Soft Matter

Cite this: *Soft Matter*, 2011, **7**, 9894

www.rsc.org/softmatter

COMMUNICATION

Continuous fabrication of monodisperse polylactide microspheres by droplet-to-particle technology using microfluidic emulsification and emulsion–solvent diffusion†

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Received 17th May 2011, Accepted 22nd August 2011
DOI: 10.1039/c1sm05910f

Monodisperse polylactide (PLA) microspheres were continuously fabricated by microfluidic emulsification and subsequent dilution in water. The diameter was precisely tuned from 6 to 50 μm by changing the flow rate of the fluids in microfluidics or the PLA concentration in the dispersed phase. The use of amphiphilic oil-soluble poly(ethylene glycol)-*b*-polylactide (o-PEG–PLA) as a matrix resulted in a highly porous microsphere morphology, and the porosity was controlled by blending PLA. Therefore, monodisperse PLA microspheres with the predetermined surface porosity were continuously produced by just enough reagents and energy.

Biodegradable polymeric microspheres have been developed for use in the chemical industry such as in coatings, inks, agrichemicals, foods, and drug delivery carriers.[1–3] In particular, polylactide (PLA) has attracted a great deal of attention from all over the world in recent decades because it is producible from renewable resources. PLA is also well known for its biodegradability and biocompatibility. It breaks down into lactic acid units that are nontoxic to the human body. In general, PLA microspheres are prepared by the emulsion–solvent evaporation technique.[4–6] However, this technique has drawbacks such as the presence of toxic solvent residues in the end products, requirement for multi-step preparation, and polydispersity in the microsphere diameter. During emulsion preparation, although dichloromethane (DCM) is commonly used as the organic solvent because of good solubility of PLA and its high volatility, which facilitates easy evaporation from the emulsion droplets, it is harmful to human health so that complete removal of it from the products is required. Surfactants used for emulsification also should be eliminated because these are non-essential to the human body. Multi-step preparation of microspheres results in greater equipment investment and the total production cost. Moreover, monodispersity in microsphere diameter enhances the quality of final products in all applications and saves further separation processes. In drug delivery applications, for example, monodisperse carriers decrease undesirable side effects and achieve precise control of drug release kinetics and encapsulation efficiency.

In recent years, preparation methods for monodisperse microspheres have been developed. For example, extrusion emulsification using a *Shirasu* porous glass membrane followed by emulsion–solvent evaporation can produce relatively uniform microspheres.[7–10] However, in fact, the polydispersity value of the droplets is higher (coefficient of variation [CV = σ/D, where σ is the standard deviation of the diameter (μm) and D is the mean diameter (μm)]; CV = 7–15%) than that in microchannel emulsification. In addition, it is difficult to control the diameter of the microspheres because the diameter and the monodispersity strongly depend on the pore size distribution of the membrane used. Furthermore, the surface anion charge of the membrane also affects the droplet production. It often restricts the choice of surfactant regardless of stability of the emulsion. On the other hand, microfluidic technology enables the fabrication of monodisperse microspheres with well-defined size (CV = 1–5%) and morphology.[11–13] Using a coaxial microcapillary fluidic device, monodisperse poly(ethylene glycol)-*b*-polylactide (PEG-*b*-PLA) polymersomes have been prepared *via* the water-in-oil-in-water (W/O/W) emulsion–solvent evaporation method by the Weitz group.[14] In the preparation of W/O/W emulsion, however, a mixture of toluene and chloroform, toxic to human health, was used as the organic phase. In addition, the prepared emulsion was required to be stored in a vessel for a few hours until the microspheres were obtained by evaporation of the organic solvent from the dispersed phase. In order to employ microfluidic technology for industrial production of functional microspheres, it is desirable to construct a simple and environmentally friendly continuous process for the preparation of monodisperse microspheres.

Herein, we report a simple and environmentally friendly process to continuously fabricate monodisperse PLA microspheres and control the diameter by droplet-to-particle technology using microfluidic emulsification and sequential emulsion–solvent diffusion. The microfluidic emulsification facilitating a monodisperse oil-in-water (O/W) emulsion enhances the marketability of products in the droplet-to-particle technology. During preparation, ethyl acetate (EA), an organic solvent approved by the FDA, was used as an oil phase because of its low toxicity compared with DCM, toluene and chloroform and its relatively high solubility in water (8.7%, w/w), and

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1sm05910f

which makes it easy to solidify the microspheres by solvent diffusion. Almost all conventional reports have employed poly(vinyl alcohol) or sodium dodecyl sulfate as a stabilizer or surfactant to prepare O/W emulsions. However, residues of these agents in microsphere products are not always good for human health in clinical applications. In order to prepare impurity-free biocompatible microspheres, we have used amphiphilic water-soluble PEG-*b*-PLA (w-PEG–PLA).[15] The PEG segment is hydrophilic and biocompatible, while, in contrast, the PLA segment is hydrophobic. Moreover, EA is a good solvent for only the PLA segment, though DCM solves both PEG and PLA segments. For these reasons, w-PEG–PLA plays a role of a surfactant in the EA/water emulsion system.[15] Eventually, a monodisperse O/W emulsion prepared by a microfluidic emulsification is simply poured into an excess amount of water, and which accelerates solvent diffusion from the oil droplets into the water and then solidifies the microspheres. This simple "dilution" process enables rapid and continuous production of micron-sized monodisperse PLA microspheres from emulsion at ordinary temperatures and pressures. To the best of our knowledge, there is no study to prepare and control monodisperse PLA microspheres with tens of micrometres in diameter.

PLA and PEG-*b*-PLA were synthesized by ring-opening polymerization of DL-lactide as previously reported.[15] 1 wt% of PLA ($M_n = 3200$, $M_w/M_n = 1.12$) was dissolved in EA, which was used as the dispersed (organic) phase. Aqueous solution of concentration 1 wt% of w-PEG–PLA ($M_n = 4400$, $M_w/M_n = 1.05$, hydrophile–lipophile balance (HLB) = 18.2) and saturated with EA was used as the continuous (aqueous) phase. These solutions were separately fed into a Y-shaped microfluidic device using a syringe pump to prepare a monodisperse O/W emulsion at the Y-junction (Fig. 1). The flow rates of the dispersed phase (Q_d) and the continuous phase (Q_c) were fixed at 60 and 600 μL h^{-1}, respectively. The obtained O/W emulsion was directly poured into 100 mL of ultrapure water *via* a Teflon tube ($\Phi = 0.5$ mm, $L = 20$ cm), which was connected to the outlet of the microfluidic device. EA was thus rapidly removed from the droplet (dispersed phase) by solvent diffusion to the large amount of water, resulting in hardened PLA microspheres.

Microfluidic emulsification enabled the preparation of a monodisperse O/W emulsion without satellite droplets, as seen in the Teflon tube (Fig. 2). The droplets did not coalesce in the tube but they collapsed when the emulsion was dropped onto a slide glass at the outlet of the tube. This means that EA, a volatile solvent, is readily

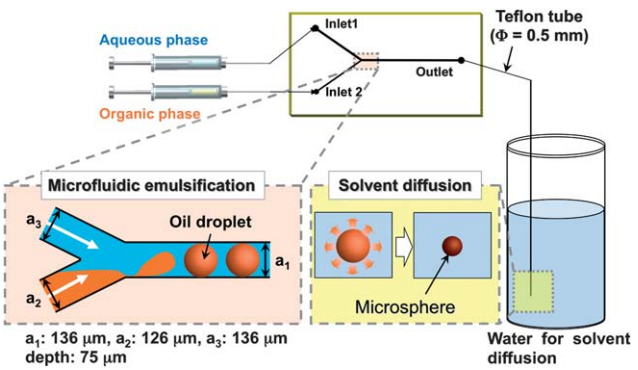

Fig. 1 Schematic of the procedure to fabricate monodisperse PLA microspheres by combining microfluidic emulsification and solvent diffusion.

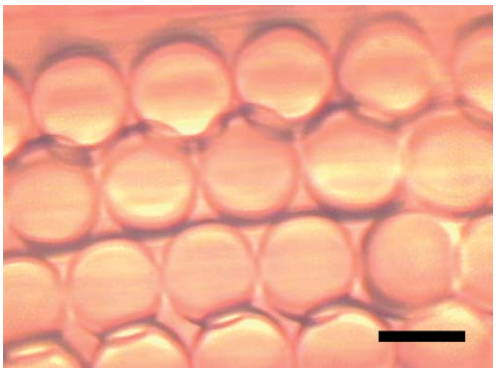

Fig. 2 Optical microscopy image of monodisperse oil droplets dispersed in the aqueous phase before solvent diffusion, captured in the Teflon tube connected from the outlet of the microfluidic device to the vessel containing water for solvent diffusion. The flow rates were $Q_d = 60$ μL $h^{-1}$and $Q_c = 600$ μL h^{-1}.

evaporated when the emulsion is exposed to air on a slide glass. Moreover, when we used an aqueous solution without EA as a continuous phase, the O/W emulsion seen in the tube was not monodisperse, even in the presence of 1 wt% of w-PEG–PLA (Fig. S1†). This result indicates that the diffusion of EA during the droplet formation at the Y-junction involves interfacial instability, giving rise to polydisperse droplets. From these results, in order to fabricate stable and monodisperse O/W emulsion, it is very important that the emulsion solution should not be exposed to the air before solvent diffusion and that the continuous phase should be saturated with EA.

The monodisperse O/W emulsion was poured into water (more than 50 times the volume of the O/W emulsion) in order to trigger the diffusion of EA from the emulsion, resulting in the rapid solidification of droplets. Consequently, monodisperse PLA microspheres were obtained. After freeze-drying, monodisperse microspheres with

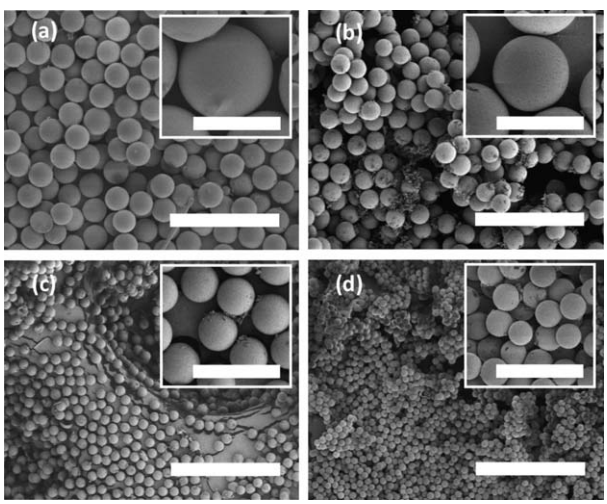

Fig. 3 SEM images of PLA microspheres prepared by solvent diffusion. The Q_c was (a) 600, (b) 1200, (c) 3000, and (d) 6000 μL h^{-1} at a fixed Q_d (60 μL h^{-1}). The concentrations of PLA and w-PEG–PLA were 1 wt%. The obtained microspheres were freeze-dried before SEM observation. Scale bars represent 100 μm. The inset is magnified view with a scale bar corresponding 20 μm.

smooth surfaces were observed by SEM (Fig. 3(a)). The mean diameter and the CV value of the microspheres were 22.4 μm and 4.2%, respectively. The diameter of the final microspheres was about five times smaller than that of the original droplets before solvent diffusion. This phenomenon indicates that volume shrinkage of the droplets is induced by solvent diffusion, which may be a promising way to prepare nanospheres.

In order to control the diameter of the microspheres, we modulated the flow rate of the continuous phase (Q_c = 300–6000 μL h^{-1}) with the flow rate of the dispersed phase (Q_d) fixed at 60 μL h^{-1}. As seen in Fig. 3(a–d), the diameter of the microspheres decreased with increasing flow rate of the continuous phase. The diameter was controlled from 6.3 to 22.4 μm. The shear force at the Y-junction in the microfluidic device is increased with increasing continuous phase flow rate, resulting in the production of smaller droplets. These droplets are then finally shrunk by solvent diffusion. Therefore, the diameter of the microspheres is smaller than that of the droplets (Fig. S3†).

The PLA concentration in the dispersed phase also affects the diameter of the microspheres. We prepared microspheres by altering the PLA concentration (1.0, 2.5, and 5.0 wt%). The mean diameter of the microspheres was increased with increasing PLA concentration (Fig. 4). This appears to be caused by a difference in the solidification rate of the microspheres. As the polymer concentration is increased, a supersaturation state of the polymer in a droplet is easily attained by solvent diffusion since the amount of polymer in a droplet increases in proportion to the concentration. Rapid solidification restricts the shrinkage of the droplets, resulting in larger microspheres. This result suggests that our approach can easily control the diameter of PLA microspheres by changing the flow rates or the concentration of the polymer in the dispersed phase.

Moreover, the control of morphology was achieved by using oil-soluble PEG-*b*-PLA (o-PEG–PLA, M_n = 14 200, M_w/M_n = 1.20, HLB = 5.6) instead of PLA as a matrix for the microspheres. Because PEG-*b*-PLA is an amphiphilic diblock copolymer and a tunable polymer with respect to solvent compatibility, we synthesized PEG-*b*-PLA with different HLB. o-PEG–PLA (HLB < 11) is soluble in EA but insoluble in water. Monodisperse PEG–PLA microspheres were fabricated likewise and the microspheres obtained had highly porous surfaces. The diameter and the CV value were 33.2 μm and 5.5%, respectively (Fig. 5(e)).

From the point of view of polymer affinity to the solvent, o-PEG–PLA dissolved in a dispersed phase would form a reverse micelle structure with a small amount of water.[5] Fabricated monodisperse oil droplets containing o-PEG–PLA are subsequently solidified by solvent diffusion. Therefore, the polymer matrix of o-PEG–PLA contains small water droplets in the PEG domains. After freeze drying, porous microspheres are obtained by the evaporation of water from the microsphere matrix.

Furthermore, the porosity of the microspheres was controlled by blending o-PEG–PLA and PLA. As the blend ratio of PLA to o-PEG–PLA was increased, the surface porosity of the microspheres was decreased and the microspheres finally showed a smooth surface when the ratio was 50/40 (w/w) (Fig. 5). These results suggest that our simple fabrication method can easily control the porosity of the microspheres with a narrow diameter distribution by altering the polymer composition in the dispersed phase, which would be available for controlling the release rate of inclusion in the microspheres.

In conclusion, we have continuously fabricated monodisperse microspheres composed of PLA or PEG–PLA by droplet-to-particle technology using microfluidic emulsification and subsequent emulsion–solvent diffusion. The diameter of the microspheres was easily tuned by the flow rate of the continuous phase during the microfluidic emulsification and the polymer concentration of the feed solution. The morphology was controlled by the polymer composition comprising the matrix. We believe that this simple fabrication technique has great potential to produce PLA microspheres with a well-defined diameter and morphology for use as a container for various hydrophobic compounds such as drugs, pigments, perfumes, and agrichemicals.

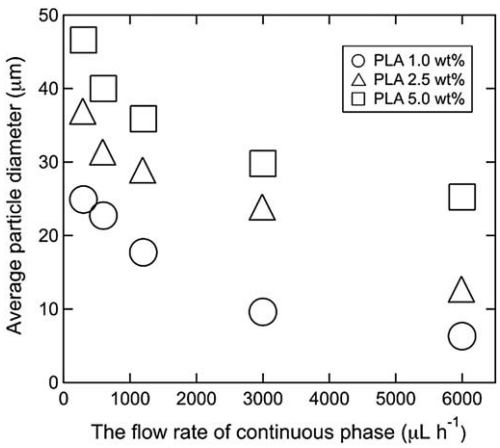

Fig. 4 Effect of the continuous phase flow rate and the concentration of PLA on the diameter of PLA microspheres. The concentration of PLA was ○: 1 wt%, △: 2.5 wt% and □: 5.0 wt%. The concentration of w-PEG–PLA in the aqueous phase was 1.0 wt%. The flow rate of the dispersed phase was 60 μL h^{-1}.

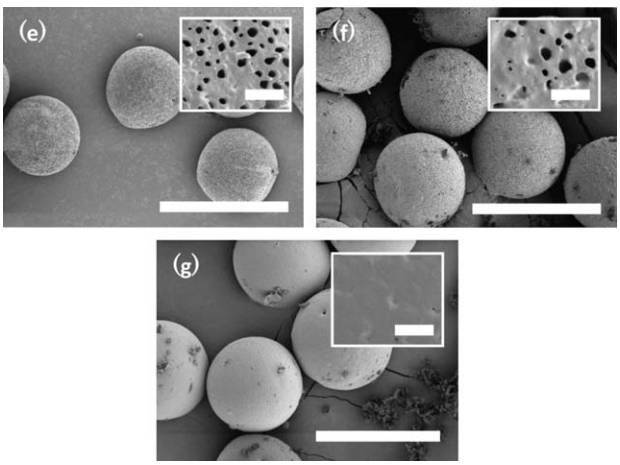

Fig. 5 SEM images of monodisperse PEG–PLA/PLA microspheres prepared by altering the content of PLA in the dispersed phase. The PEG–PLA/PLA ratios (w/w) were (e) 50/0, (f) 50/20, and (g) 50/40. The concentration of w-PEG–PLA in the aqueous phase was 1 wt%. The flow rates were fixed at Q_d = 60 μL h^{-1} and Q_c = 600 μL h^{-1}. Scale bars represent 50 μm. The inset shows the surface of the microspheres with a scale bar corresponding 1 μm.

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