

Double Emulsion Droplets as Microreactors for Synthesis of Magnetic Macroporous Polymer Beads

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Abstract An easy method is presented to fabricate monodisperse magnetic macroporous polymer beads (MMPBs). Water-in-oil high internal phase emulsion (HIPE) is prepared by emulsifying aqueous iron ions solution in an oil phase containing monomers. The HIPE is introduced into a simple microfluidic device to fabricate monodisperse (water-in-oil)-in-water double emulsion droplets. The droplets serve as microreactors to synthesize Fe₃O₄ nanoparticles and are on-line polymerized to form MMPBs. The prepared MMPBs display uniform size, interconnected porous structure, superparamagnetic behavior and uniform distribution of Fe₃O₄ in polymer matrix. The MMPBs are characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), vibrating sample magnetometry (VSM). We believe that this method is a universal technique in preparing macroporous nanocomposite beads.

Keywords: Double emulsion droplets; Magnetic macroporous polymer beads; Microreactor.

INTRODUCTION

Magnetic macroporous polymers, which incorporate magnetic nanoparticles into macroporous polymer matrix, have promising applications in bioseparation, catalyst supports and bioengineering fields. Magnetic inorganic nanoparticles served as stabilizer of polymerized high internal phase emulsions (polyHIPE) is an effective method to prepare magnetic macroporous polymer monoliths. Bismarck *et al.*^[1] applied two types of oleic acid-modified iron oxide nanoparticles as emulsifiers of polyHIPE to prepare magnetic macroporous polymers. Krajnc *et al.*^[2] prepared magnetic polystyrene stabilized by a combination of a surfactant and maghemite/magnetite (uncoated or coated with oleic acid) nanoparticles and then cured thermally. Ghosh *et al.*^[3] exploited iron oxide nanoparticles coated with oleic acid or acrylic acid to prepare porous hybrid nanocomposites *via* polymerization in highly concentrated water-in-oil emulsions. Li *et al.*^[4] reported poly(styrene-divinylbenzene) macroporous nanocomposites solely stabilized by magnetite nanoparticles surface-modified with 12-acryloxy-9-octadecenoic acid. Mert *et al.*^[5] used humic acid modified iron oxide magnetic nanoparticles as stabilizers to prepare magnetic macroporous monoliths. In previous works, composite materials were only fabricated with advance prepared magnetic nanoparticles, which usually causes aggregation of nanoparticles and also leads to complication of the preparation process. Moreover, most present preparations focus on magnetic macroporous polymer in the shape of monoliths. However, beads are the most common

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materials in current support and separation fields. It is necessary to develop a simple method to prepare MMPBs with controlled size and morphology. Droplet-based microfluidic device is a powerful tool to generate micrometer size droplets between two immiscible phases. These independent droplets are promising microreactors and templates for synthesizing various advanced particle materials with monodispersity and controlled morphology, including double or multiple emulsions^[6–9], spherical particles^[10, 11], Janus particles^[12–14], microcapsules^[15, 16] and vesicles^[9, 17]. Recently, Cooper *et al.*^[18] prepared macroporous beads by simple injection of HIPE droplets into a glass column using a syringe pump, which allows the synthesis of macroporous beads by combining droplet-based microfluidic technique and polyHIPE process. Dubinsky and co-workers^[19] synthesized macroporous particles by porogen technique in a microfluidic device. Gokmen and co-workers^[20] fabricated monodisperse macroporous beads and rods by polyHIPE in a microfluidic device. However, they obtained blank macroporous beads. The additional processes were needed to functionalize the beads. There is no report on preparing macroporous nanocomposite beads through droplet-based microfluidic devices.

In this work, we present an easy method to fabricate monodisperse magnetic macroporous beads (MMPBs). (Water-in-oil)-in-water double emulsion droplets are fabricated in a microfluidic device. The droplets are on-line polymerized and converted into macroporous polymer beads; and meanwhile the droplets are served as microreactors to synthesize Fe₃O₄ nanoparticles embedded into the macroporous polymer matrix. The prepared MMPBs display uniform size, interconnected porous structure, superparamagnetic behavior and uniform distribution of Fe₃O₄ in polymer matrix.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EGDMA), purchased from TCI and Alfa Aesar Company respectively, were passed through an alumina column before use to thoroughly remove the inhibitors. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) was obtained from Alfa Aesar Company. Poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) copolymer (PPO-PEO-PPO, M_n 2800) was supplied by BASF Corporation. FeCl₃·6H₂O, FeCl₂·4H₂O, and NaOH were purchased from local suppliers and used as received.

Preparation of Water-in-oil HIPE

The water phase was prepared in advance by dissolving appropriate amount of FeCl₃·6H₂O and FeCl₂·4H₂O in deionized water (Table 1). The oil phase comprising 1.02 mL GMA, 0.68 mL EGDMA, PEO-PPO-PEO (15% *W/V* of organic phase) and DMPA (2% *W/V* of monomer) was added to a 50 mL three-neck round-bottomed flask. The flask was fitted with a D-shaped paddle driven by an overhead stirrer and entirely covered with a piece of aluminum foil to avoid light penetration. The oil phase was stirred at 400 r/min for 20 min, and then the water phase (78% *V/V* according to total volume of emulsion) was added dropwise into the oil phase under constant stirring. After water phase addition, stirring was continued for 5 min to produce homogeneous water-in-oil HIPE.

Table 1. Iron ions concentration in internal phase of HIPE

Sample	Iron ions concentration (mol/L)	
	Fe ³⁺	Fe ²⁺
B1	0	0
B2	0.01	0.009
B3	0.02	0.018
B4	0.05	0.045
B5	0.1	0.09

Preparation of MMPBs in Simple Microfluidic Device

The prepared HIPE was immediately loaded into a syringe with a blunt needle. The needle was inserted into a transparent silicone tube (internal diameter = 1 mm) and pointed in the same direction of the water phase flow.

The tip of needle was kept in the center of the tube. HIPE was then injected into the silicone tube at a flow rate of 0.20 mL/h using a syringe pump (LONGER, LSP01-1A). NaOH solution (1% *W/V*) was pumped into the tube at 1.20 mL/min flow rate. Spherical HIPE droplets were formed at the tip of the needle. The droplets passed through the transparent tube and then exposed to UV light (high-pressure mercury lamp, 250 W) for approximately 30 s. MMPBs were collected at the end of the tube and then stirred in water bath at 80 °C for at least 15 min to polymerize further. The MMPBs were thoroughly washed with warm water and methanol three times and dried under vacuum at room temperature overnight.

Characterization

The morphologies of the MMPBs were investigated using a Philips XL 30 FEG scanning electron microscope (SEM). An MMPB was randomly selected and sectioned with a razor blade to show the internal structure. The samples were firmly mounted on aluminum studs and sputter coated with gold film before SEM imaging. A total of 20 MMPBs on SEM micrograph were randomly measured to determine the average diameter of MMPBs. Fourier transform infrared spectra (FTIR) were recorded from 4000 cm^{-1} to 400 cm^{-1} spectral range on a Bruker IFS-66 spectrometer using KBr pellets method. X-ray diffraction (XRD) patterns were obtained using powder Philips PW1700 diffractometer with $\text{CuK}\alpha$ radiation ($\lambda = 0.15406 \text{ nm}$) to analyze the crystal structure of magnetic particles formed in the MMPBs. Magnetic properties of the MMPBs with different magnetic particle contents were evaluated using a vibrating sample magnetometer (VSM, Lake Shore 7410) at room temperature. Samples for transmission electron microscopy (TEM) were ground in water. The obtained suspensions were deposited onto a carbon grid and dried at room temperature. The images were observed using a JEOL JEM-1011 TEM operated at 100 kV.

RESULTS AND DISCUSSION

Double emulsion droplets are fabricated in a microfluidic device and are served as microreactors to prepare monodisperse MMPBs. As illustrated in Fig. 1, water-in-oil HIPE is prepared by emulsifying aqueous iron ions solution in an oil phase which contains GMA and EGDMA as monomers, PEO-PPO-PEO copolymer as surfactant and DMPA as photoinitiator. The prepared HIPE is loaded in a syringe and injected into a microfluidic tube. NaOH solution flows through the tube and is used as a carrier continuous phase. (Water-in-oil)-in-water double emulsion droplets are formed at the tip of the needle because of the surface tension of HIPE and the shearing force of the continuous phase^[21]. The droplets are polymerized by UV irradiation and finally converted into solid porous beads. Meanwhile, NaOH solution diffuses into the internal phase of droplets and reacts with iron ions. Magnetic nanoparticles are formed and embedded into the macroporous polymer matrix based on co-precipitation mechanism in the following equation:



MMPBs are collected at the end of the microfluidic tube. Five samples with different concentrations of iron ions are synthesized as shown in Table 1.

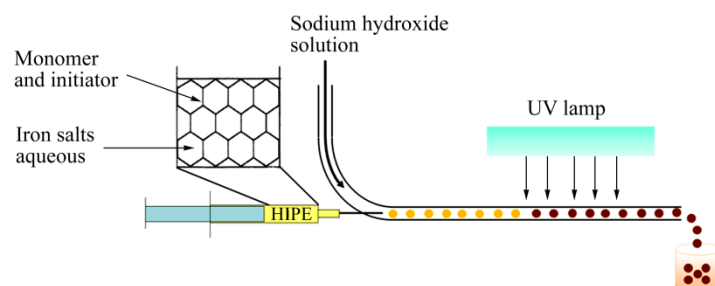


Fig. 1 MMPBs preparation by injecting HIPE into microfluidic device

In this process, the flow rate is important for obtaining high-yield and monodisperse MMPBs. Reducing the flow rate of HIPE results in a lower yield of MMPBs. By contrast, a fast flow rate of HIPE leads to a transition from dripping to jetting and production of polydisperse droplets^[21]. Increasing the flow rate of continuous phase decreases UV curing time and results in incomplete MMPB polymerization. Therefore, to harvest high-quality and high-yield monodisperse MMPBs, optimization of the flow rate of HIPE phase and carrier continuous phase is needed. A flow rate of 0.20 mL/h was selected for HIPE, and the continuous phase of NaOH solution was pumped into microfluidic tube at 1.20 mL/min.

The diameter of beads is adjusted by changing the size of syringe needle. As shown in Fig. 2(a–c), the uniform MMPBs with diameters of $(744 \pm 40) \mu\text{m}$ (relative standard deviation (RSD) = 5.4%), $(616 \pm 20) \mu\text{m}$ (RSD = 3.2%) and $(424 \pm 23) \mu\text{m}$ (RSD = 5.4%) are produced using 22G (gauge size), 23G, and 25G syringe needles (corresponding to inner diameter 390 μm , 320 μm and 230 μm). Larger or smaller beads can also be prepared by changing syringe needles. However, beads with less than 200 μm in diameter are difficult to be obtained through this method because a narrow needle might lead to unstable HIPE demulsification. The MMPBs show uniform size independent of the diameter of syringe needle used, and micrometer-sized pores are found on the entire surface of MMPBs (Fig. 2d). These MMPBs are significantly different from porous beads prepared by porogen technique, which shows a “skin” without pore structure on the surface^[19]. Figures 2(e)–2(f) show that the internal structure of the beads is a typical morphology of polyHIPE materials with interconnected spherical voids. The voids are negative images of originally present emulsified internal phase, which are interconnected with their neighbors through “windows” in the walls. The spherical voids have an average size of $(17.7 \pm 3.5) \mu\text{m}$ (RSD = 19.8%), which is calculated from the SEM images using the following equation^[22, 23].

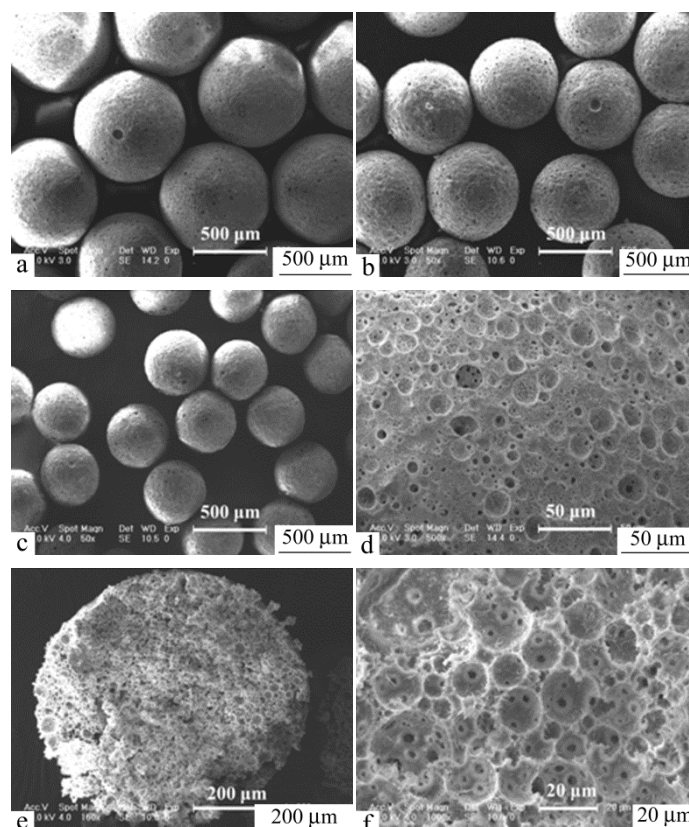


Fig. 2 SEM images of individual MMPBs (Sample B2 in Table 1): (a) whole beads (22G needle), (b) whole beads (23G needle), (c) whole beads (25G needle), (d) magnified image of bead surface showing pore structure, (e) sectioned “half-bead” showing internal pore structure and (f) magnified image of a fracture surface in a cross-section

$$R = \frac{2r}{\sqrt{3}}$$

where R is the equatorial value of void size and r is the diameter value measured on the images. The average “windows” size is $(2.8 \pm 0.6) \mu\text{m}$ (RSD = 21.4%) measured from SEM images. Normally, this value is slightly lower than the real value because the “windows” are randomly distributed on the surface of the spherical voids. Due to the numerous interconnected pores, NaOH solution in continuous phase could easily diffuse into HIPE droplets and reacted with Fe^{3+} and Fe^{2+} , thereby forming the MMPBs.

The chemical structures of the MMPBs were investigated using an FTIR spectrometer. Blank beads (Sample B1) and MMPBs (Sample B2) were analyzed. As shown in Fig. 3, the characteristic peak at 1740 cm^{-1} corresponds to the C=O stretching vibration, and the peaks at 1250 , 910 , and 850 cm^{-1} correspond to epoxy groups caused by the symmetric, asymmetric stretching, and deformation vibrations, respectively. The abundant epoxy groups in beads provide opportunity to convert into amino^[24], carboxyl^[25], azide^[20] groups, which are beneficial for biomolecule or ligand conjugation. With the addition of iron ions into the internal phase, Fe—O bond at 580 cm^{-1} was found in the sample B2 spectra, thereby indicating formation of iron oxide nanoparticles^[26].

To verify the composition of iron oxide nanoparticles in the polymer beads, XRD patterns were measured. Some distinctive peaks at 2θ values of 30.3 , 35.7 , 43.5 , 53.7 , 57.2 , and 63.0 are found (Fig. 4), which are consistent with the characteristic peaks of the magnetite Fe_3O_4 crystallite (powder diffraction file, JCPDS card no.74-0478). The diameter of Fe_3O_4 nanoparticles are calculated using Debye-Scherrer equation,

$$D = K\lambda/\beta\cos\theta$$

where D is the crystalline grain size, K is Scherrer constant, λ is the X-ray wavelength, β is the peak width of half-maximum, and θ is the Bragg diffraction angle. The crystallite size obtained from this equation is approximately 13 nm.

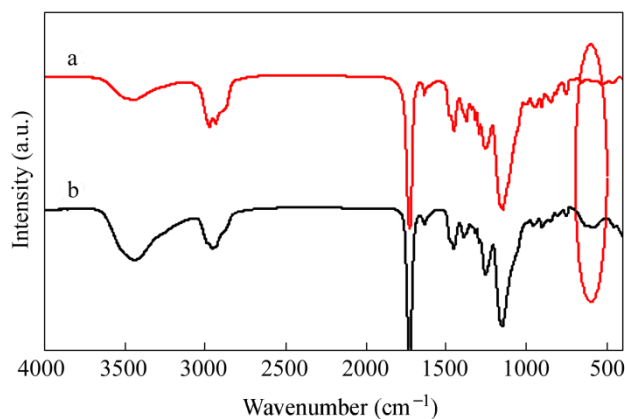


Fig. 3 FTIR spectra of (a) blank beads (B1 of Table 1) and (b) MMPBs (B2 of Table1)

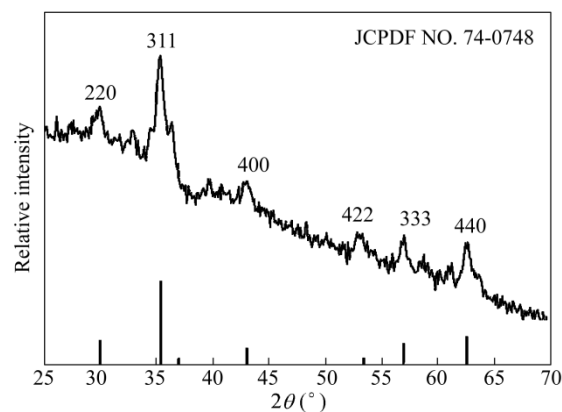


Fig. 4 XRD patterns of MMPBs (Sample B4 in Table 1)

The distribution of Fe_3O_4 nanoparticles inside the polymer walls has been observed by TEM (Fig. 5). Black dots represent nanoparticles which are embedded in the gray polymer walls. The light regions represent “windows” in the walls. In previous researches^[1–4], nanoparticles were mixed with external phase of HIPE in advance, which normally lead to aggregation of nanoparticles at the oil/water interface. However, in the present study, Fe_3O_4 nanoparticles are uniformly distributed throughout the polymeric matrix, which is favorable for the mechanical properties and application of MMPBs. The average diameter of magnetic nanoparticles is about $(13.2 \pm 1.5) \text{ nm}$ (RSD = 11.4%) calculated from TEM photographs, which is consistent with the XRD results.

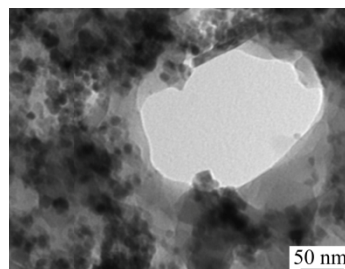


Fig. 5 TEM images of MMPBs (Sample B2 in Table 1)

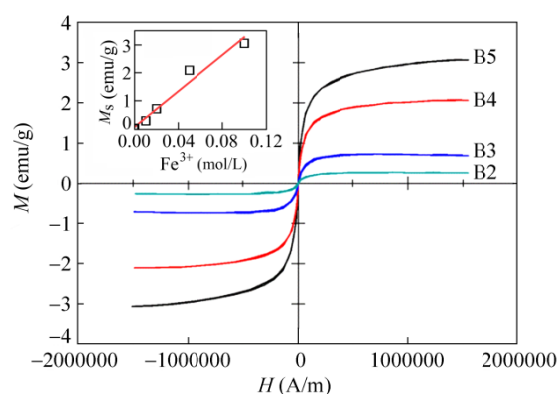


Fig. 6 Hysteresis loops of MMPBs measured at $T = 300$ K
The inset shows the relationship between saturation magnetization and concentration of iron ions.

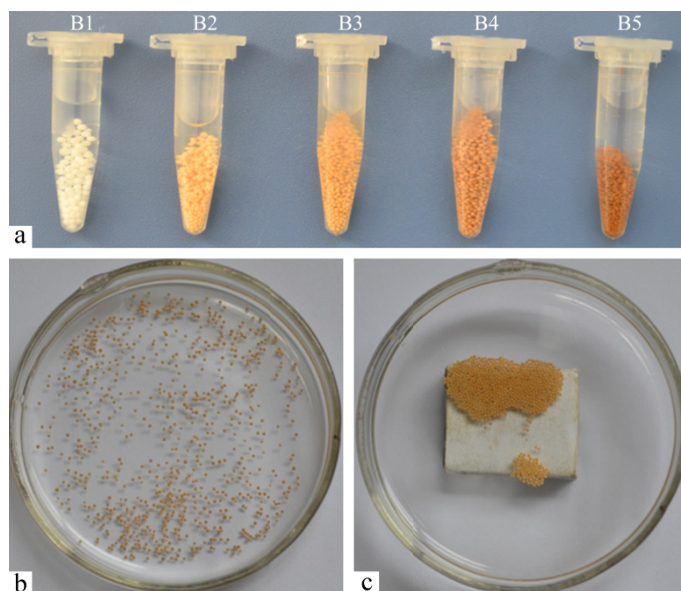


Fig. 7 Image of MMPBs: (a) samples B1 to B5 of Table 1, (b) without magnetic field and (c) with magnetic field

Magnetic properties of MMPBs were characterized using VSM (Fig. 6). Neither remanent magnetization nor hysteresis is found in the magnetization-demagnetization curves, which indicates the superparamagnetic behavior of MMPBs. The saturation magnetization of MMPBs could be easily adjusted by changing the

concentration of iron salts in the aqueous internal phase. Increasing the concentration of iron salts resulted in gradual color changes of MMPBs from white to brown (Fig. 7a). Magnetic nature of MMPBs is also demonstrated with the use of the images of magnetic attraction of MMPBs in a Petri dish. MMPBs dispersing in the dish without magnet are shown in Fig. 7(b). When a magnet was applied at the bottom of the dish, MMPBs were instantly adsorbed by the magnet (Fig. 7c). After removing the magnet, the MMPBs disperse again.

CONCLUSIONS

In summary, a simple method has been developed to fabricate MMPBs. (Water-in-oil)-in-water double emulsion droplets are prepared by injecting HIPE into a simple microfluidic device. The droplets are served as microreactors to synthesize Fe_3O_4 nanoparticles and are polymerized to form MMPBs. FTIR, SEM, TEM, XRD, and VSM are used to confirm the chemical structure, porous morphology and magnetic properties of MMPBs. Size of beads could be adjusted by changing the size of syringe needle. And the saturation magnetization of MMPBs increases linearly with increasing concentration of iron ions. We believe this is a versatile method, which means other functional macroporous nanocomposite beads could be fabricated by the same procedure.

REFERENCES

- 1 Vilchez, A., Rodríguez-Abreu, C., Esquena, J., Menner, A. and Bismarck, A., *Langmuir*, 2011, 27(21): 13342
- 2 Kovačič, S., Ferk, G., Drofenik, M. and Krajnc, P., *React. Funct. Polym.*, 2012, 72(12): 955
- 3 Ghosh, G., Vilchez, A., Esquena, J., Solans, C. and Rodríguez-Abreu, C., *Mater. Chem. Phys.*, 2011, 130(1): 786
- 4 Li, T., Liu, H., Zeng, L., Yang, S., Li, Z., Zhang, J. and Zhou, X., *J. Mater. Chem.*, 2011, 21(34): 12865
- 5 Mert, E.H., Yıldırım, H., Üzümcü, A. T. and Kavas, H., *React. Funct. Polym.*, 2013, 73(1): 175
- 6 Okushima, S., Nisisako, T., Torii, T. and Higuchi, T., *Langmuir*, 2004, 20(23): 9905
- 7 Utada, A., Lorenceau, E., Link, D., Kaplan, P., Stone, H. and Weitz, D., *Science*, 2005, 308(5721): 537
- 8 Seo, M., Paquet, C., Nie, Z., Xu, S. and Kumacheva, E., *Soft Matter*, 2007, 3(8): 986
- 9 Shum, H.C., Lee, D., Yoon, I., Kodger, T. and Weitz, D. A., *Langmuir*, 2008, 24(15): 7651
- 10 Xu, S., Nie, Z., Seo, M., Lewis, P., Kumacheva, E., Stone, H.A., Garstecki, P., Weibel, D.B., Gitlin, I. and Whitesides, G.M., *Angew. Chem.*, 2005, 117(5): 734
- 11 Ziemecka, I., van Steijn, V., Koper, G.J., Rosso, M., Brizard, A.M., van Esch, J.H. and Kreutzer, M.T., *Lab on a Chip*, 2011, 11(4): 620
- 12 Nie, Z., Li, W., Seo, M., Xu, S. and Kumacheva, E., *J. Am. Chem. Soc.*, 2006, 128(29): 9408
- 13 Nisisako, T., Torii, T., Takahashi, T. and Takizawa, Y., *Adv. Mater.*, 2006, 18(9): 1152
- 14 Zhao, Y., Gu, H., Xie, Z., Shum, H.C., Wang, B. and Gu, Z., *J. Am. Chem. Soc.*, 2012, 135(1): 54
- 15 Nurumbetov, G., Ballard, N. and Bon, S.A., *Polym. Chem.*, 2012, 3(4): 1043
- 16 Wang, J., Hu, Y., Deng, R., Xu, W., Liu, S., Liang, R., Nie, Z. and Zhu, J., *Lab Chip*, 2012, 12(16): 2795
- 17 Shum, H.C., Kim, J.-W. and Weitz, D.A., *J. Am. Chem. Soc.*, 2008, 130(29): 9543
- 18 Zhang, H. and Cooper, A., *Chem. Mater.*, 2002, 14(10): 4017
- 19 Dubinsky, S., Zhang, H., Nie, Z., Gourevich, I., Voicu, D., Deetz, M. and Kumacheva, E., *Macromolecules*, 2008, 41(10): 3555
- 20 Gokmen, M. T., Van Camp, W., Colver, P.J., Bon, S.A. and Du Prez, F.E., *Macromolecules*, 2009, 42(23): 9289
- 21 Utada, A. S., Fernandez-Nieves, A., Stone, H.A. and Weitz, D.A., *Phys. Rev. Lett.*, 2007, 99(9): 094502
- 22 Barbetta, A. and Cameron, N.R., *Macromolecules*, 2004, 37(9): 3188
- 23 Carnachan, R.J., Bokhari, M., Przyborski, S.A. and Cameron, N.R., *Soft Matter*, 2006, 2(7): 608
- 24 Ma, Z., Guan, Y. and Liu, H., *J. Polym. Sci. Part A: Polym. Chem.*, 2005, 43(15): 3433
- 25 Horák, D., Rittich, B. and Španová, A., *J. Magn. Magn. Mater.*, 2007, 311(1): 249
- 26 He, H., Zhang, Y., Gao, C. and Wu, J., *Chem. Commun.*, 2009, 48(13): 1655