-drying [8,9], the spherical particles were condensed from the solution containing droplets, thus the size and shape homogeneity of these particles are dominated by the droplet size dispersion, droplet stability, solute precipitating or coacervating during condensation process. For solutions within which the solutes are usually homogenously dissolved with less aggregation, especially for low molecular weight polymer materials, the dispersion of the droplets affects the most the homogeneity of the condensed particles. Keeping the droplets stable and maintaining the condensing in more equilibrium condition can improve the both size and shape uniformity of the condensates. Comparing with emulsification evaporation and spray-drying technique, the droplet condensing time during emulsification–diffusion is much shorter than that during solvent evaporation process, and the droplets stability is higher than that during air spray-drying process. Shorter condensing time reduces the portion of the merged droplets and higher stability reduced the portion of broken droplets. Thus, by generating the monodisperse droplets of dispersed phase in the continuous phase and keeping it stable during condensing process, it is possible to fabricate nanospheres using emulsification–diffusion technique with much improved monodispersity.

As a typical application, drug containing particles with homogenous size and shape exhibit less fluctuation of the releasing velocity. The particles obtained using mechanical or ultrasonic emulsification generally have large size dispersion [10–12]. Several microfluid based designs have been developed to produce monodisperse liquid droplets. The two most commonly used structures are the T junction [13] and flow-focusing device (FFD) [14]. These devices can be either fabricated using microfabrication technology [15] or constructed using macroscopic elements [16]. For FFD, gas or liquid is driven out of a capillary tube into a continuous liquid phase. The end of the capillary tube is positioned coaxially against an orifice. The flow velocity of the external liquid through the orifice is higher than that of the central fluid flows out of capillary tube. The contraction effect of the external fluid focuses the continuous stream out the end of capillary tube passing through the orifice, which was broken into discrete droplets. Under certain conditions, these droplets can be monodisperse [14]. Here we report our study on the design of a flow-focusing device and the fabrication of monodisperse hydrophilic spheres. We choose low molecular weight ε-polylysine as a model material with the consideration that lower molecular weight can decrease the size fluctuation at hundreds nanometers

* Corresponding author. Tel.: +86 25 83790951; fax: +86 25 83790951.
E-mail address: balong@seu.edu.cn (L. Ba).

0927-7757/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2011.03.013

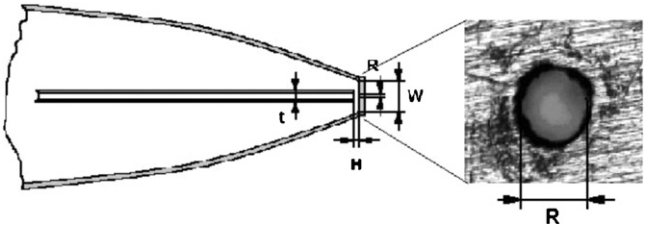

Fig. 1. Illustration of "focusing-flow" microfluid device, the designed geometry parameters is: R = 94 μm, t = 210 μm, H = 65 μm, W = 5 mm; right image is the optical microscopy of the orifice on copper sheet.

scale of the condensates. The fabrication conditions and the morphology of the monodisperse micro- or nano-spheres condensed from water-in-oil emulsion will be reported in the following text.

2. Materials, microfluid device and experiments

Polylysine (85%, MW 4–10 K) was obtained from domestic market. Ethyl acetate, propylene carbonate, glyceryl monostearate and 25 w/v% glutaraldehyde aqueous solution were obtained from Sinopharm Chemical Reagent Co. Ultrapure water (18.2 MΩ) was used for all aqueous solutions. 6-Carboxyfluorescein (6-CF) (Sigma) was used as fluorescence dye.

For typical flow-focusing geometry, two immiscible fluids flow through coaxial tubes near an orifice under pressure. The fluid with lower flow rate in the centered capillary tube was driven through an aligned orifice. The faster flow rate of the enveloping continuous fluid causes a "focusing" effect, which breaks a continuous stream out of the capillary tube into discrete droplets. A basic model has been made for the oil-in water system [17]. In the model, the droplet breakup occurs when the shear stress applied by the continuous oil phase overcomes the interfacial stress σ/R (σ is the interfacial tension between the water and oil phases, and R is the radius of curvature at the interface). This model exploits the shear-rupturing mechanism, without considering the influence of turbulence flow at the exit of the orifice introduced by the shift and rotation movement of the droplets. The ratio between the two stresses (shear stress τ and interfacial tension σ) is defined as the Capillary number [18]:

$$Ca = \frac{\tau}{\sigma/R} = \frac{\mu\dot{\gamma}R}{\sigma} \quad (1)$$

where μ is the dynamic viscosity of the continuous phase and $\dot{\gamma}$ is the shear rate, given by $Q_0/\pi r^3$ (Q_0 is the flow rate of the discrete phase, r is the radius of the orifice). By assuming that the droplets were created by breaking a focused stream which is confined by an orifice, and a confined droplet was broken into two equal size droplets by shear stream, the diameter of the droplets can be expressed as [17]:

$$D \approx \frac{2^{2/3} Ca\ \sigma\pi r^3}{\mu Q_0} \quad (2)$$

Based on the model, we designed the parameters of the FFD as shown in Fig. 1. The diameter of the capillary tube is 210 μm, while the orifice diameter is 94 μm. The flow rate of the discrete phase we used in the experiment varies from 10 to 80 ml h^{-1}. We use ethyl acetate as continuous phase in the experiments. The flow rate of continuous phase Q_c was kept to 350 ml h^{-1}. The material of the outer tube and the capillary tube is glass which was hydrophobilized by silane surfactant. A 60 μm thick copper plate with a 94 μm round orifice drilled using an excimer laser micromachining system (Optec MicroMaster) was mounted on the end of the enveloping glass tube. For the convenience of cleaning (since the orifice can be blocked by debris in liquid, the copper plate need frequent refreshing), the plate was mechanically pressed to the end of glass tube using a mountable cap and sealed with rubber membrane. This orifice and the capillary tube were kept coaxial. The gap between the end of the capillary tube and surface of the copper plate was measured as 65 μm. Two commercial syringe pumps were used to generate continuous and discrete flows. The droplets driven into continuous phase were recorded by using a high-speed charge coupled device (CCD) camera (FASTCAM-ultima 512). 1000 frames of 512 × 512 pixels or 2000 frames of 256 × 256 pixels can be recorded per second. The fluids used for high speed imaging analysis are pure water and pure ethyl acetate.

The polylysine solutions with different concentrations were used as dispersed phases. 1 g polylysine was dissolved in 10 ml ultrapure water. This solution was then dissipated 50 and 2500 times for comparison. Q_c and Q_0 were chosen as 350 ml h^{-1} and 10 ml h^{-1}. The stream of ethyl acetate carrying polylysine/water droplets was driven into a beaker within which 15 ml ethyl acetate, propylene carbonate and glutaraldehyde mixture was placed. In the mixture, the volume ratio of ethyl acetate and propylene carbonate was tuned to 1:0.55. The glutaraldehyde was dissolved into ethyl acetate by mixing with 25 w/v% glutaraldehyde aqueous solution with ethyl acetate at volume ratio of 6:1 and dehydrated using acrylic resin. The concentration of glutaraldehyde in mixture was tuned to 0.6 wt%. 1 w/v% glyceryl monostearate was dissolved in ethyl acetate as surfactant. The solution in the beaker was stirred during and after droplets loading process at speed of 600 rpm. After injecting the stream into solvent in the beaker for 15 s and keeping stirring for 2 h, the precipitates were collected by centrifuging. The washed solid particles were observed using an optical microscope (Olympus IX51) and a Scanning Electron Microscope (SEM) (Zeiss Ultraplus). A mixture of water soluble luminescent dye and polylysine (0.5 ml, 1 × 10^{-6} mmol 6-CF water solution mixed with 10 ml 5 w/v% polylysine water solution) was also used as dispersed phase, with the idea of mimicking the drug dispersed in the spherical particles and observing its stability within aqueous solution. The particle size distribution was measured from SEM images. The size distribution of cross-linked polylysine spheres obtained from mechanically stirring was also measured.

3. Results and discussion

The high speed video images of the droplets in the ethyl acetate solution in the vicinity of the orifice were shown in Fig. 2. The images were taken at time interval of 1 ms or 500 μs. Water dripped out of capillary tube was pressed into narrow stream and then broken into discrete droplets by Rayleigh instability [19]. It can be seen that for Q_0 of 10 and 30 ml h^{-1}, the droplets driven out of orifice have almost equal size. When the Q_0 was increased to 80 ml h^{-1}, the water forms continuous stream with diameter much less than that of orifice. In the system of silicone in water [20] and water in mineral oil [21] systems, it was experimentally observed that the formation and size of the monodispersive water droplets depends on both the flow rates and their ratio of dispersed and continuous phases. Comparing our experiment conditions with above two systems, the flow rates of the dispersed phase and continuous phase of our experiment are close to that of water in oil system and much higher than that of oil in water system. The monodispersive droplets were formed in very similar Q_0/Q_c ratio ranges. This means that the scale invariance is valid for various fluid combinations and FFD geometries. By analyzing the size fluctuation of the droplets in the vicinity of the orifice among 1000 frames recorded in 1 s, it gives the diameter variation is less than 5% (less than the resolution limit of the high speed imaging CCD camera) for Q_0 = 10 ml h^{-1}. The increased Q_0 changes the discrete droplets into a near continuous stream with narrowed diameter (Fig. 2b).