Continuous Synthesis of Copolymer Particles in Microfluidic Reactors

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Polymer particles find numerous applications in diagnostics, chromatographic strip tests, latex agglutination assays, and suspension array tests. Detection, immobilization, and separation of DNA,[1] cells,[2] and proteins[3] requires microbeads carrying surface functionalities, e.g., carboxylic, amino, or aldehyde groups. The preparation of monodispersed functionalized microbeads in the size range from several micrometers to ca. 100 μm is generally achieved via suspension and dispersion polymerization, the variants of emulsion polymerization,[4−7] or by means of membrane emulsification accompanied by monomer polymerization.[8,9] These methods are frequently time-consuming (e.g, they may include several stages), or they lack sufficient control over particle size distribution.[10−13] In addition, in dispersion and emulsion copolymerization a difficulty in control over particle compositions exists due to the different reactivity of monomers.

In this Note we describe a microfluidics-based method for the rapid continuous "on-fly" preparation of copolymer particles in the size range from 100 to 150 μm and coefficient of variance (CV) below 2%. The method can be extended to the production of microbeads with dimensions from 10 to 1000 μm by changing the design of the microfluidic device. The described strategy employs the generation of extremely monodispersed droplets from a mixture of two monomers in a microfluidic flow-focusing device (MFFD). These droplets are then in situ solidified by means of continuous UV-initiated free-radical polymerization. Several groups reported emulsification of monomer liquids in microfluidic devices, accompanied by their thermally-initiated or UV-initiated *batch* polymerization.[14,15] Recently, Kumacheva and Whitesides[16] conducted *continuous* in-situ photopolymerization of monomer droplets emulsified in a microfluidic flow-focusing device[17] to obtain up to 10^5 particles/min.

Here we extended this approach to the production of copolymer particles with controlled size and low polydispersity. We note that copolymerization is not a trivial extension of our previous work: the incorporation of hydrophilic comonomers in a host monomer liquid has several important consequences for the emulsification process and for the appropriate selection of the material of MFFD.

The microfluidic device was fabricated in polyurethane (PU) elastomer using standard soft lithography.[16,18] Masters were prepared with features of SU-8 100 photoresist (MicroChem) in bas-relief on silicon wafers. We produced the monomer droplets by emulsifying a mixture of triproplylene glycol diacrylate (TPGDA) and acrylic acid (AA) in a 2 wt % aqueous solution of sodium dodecyl sulfate (SDS).

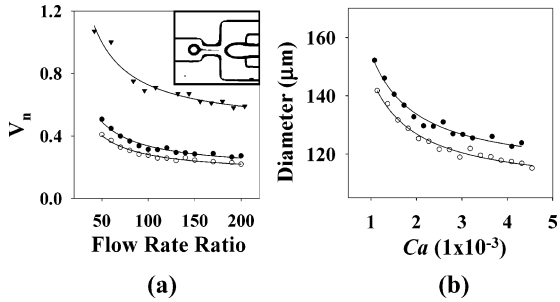

Figure 1. (a) Variation in normalized droplet volume, V_n, plotted versus the ratio of flow rates of water and monomer phases. Flow rate of monomer mixture is 0.01 mL/h. Concentration of AA in monomer mixture, c_{AA} (wt %), is 0 (▼), 5 (●), and 8 (○). Inset: optical microscopy image of the newly formed TPGDA/AA droplet produced in the orifice of MFFD. (b) Variation in droplet diameter plotted as a function of capillary number, c_{AA} (wt %): (●) 5; (○) 8. Flow rate of monomer liquid is 0.01 mL/h. The height and the width of the orifice were 86 and 70 μm, respectively, in the emulsification of TPGDA and 180 and 90 μm, respectively, in the emulsification of TPGDA/AA monomer mixture. The value of *Ca* was calculated using the flow rate of the continuous phase.

The mechanism of formation of extremely monodispersed droplets by breakup of the liquid thread in the orifice of MFFD has been described elsewhere.[17] The strategy relies on the mechanism of droplet formation in confinement; this mechanism is different than the breakup of liquid jets in unbound conditions or the mechanism of droplet formation by using membrane emulsification.[8,9] A liquid mixture of monomers and an aqueous SDS solution were supplied to the MFFD via polyethylene tubing attached to syringes operated by two digitally controlled syringe pumps. The continuous phase was supplied from two sides of the MFFD, and the droplet phase was supplied from a center channel. A pressure gradient along the long axis of the MFFD forced the liquids into a narrow orifice where an unstable cylinder-shaped thread of the monomer mixture broke up to release droplets. Figure 1, inset, shows an optical microscopy image of a newly formed droplet.

The monomer mixture supplied to the MFFD comprised from 0 to 15% of acrylic acid. We note that when the concentration of AA in the TPGDA/AA mixture was c_{AA} = 10 wt %, the droplets showed a trend to adhere to the walls of MFFD. While this problem could be solved by fabricating the MFFD in poly(dimethylsiloxane), for c_{AA} > 15 wt % the monomer thread did not break in a large range of flow rates of the continuous phase. Moreover, in the course of emulsification water-soluble acrylic acid diffused from the monomer mixture into the aqueous phase.

For 0 < c_{AA}< 8% the dimensions of droplets were controlled by the ratio of flow rates of the continuous and droplet phases and the properties of monomer mixture (that is, its viscosity and interfacial tension with a continuous phase). Figure 1a shows the variation in droplet volume with the change in ratio of flow rates of the continuous and monomer phases.[19] The volume of droplets, V_n, was normalized by d^3, where d is the width of the rectangular orifice. With increasing flow rate ratio the value of V_n gradually decreased. For

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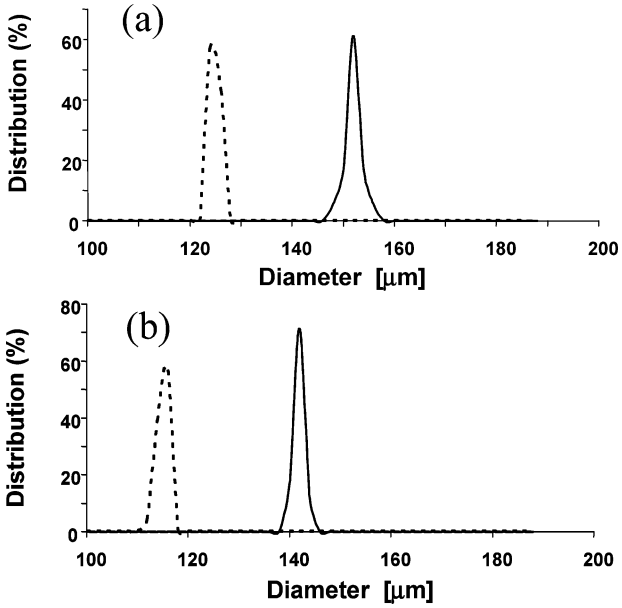

Figure 2. Distribution of diameters of monomer droplets produced from (a) monomer mixture with 5 wt % AA and (b) 8 wt % of AA at flow rate ratios 50 (—) and 200 (- - -). Flow rate of an aqueous phase was 0.01 mL/h.

example, for c_{AA} = 8 wt %, when the flow rate ratio increased from 50 to 200 the value of V_n decreased from 0.5 to 0.27, corresponding to the reduction in average droplet diameter from 153 to 123 μm.

In the second series of experiments we examined the effect of composition of the TPGDA/AA mixture on droplet dimensions. For a particular ratio of flow rates of the monomer and continuous phases an increase in c_{AA} from 5 to 8 wt % resulted in a notable decrease in the normalized droplet volume. We attribute this effect to the variation in viscosity and interfacial tension between the continuous and dispersant phases for the two monomer mixtures used. Since these properties changed simultaneously, we related the variation in the ratio of viscous stress to the stress due to interfacial tension via a capillary number $Ca = \mu v/\gamma_{12}$, where v is the characteristic velocity of the aqueous phase, μ is the dynamic viscosity of the droplet phase, and γ_{12} is the value of interfacial tension between the monomer and water phases. The monomers with 0, 5, and 8 wt % of AA had viscosity 13.2, 12.6, and 12.2 cP; the values of interfacial tension with the continuous phase were 4.0, 3.7, and 3.4 mN/m, respectively. Increase in concentration of acrylic acid in the mixture from 5 to 8 wt % led to ca. 6% increase in Ca. This change resulted in a 7 ± 1% decrease in droplet diameter (Figure 1b).

The monomer droplets were highly monodispersed in the entire range of flow rate ratios used in the current work. Above the flow rate ratio of 200 droplet polydispersity increased. Figure 2 shows the distribution of diameters of droplets comprising 5 and 8 wt % of acrylic acid. The droplets had a coefficient of variance (defined as the standard deviation in diameter divided by mean droplet diameter) not exceeding 2%.

The production of copolymer particles was achieved by UV-initiated free-radical polymerization of the comonomer mixture. We mixed 4 wt % of photoinitiator 1-hydroxycyclohexyl phenyl ketone (HCPK) with a TPGDA/AA monomer mixture. The droplets flowing through the downstream channel of the MFFD were irradiated with a UV light (400 W at 330−380 nm, UVAPRINT 40C/CE, Dr. K. Hönle GmbH UV-Technologie, Germany). The time of photopolymerization was controlled by droplet velocities in the microfluidic reactor, and it was generally from 10 s to several minutes. Monomer conversion to polymer was close to 100%. The efficiency of particle production was up to 250 particles/s. Figure 3a shows a typical SEM image of the resulting poly(TPGDA/AA) particles. The resulting copolymer microbeads had a spherical shape and a smooth surface. The particles were highly monodispersed, with coefficient of variance below 2%, that is, similar to CV of the corresponding monomer droplets. The surface of particles obtained from the TPGDA/AA mixture with 5 wt % of AA (characterized by X-ray photon spectroscopy[20]) contained 12.3 mol % of acrylic acid.

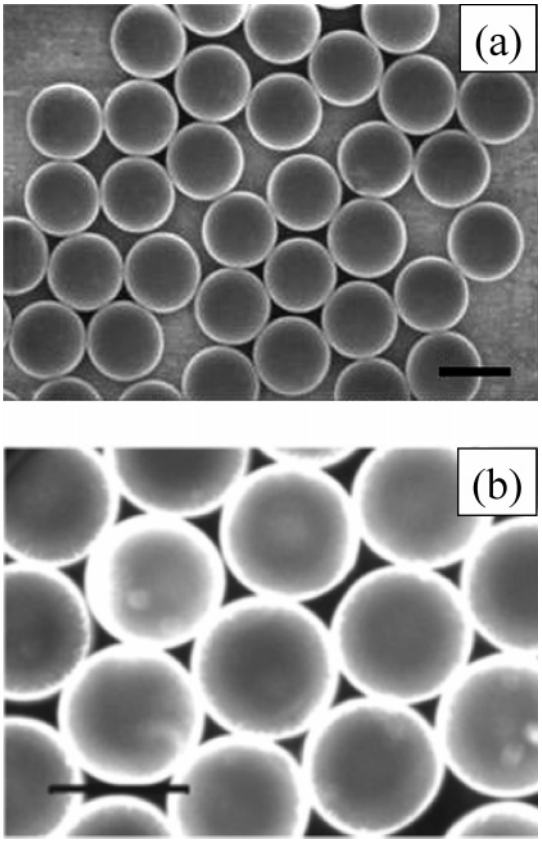

Figure 3. (a) Typical SEM images of poly(TPGDA/AA) particles prepared in the MFFD by polymerizing TPGDA/AA monomer mixture comprising 5 wt % of AA. (b) Fluorescent microscopy image of copolymer particles shown in (a) following their bionconjugation with FITC−BSA. Scale bar is 100 μm.

This amount of carboxylic groups on the surface of copolymer microbeads was sufficient for the immobilization of biomolecules. Bioconjugation of poly(TPGDA/AA) particles synthesized in the microfluidic reactor was demonstrated for bovine serum albumin covalently labeled with a fluorescein isothiocynate (FITC−BSA). The bioconjugation was achieved by first attaching the FITC−BSA to the polymer particles for 1 h at 30 °C in a phosphate buffer at pH = 6.0. Following this step, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) was added to the dispersion of poly(TPGDA/AA) microbeads bearing FITC−BSA; the system was then mixed for 1 h at 30 °C.[21] After sonicating and sedimenting the resulting microbeads, we resuspended them in deionized water. A series of control experiments were conducted to prove that FITC−BSA attached to the microbead surface: we heated micro-

beads with (i) FITC−BSA, (ii) EDC, and (iii) EDC and FITC−BSA. Attachment of fluorescent FITC−BSA to the microbead surface occurred only in case iii. Figure 3b shows a typical fluorescent microscopy image of the copolymer microbeads synthesized using in a MFFD reactor and conjugated with FITC−BSA.

In summary, we described a strategy for the rapid continuous synthesis of carboxylated copolymer particles in a microfluidic reactor. We achieved good control over particle dimensions by varying the flow rates of the continuous aqueous phase and monomer mixture and by varying the composition of droplet phase. The diameter of microbeads can be further reduced by decreasing the width and the height of the orifice of MFFD. The described method allows for the production of extremely monodispersed particles with coefficient of variance below 2%. Although polymerization within a MFFD reactor resembles suspension or miniemulsion polymerizations, it allows one to avoid many of the complications associated with these processes such as breadth of the particle size distribution or need for an osmotic costabilizer.

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Supporting Information Available: Description of droplet preparation, experimental details of XPS experiments, and viscosity measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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