

***In Situ* Microfluidic Synthesis of Monodisperse PEG Microspheres**

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Abstract: This study presents a microfluidic method for the production of monodisperse poly(ethylene glycol) (PEG) microspheres using continuous droplet formation and *in situ* photopolymerization in microfluidic devices. We investigated the flow patterns for the stable formation of droplets using capillary number and the flow rate of the hexadecane phase. Under the stable region, the resulting microspheres showed narrow size distribution having a coefficient of variation (CV) of below 1.8%. The size of microspheres (45–95 μm) could be easily controlled by changing the interfacial tension between the two immiscible phases and the flow rates of the dispersed or continuous phase.

Keywords: microfluidics, droplets, microspheres, monodispersity, PEG

Introduction

Polymer particles having size in the range of 1–100 μm are widely used in the coatings, adhesive, toners, inks, paper, chromatographic columns, and supports for synthetic catalysts or biomolecules containing DNA, proteins, and cells.^{1–5} In many of these applications, there are great needs for the synthetic method having a capability of precise control of their size and narrow size distribution.^{5,6} To satisfy the demand of specific properties from versatile applications, the preparation methods have been extensively developed to find efficient production way. In general, polymer particles having micron size could be produced by several approaches including emulsion, suspension, precipitation and dispersion polymerization, membrane emulsification, and multistage process.^{6–9} Although the preparation of monodisperse polymer beads is well established, the resulting beads shows wide size distribution (high polydispersity). In addition, most of these conventional methods are very material-specific or time and labor consuming due to tedious several steps. Thus, the synthetic method of micron sized beads with a narrow size distribution of the produced microspheres is still challenging area.

Recently, several methods have been reported to synthesize monodisperse microparticles. Especially, membrane emulsification technique based on the ejection of discontinuous phase through a porous membrane has been presented to produce monodisperse droplets.^{10,11} With similar concept, microfluidic reactors for the generation of monodisperse emulsion droplets were proposed because the produced emulsion droplets could be easily controlled by the com-

bination of driving pressures of the two immiscible fluids and geometry of microchannels.^{5,12–14}

The purpose of the presenting study is to demonstrate *in situ* production of highly monodisperse microspheres in the continuous microfluidic devices by photopolymerization of confined droplets of prepolymer generated on the mechanism of shear force driven break-off. The proposed method produces monodisperse PEG microspheres with rapid control of sizes from 45 to 95 μm . Firstly, the microfluidic device formed monodisperse droplets of PEG prepolymer and then performed polymerization of these droplets *in situ*.

Experimental

Materials. Polyethylene glycol diacrylate (PEG-DA, $M_n = 575$), *n*-hexadecane, sorbitan monooleate (Span 80) were purchased from Sigma-Aldrich chemicals (MO, USA). 2-Hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur1173) was provided from Ciba specialty chemicals. The SU-8 photoresist and developer solution for fabricating silicon mold were purchased from Microchem (MA, USA). The poly(dimethylsiloxane) (PDMS) used was obtained from Dow Corning (MI, USA).

Fabrication of Microfluidic Chip. Silicon master was fabricated with a SU-8 photoresist using photolithography. The microfluidic device was fabricated by replica micro-molding method. Mixture of PDMS prepolymer and curing agent (10:1, Sylgard 184, Dow Corning Co.) was thoroughly stirred then degassed in a vacuum pump. The degassed PDMS mixture was then poured onto the silicon master and cured at 65 °C. After curing, the PDMS replica was peeled away from the silicon master. The PDMS replica was bonded to a procured PDMS spin-coated slide glass. The

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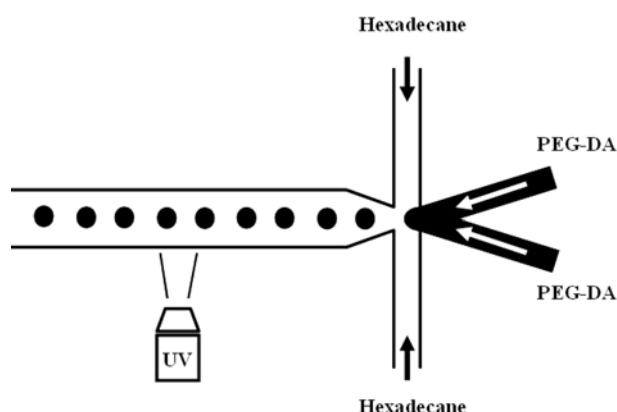


Figure 1. Schematic diagram of microfluidic device used to synthesize monodisperse PEG microspheres. The geometry of microfluidic devices contains the hydrophobic hexadecane fluid as immiscible phase with the prepolymer of PEG phase. Droplets were formed by shear force between hexadecane and PEG prepolymer solution, and then flowed into UV irradiation region for photopolymerization. The microfluidic dimensions of width and height are 200 and 100 μm , respectively.

dimensions of microfluidic device were $200 \times 100 \mu\text{m}$ (Width \times Depth).

In situ Microfluidic Synthesis of Monodisperse Microspheres. Figure 1 shows a schematic diagram of typical flow-focusing microfluidic devices. Two kinds of immiscible fluids were supplied into microchannel using microsyringe pump (Harvard Apparatus PHD2000, USA). The hexadecane as continuous phase was injected from two side of the microchannel, and disperse phase comprising PEG-DA prepolymer and photoinitiator was supplied from central line. Once emulsions pinched off at the T-junction were irradiated with ultraviolet source, it immediately solidified into solid microspheres. Span80 was used for preventing attachment of gel stated microspheres during UV polymerization as decreasing size of emulsions. The device for generation of microsphere was placed on fluorescence microscope equipped with UV light provided from mercury lamp. UV light was well-optimized to irradiate specific region without any mask for blocking ultraviolet. The intensity of ultraviolet amplified in the highly magnified objective lens, whereas the area of irradiation was much narrower. As our experimental condition, 40x of objective lens was selected. Under the condition, PEG-DA microspheres could be rapidly solidified in very short exposure time (≈ 75 ms).

Analytical Instruments. The inverted microscopy (Nikon, TE2000, Japan) equipped monochrome CCD camera (Photometrics, Coolsnap cf, USA) is used to observe the formation of microemulsion and irradiation process of ultraviolet. The size distribution of microsphere was analyzed using image J (<http://rsb.info.nih.gov/ij/>) and Image-pro (Media cybernetics). The size distribution of the microsphere along with their coefficient of variation (CV, defined as the stand-

ard deviation in the measured diameter divided by the average diameter) were calculated from measured value of produced microspheres.

Results and Discussion

Generation of PEG Droplet in the Microfluidic Device.

As shown in Figure 1, the microfluidic channel fabricated with PDMS consists of two inlets of continuous fluids with hydrophobic hexadecane solution and two inlets of hydrophilic PEG prepolymer as a dispersed phase. Hexadecane and PEG prepolymer solution are immiscible because of two distinctive properties such as hydrophilic or hydrophobic and then they can easily form W/O emulsion in the microfluidic channel. Disperse phase having PEG prepolymer and photoinitiator was supplied from two central Y-shaped microchannel, and hexadecane phase as continuous phase was injected perpendicularly from two side microchannels, where W/O emulsion are reproducibly formed by rupturing of hydrodynamic instability under the our experimental condition (Figure 2). Figure 2 shows that droplets of PEG prepolymer are clearly pinched off from T-junction and surrounded in continuous hexadecane without attachment on the PDMS wall. The formation of droplets at a junction is governed by the competition between the viscous stress and the surface tension stress.^{5,15-17} The capillary number ($Ca=U\mu/\gamma$) is a value showing the relative importance of these two effects, where U is the flow rate of continuous phase, μ is the viscosity of the continuous phase, and γ is the interfacial tension between the hydrophilic and continuous phases. Ca can be modified by varying Q_{hs} , the flow rate of the hexadecane continuous phase, which results from a change in U through the relationship, $U=Q_h/A$, where A is the cross-sectional area of the microchannel.

A diagram of flow patterns was used for the investigation of droplet formation in microfluidic device with hydrodynamic factors. We depicted the flow patterns of our system in Ca and Q_m coordinates to investigate the condition for generation of monodisperse droplets. As shown in Figure 2, three kinds of flow patterns were clearly observed after the junction: stable droplets (A), unstable flows (B), and elongation flow pattern (C). Figure 2 shows that it is difficult to generate stable microdroplets at low flow rate of Q_p ($< 0.2 \mu\text{L}/\text{min}$), W/O emulsions could not separate uniformly from junction due to unstable hydrodynamic pressure of PEG prepolymer phase, and sometimes be happened to reverse flow when the flow of hexadecane phase relatively increased (regime C). Stable and monodisperse emulsions were generated in limited range of Ca ($0.5 \times 10^{-2} \sim 0.5 \times 10^{-1}$) and flow rate of dispersed phase ($0.2 \sim 1.1 \mu\text{L}/\text{min}$) (regime B). As the flow rate of the prepolymer phase is decreased from 1.1 to $0.2 \mu\text{L}/\text{min}$, the region of stable droplet formation decreases because the Ca range required for the stable formation of droplets is limited, and uniform droplets could not be

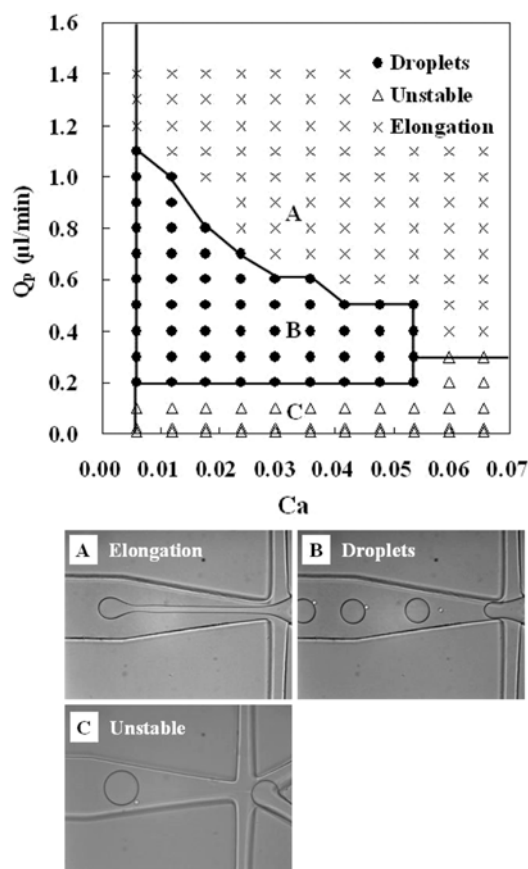


Figure 2. The flow diagram as a function of the flow rate of PEG prepolymer (Q_p) and of the capillary number (Ca) in the flow focusing microfluidic device. Three distinct regions were observed: (A) elongation flow of thread, (B) stable droplets, (C) unstable droplet formation. The prepolymer phase consisted of PEG-DA (95 wt%) and photoinitiator (5 wt%). The continuous phase consists of hexadecane, and Span 80 (stabilizer; 5 wt%).

obtained at high continuous phase flow rates ($Ca > 0.5 \times 10^{-1}$). If the value of Ca is gradually increased at a constant value of Q_p , a critical capillary number is reached where the droplets of the PEG begin to break off. This is the onset of the transition from the unstable droplet phase (regime B) to stable droplet phase (regime A). And monodisperse droplets could not be generated at high flow rate of hexadecane phase ($Ca > 0.5 \times 10^{-1}$). At higher flow rate of prepolymer phase ($Q_p > 1.1 \mu\text{L}/\text{min}$), droplets did not separate by hexadecane phase and flow formation was changed to cylindrical elongated thread along the microchannel because the shear force of hexadecane phase was not high enough to break off as spherical emulsions at the junction (regime A). Although we generated monodisperse W/O emulsions in microchannel, it should be considered whether size of microemulsion was excess over the height of microchannel. Under ultraviolet irradiation, photopolymerization of PEG-DA induces the blocking problem along the microchannel when emulsions

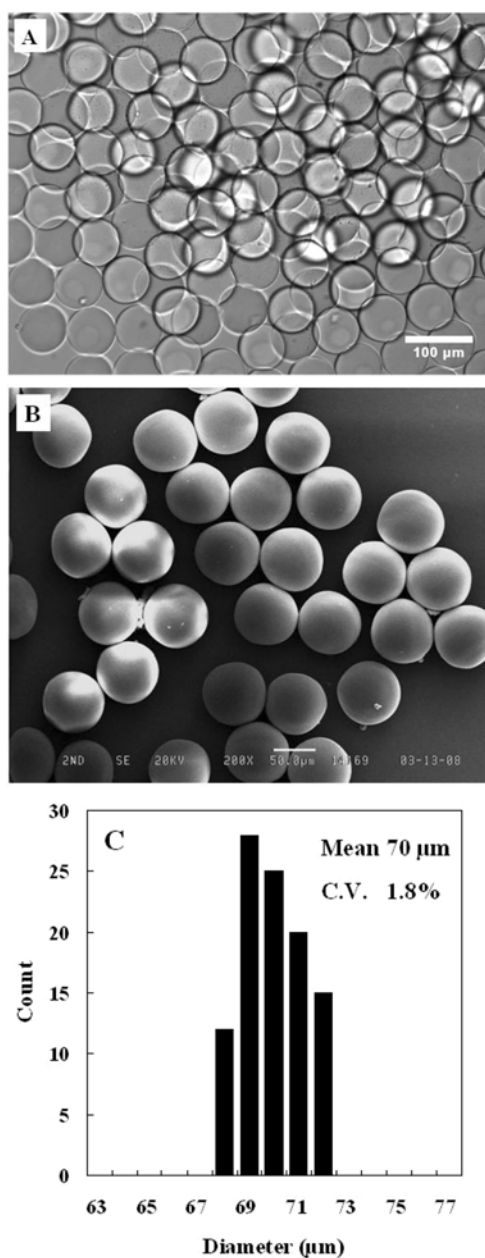


Figure 3. (A) Optical microscopy image of the monodisperse PEG microspheres suspended in hexadecane. (B) SEM image of PEG microspheres produced from the microfluidic device. The microspheres were formed at $Q_p=4.0 \mu\text{L}/\text{min}$ with hexadecane phase and $Q_c=0.14 \mu\text{L}/\text{min}$ (PEG-DA prepolymer). (C) Size distribution of microspheres collected in hexadecane (Mean diameter: $70 \mu\text{m}$, C.V: 1.8%) at both $4.0 \mu\text{L}/\text{min}$ of Q_p and $0.14 \mu\text{L}/\text{min}$ of Q_c , C.V was defined as the standard deviation of the diameter of microspheres divided by the mean diameter.

were attached on the PDMS wall. Thus, Span80 was added 3~5% concentration in hexadecane phase to decrease the size of emulsion as manipulating interfacial tension.

Characterization of PEG Microspheres. Figure 3(A) show optical microscopy image of the resulting microspheres after

the UV photopolymerization and all PEG microspheres produced in our microfluidic device have a narrow size distribution. Above result confirmed that our proposed microfluidic method successfully produced regular size of microspheres and the microspheres were easily separated without coalescence in the microfluidic device. Although the UV irradiation time is extremely short (< 100 ms) in considering of flow rate and residence time at photopolymerization region, the produced PEG microspheres are stable because continuously successful photopolymerization of prepolymer droplets is performed in the microfluidic device. Typical SEM image of PEG microspheres produced in microfluidic device also proved the successful photopolymerization (Figure 3(B)). The producing microspheres were highly stable and retained the spherical structure without deformation (Figure 3(B)). In addition, Figure 3(C) showed that the coefficient of variance (CV) was below 1.8%, which proved that the resulting microspheres were highly monodisperse according to the US National Institute of Standards and Technology (NIST) definition of monodispersity as a narrow size distribution having a CV $< 5\%$.^{5,18,19}

The polymerization of PEG microspheres was confirmed by analysis with Fourier transform infrared spectroscopy (FTIR) because it is a powerful tool used to identify specific chemical bonds simply by interpreting the infrared absorption spectrum.^{3,20} Since the activated radicals initiates the polymerization by attacking the carbon-carbon double bonds (C=C) presents in two acrylate groups of PEG-DA prepolymer, the photopolymerization results not only in linear chain growth but also in crosslinked network, which produce insoluble polymer structures. Figure 4 shows the FTIR spectra of PEG-DA prepolymer and of the PEG microsphere synthesized in the microfluidic device. FTIR spectrum obviously confirmed the cross-linking of PEG-DA prepolymer because characteristic peak of the terminal C=C stretch (1635 cm^{-1}) was dramatically decreased before and after UV irradiation (Figure 4). The FTIR spectrum

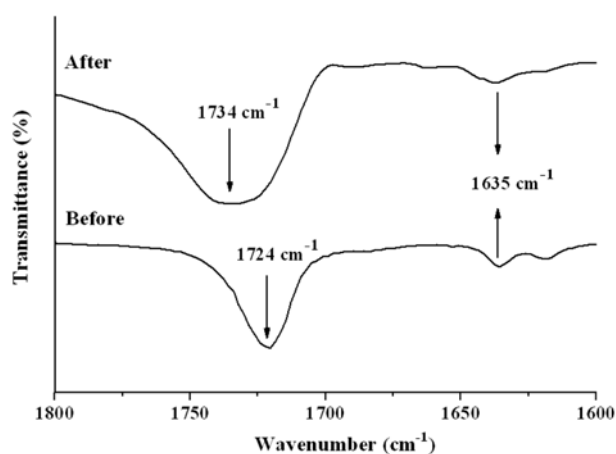


Figure 4. FTIR spectra of prepolymer (PEG-DA) and polymerized PEG microspheres prepared by microfluidic method.

indicates the degree of C=C conversion is about 90%, which reveals that the *in situ* photopolymerization in microfluidic device is rapid and efficient. In addition, the peak of carbonyl stretching resonance shifts from 1724 to 1734 cm^{-1} upon cross-linking was found because of the degradation of ester bonds in PEG acrylate. This observation of characteristic peaks of polymerized PEG-DA gives an evidence of the successful microfluidic approach for synthesis of PEG-DA microspheres.

Effect of Interfacial Tension and Flow Rate on the Microsphere Size. We investigated the effect of interfacial tension between hexadecane phase and PEG-DA prepolymer phase, and expressed the mean diameter of generated microspheres as a function of Q_h at different concentrations of surfactant (Span 80) in hexadecane phase (Figure 5). As increase of amount of surfactant dissolved in continuous phase leads the decrease of interfacial tension between two immiscible phases, the size of microspheres is reduced because the flow thread of hydrophilic PEG prepolymer makes thin shape. However, when a surfactant was dissolved into continuous phase below 2%, high interfacial tension between two of immiscible fluid gave rise to generate irregular particles which were deformed from attachment on the PDMS wall during polymerization. Finally, we have optimized the concentration of surfactant ($>3\text{ wt}\%$) for generating monodisperse microspheres having their narrow size distribution. When the flow rate of the continuous hexadecane phase increased from 2 to $6\text{ }\mu\text{L}/\text{min}$, the mean diameters of PEG microspheres reduced from 95 to $45\text{ }\mu\text{m}$, respectively. The high flow rate of the continuous phase ($>6\text{ }\mu\text{L}/\text{min}$) induces a transition regime from stable droplets to unstable or elongation of droplets.

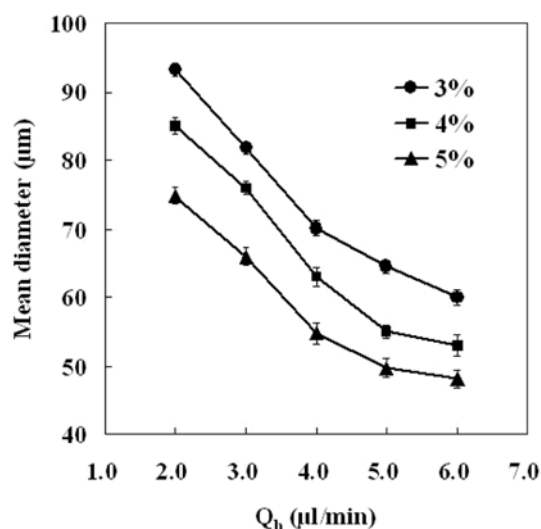


Figure 5. Dependence of the size of microspheres at the different concentration of Span 80 (3~5%) while the range of flow rate of hexadecane phase (2~6 $\mu\text{L}/\text{min}$) at constant flow rate of PEG prepolymer (Q_p : $0.2\text{ }\mu\text{L}/\text{min}$) was changed.

Thus, the mean diameter of the microspheres could be gently manipulated as controlling flow rate of hexadecane phase and interfacial tension, ranging from 40 to 95 μm in our experiments. In addition, the produced microspheres in the stable regime of droplet formation show high monodispersity (below 2% CV).

Conclusions

The microfluidic technique has been demonstrated a simple but efficient tool to generate PEG microspheres. Using continuous droplet formation and *in situ* photopolymerization, we have synthesized microspheres with sizes from 45 to 95 μm and high monodispersity. The average microspheres could be easily manipulated by the change of interfacial tension, and the flow rate of the droplets and continuous phases.

Microfluidic approach has several advantages: 1) It produces microspheres with a very narrow size distribution. 2) The simple change of flow rate and interfacial tension provide easy control of produced microspheres. 3) The proposed method can be scaled up to mass production of microspheres if microfluidic devices are operated in parallel or filed up. 4) the microfluidic device can be applied with various materials hydrogel, metals, chemicals, and other polymers. Furthermore, we might present co-polymerization as adding functional monomer for improvement of properties and biological application such as biosensor with proposed method.

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