AIP | Biomicrofluidics

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Citation: Biomicrofluidics **6**, 034104 (2012); doi: 10.1063/1.4738586
View online: http://dx.doi.org/10.1063/1.4738586
View Table of Contents: http://bmf.aip.org/resource/1/BIOMGB/v6/i3
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Droplet based microfluidic fabrication of designer microparticles for encapsulation applications

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(Received 19 April 2012; accepted 2 July 2012; published online 20 July 2012)

Developing carriers of active ingredients with pre-determined release kinetics is a main challenge in the field of controlled release. In this work, we fabricate designer microparticles as carriers of active ingredients using droplet microfluidics. We show that monodisperse droplet templates do not necessarily produce monodisperse particles. Magnetic stirring, which is often used to enhance the droplet solidification rate, can promote breakup of the resultant microparticles into fragments; with an increase in the stirring time, microparticles become smaller in average size and more irregular in shape. Thus, the droplet solidification conditions affect the size, size distribution and morphology of the fabricated particles, and these attributes of the microparticles strongly influence their release kinetics. The smaller the average size of the microparticles is, the higher the initial release rate is. The release kinetics of drug carriers is strongly related to their characteristics. The understanding of this relationship enables the fabrication of tailor-designed carriers with a specified release rate, and even programmed release to meet the needs of applications that require a complex release profile of the active ingredients. © *2012 American Institute of Physics*. [http://dx.doi.org/10.1063/1.4738586]

INTRODUCTION

An emulsion is a mixture of two immiscible liquids, in which one liquid is dispersed in the form of droplets in another liquid that forms the continuous phase. They are frequently used as a precursor to fabricate particles or capsules for industrial applications. In particular, emulsion-based delivery systems designed for the encapsulation, protection and release of drugs and other active ingredients, are of increasing interest to pharmaceutical research and other biomedical applications. Among these systems, polymer micro/nanoparticles are promising vehicles due to their potential to release active ingredients in a controlled manner.[1–9] To prepare polymer micro/nanoparticles, the polymer and the drug are first dissolved in an organic solvent to form the dispersed, droplet phase. This phase is then mixed with an aqueous continuous phase to form an oil-in-water emulsion. As a final step, the emulsion droplets are solidified to form polymer micro/nanoparticles with drugs encapsulated in the resultant polymer matrix. To solidify the droplets, the organic solvent has to be removed. Moreover, for most biomedical applications the organic solvent is often harmful. Therefore, the solvent removal step is critical. Among typical solvent removal methods, including evaporation, lyophilization, reverse extraction, precipitation, and dialysis (solvent exchange), evaporation is frequently used due to its simplicity.[10] Under room conditions, evaporation proceeds relatively slowly; to enhance the rate of particle production, the precursor emulsions are often mechanically agitated to increase the effective air/solvent interfacial area, leading to faster solvent evaporation. Besides, with conventional

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emulsion techniques, mechanical agitation also helps to introduce the shear to emulsify the immiscible liquids involved, and prevent the droplets from undesired coalescence. However, mechanical agitation does not provide exquisite control over the characteristics of formed droplets, such as droplet size and size distribution. Hence, the characteristics of the resultant polymer particles are also not precisely controlled.

Recently, developments in droplet microfluidics enable the generation of monodisperse and size-controlled single/multiple emulsion droplets.[11–20] This provides a unique opportunity to fabricate monodisperse and size-controlled polymer micro/nanoparticles for controlled release study. However, monodisperse polymer particles are not always observed. This is mainly because the subsequent solvent evaporation and droplet solidification step, usually in the form of mechanical agitation, can disturb the droplet templates and distort the shape and size uniformity of the resultant solid particles. The solvent evaporation step can also affect the surface morphology and other characteristics, such as encapsulation efficiency.[21] This effect of the solvent evaporation step on the properties of the resultant particles has yet to be systematically studied.

In this work, we take advantage of monodisperse oil-in-water emulsion droplet templates produced using microfluidic techniques, and investigate the effect of the droplet solidification step alone on the characteristics of the resultant drug-loaded poly (lactic-co-glycolic acid) (PLGA) microparticles, such as size and release kinetics. We demonstrate that monodispersity of the droplet templates does not guarantee the uniformity of the final microparticles. Mechanical agitation introduced during the solidification process raises the polydispersity of the final PLGA microparticles; this significantly affects the release characteristics of the PLGA microparticles. We further explore the relationship between size and release kinetics of the final microparticles as drug carriers. Our results provide guidelines on how to tune fabrication conditions for fabricating microparticles with appropriate characteristics for the target applications.

EXPERIMENT SECTION

We use a capillary microfluidic device to generate monodisperse oil-in-water single emulsions.[11–20] In a typical capillary microfluidic single-emulsion device, two cylindrical capillaries (World Precision Instrument Inc.), which have an inner diameter and an outer diameter of 0.58 mm and 1 mm, respectively, are tapered using a micropipette puller (P-97, Sutter Instrument, Inc.). The tips of the capillaries are polished to desired diameters using a sand paper. Typical tip diameters of the injection and collection capillaries are 25 μm and 100 μm, respectively. Then, the polished round capillaries are coaxially aligned inside a square capillary (AIT glass). The device is used for generating monodisperse oil-in-water single emulsions.

To prepare the emulsion templates, we use dichloromethane (DCM) with 8% (w/v) PLGA (50:50) and 0.8% (w/v) rifampicin as the oil dispersed phase. In this study, we choose rifampicin, which is a drug for treating tuberculosis and can potentially be benefited from the approach, as a model to investigate the encapsulation and controlled release of active ingredients. PLGA, a common material in drug encapsulation and controlled release study, is known for its excellent biocompatibility and tunable degradability.[5–9] DCM is a frequently used organic solvent for PLGA and evaporates quickly at room temperature. The aqueous continuous phase consists of deionized water with 1% (w/v) poly(vinyl alcohol) (PVA), which is added as a surfactant to prevent oil droplets from coalescence.

The oil dispersed phase is pumped through one round capillary, which is known as the injection capillary. The aqueous continuous phase flows in the opposite direction through the region between the other round capillary, often known as the collection capillary, and the outer square capillary. The resultant jet breaks up into monodisperse oil-in-water (O/W) single-emulsion drops at the orifice of the collection capillary. The flow rates of the dispersed and the continuous phases in all experiments are fixed at 800 and 2000 μl/h, respectively.

After collection, the emulsion droplets are magnetically stirred to enhance the evaporation of DCM in the oil droplets. After complete evaporation of DCM, the emulsion droplets are solidified to form PLGA microparticles with drugs encapsulated in the resultant polymer matrix. To investigate the effect of the extent of stirring, we stir the emulsions magnetically (IKA) at

different rotational speeds of 100 rpm and 800 rpm, respectively, for different lengths of time. At 100 rpm, the corresponding energy outputs for stirring time of 2.5 h, 15 h, 22 h, and 45 h are 0.72 kJ, 4.32 kJ, 6.34 kJ, and 13.25 kJ, respectively; whereas, at 800 rpm, the corresponding energy outputs are 5.76 kJ, 34.56 kJ, 50.69 kJ, and 106.0 kJ, respectively. A schematic illustration of the experimental set-up is shown in Figure 1(a). For the purpose of comparison, we also evaporate the solvents without stirring under room temperature for at least 2 days to make sure all DCM has been evaporated and the PLGA particles are completely solidified.

The morphology of the resultant PLGA microparticles is characterized using an optical microscope (Nikon Eclipse 80i) and a scanning electron microscope (Hitachi S3400N VP SEM). To obtain the size distribution of the microparticles, we measure at least 100 microparticles for each diameter by analyzing microscope images using the open-source image analysis software, Image J.

To investigate the *in vitro* drug release profile of drug-loaded PLGA microparticles, PLGA microparticles that had been loaded with 1 mg rifampicin were dispersed in 1 ml phosphate-buffered saline (PBS) solution (*p*H 7.4) in centrifuge tubes and shaken at 90 rpm at 37 °C. At each predetermined time interval, dispersions were centrifuged and 200 μl of supernatants were collected. The supernatants were then filtered and assayed by UV method at 473 nm with a microplate reader. The PBS solution was replaced with fresh solution at each time point after assaying the rifampicin.

RESULTS AND DISCUSSION

We produce monodisperse oil-in-water single emulsion droplets continuously using droplet microfluidic devices shown in Figure 1(b). We control the size of droplets produced by changing the flow rates of the phases and the geometry of the capillary device. The size of the oil droplet increases with increasing inner phase flow rate, increasing diameter of injection and collection capillary tips, and with decreasing continuous phase flow rate.[22–24] The monodisperse emulsion droplet templates with diameters of about 60 μm are shown in Figure 1(c). The size uniformity of the droplet templates represents the maximum uniformity that the resultant microparticles can have. The emulsion templates are often stirred to enhance the rate of solvent

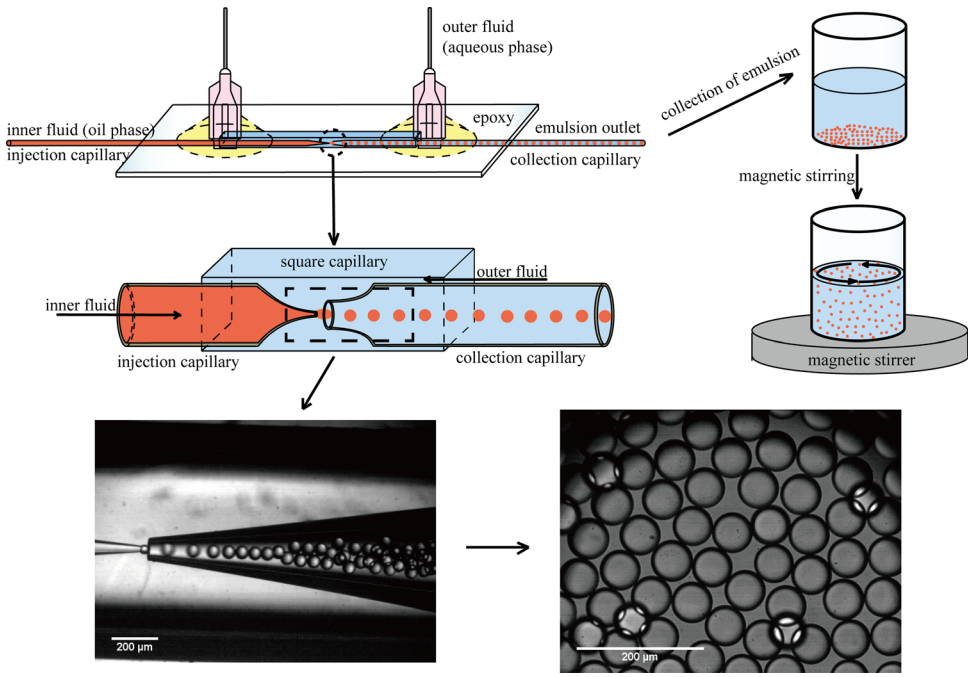

FIG. 1. (a) Schematic of experimental setup; (b) microfluidic generation of monodisperse oil-in-water single emulsion droplets; (c) typical monodisperse droplet templates produced using capillary microfluidic devices (enhanced online). [URL: http://dx.doi.org/10.1063/1.4738586]

evaporation. As conventional emulsion techniques typically rely on turbulent mixing for emulsifying the precursor solution, the resultant emulsion templates tend to be polydisperse in size. Therefore, the stirring step, which may break up the emulsion templates, does not significantly alter the size distribution of the final microspheres. As a result, the effect of the stirring conditions on the size distribution of the resultant microspheres is rarely studied. However, with the advent of microfluidic emulsification, the emulsion templates can achieve a narrow size distribution; the extent of stirring during the solidification process raises the polydispersity of the final microparticles, as illustrated schematically in Figure 2; this change in size and size distribution of the microparticles significantly affects their release characteristics.

In our work, although monodisperse PLGA/DCM-in-water emulsion droplets are produced using droplet microfluidics, polydisperse PLGA microparticles are observed after magnetic stirring is applied to promote solvent evaporation. There are two possible routes by which the polydispersity increases, as schematically illustrated in Figure 3(a). First, shear introduced by the magnetic stirrer may lead to simultaneous coalescence and break-up of droplets before particles are formed, thus disrupting the size distribution of the droplet templates. Thus, the resultant microspheres are polydisperse. Second, the magnetic stirring can continue to break down the solidified polymer microparticle; thus, the final products will be not only polydisperse in size, but also fragmented in shape. The difference between these two routes can be identified by the shape of the particles. In our experiments, polydisperse fragmented microparticles are observed. To confirm the second route, we solidify monodisperse PLGA/DCM droplets under room temperature without any stirring, and the resultant PLGA microspheres are monodisperse. The diameter of these microspheres is about 34.2 μm with a standard deviation of 1.75, as shown in Figures 3(b) and 3(c). This indicates that the droplet have shrunken by nearly 60% after solvent evaporation. Then, we stir a suspension of these monodisperse microspheres using a magnetic stirrer. After stirring for 2.5 h, indeed, polydisperse fragmented microparticles are observed; the average diameter of these polydisperse microparticles is 19.9 μm with a standard deviation of 14.9, as shown in Figure 3(d). This confirms that microparticles can be broken up into small fragments even after solidification. Therefore, minimal stirring should be used if monodisperse microspheres are desired.

To investigate the effect of the extent of stirring, we separately vary the stirring speed and the stirring time during fabrication of the particles. From our observations, varying stirring speed does not significantly affect the morphology of microparticles. However, when stirring time is increased, the fraction of broken PLGA microparticles increases, as shown in Figure 4(a). With

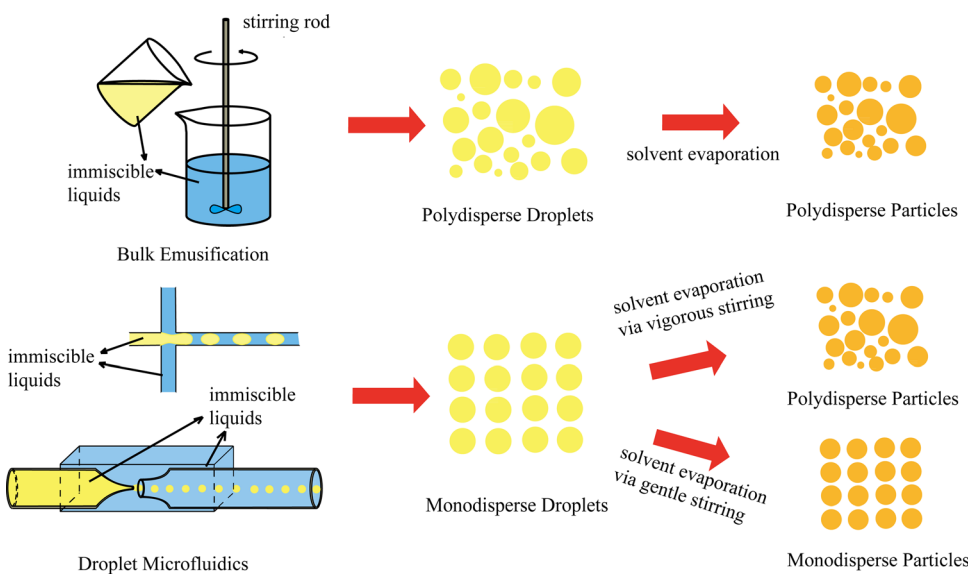

FIG. 2. Schematic showing the effect of solvent evaporation on the particle size and size distribution with bulk emulsification techniques (top) and droplet microfluidics (bottom).

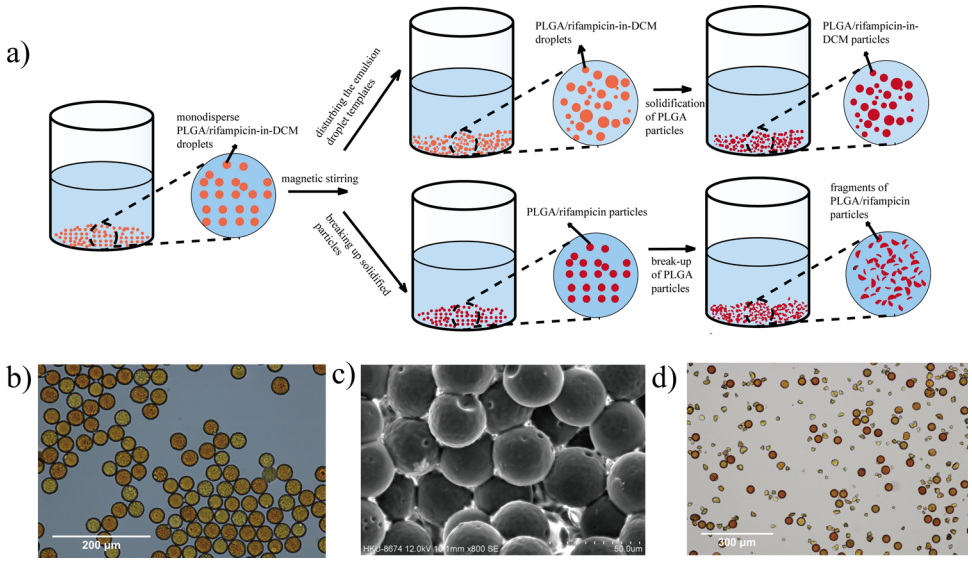

FIG. 3. (a) Schematic of two possible routes by which the polydispersity of microparticles increases; (b) optical microscope image and (c) SEM image of monodisperse microspheres formed by solvent evaporation from emulsion droplet templates without stirring; (d) optical microscope images of suspensions of monodisperse solidified microspheres stirred at 100 rpm.

a further increase in stirring time, the PLGA particles become more irregular in shape and smaller in size, as shown in Figure 4(b). Noticeably, after 46 h of stirring, only fragmented pieces of PLGA polymers are left, as shown in Figure 4(c). Since all microparticles become fragmented pieces as stirring time is increased, the polydispersity of microparticles appears to have decreased. As a result, the average size and standard deviation of microparticles decrease with the increase of stirring time, as shown by the plot in Figure 4(d). These results suggest that the breakup of the solidified particles while being stirred is the major factor in affecting the size and size distribution of the final particles.

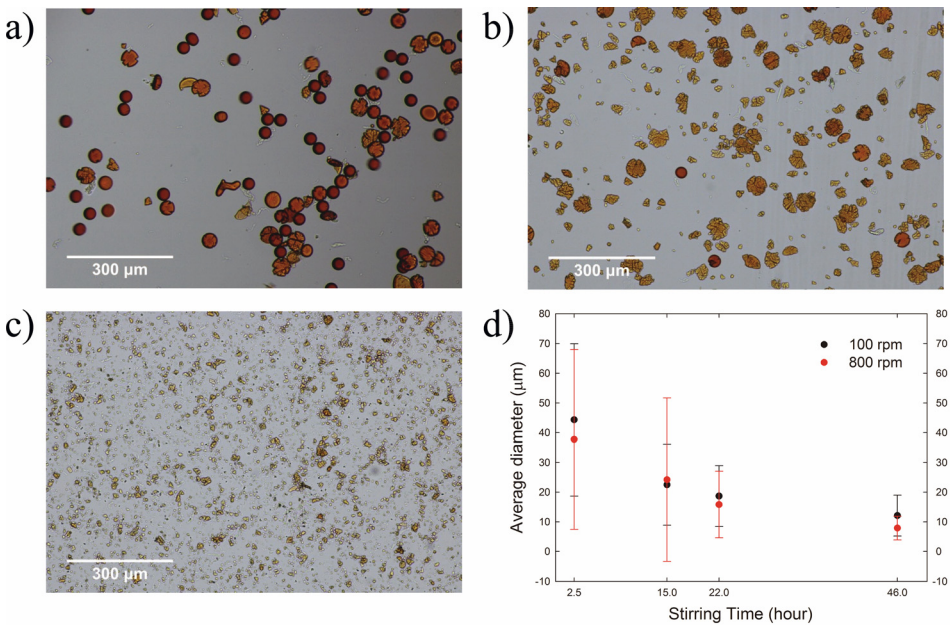

FIG. 4. Optical microscope images of microparticle suspensions stirred at (a) 800 rpm for 2.5 h, (b) 15 h, (c) 46 h, (d) a plot of the average diameter of PLGA microparticles (with the error bars denoting a standard deviation for each data point) as a function of stirring time using a magnetic stirrer.

When microparticles are used for drug encapsulation, the release kinetics of the drug from the particles should depend critically on their morphology. To investigate the effect of the fabrication conditions on the release kinetics of the microparticles, we encapsulate a drug, rifampicin, as a model compound in the microparticles that are prepared under different conditions; we measure their release kinetics subsequently. The drugs are distributed and physically trapped in the PLGA polymer matrices.[25–27] The encapsulation of drugs in PLGA microparticles can extend the release time of the drug and provide a relatively long-term therapeutic effect. Therefore, it is particularly advantageous for drugs used in treatment of chronic diseases, such as rifampicin. Although rifampicin is chosen as a model in our study, our results should be applicable to other drugs with similar entrapment mechanisms.

Typically, the release of drug from PLGA microparticles follows a triphasic release pattern as shown in Figure 5(a).[28] Drugs encapsulated inside the PLGA microspheres are released from the surface of microspheres at the beginning; this usually leads to an initial burst release. After that, the drugs diffuse continuously from the interior of the microsphere to the surroundings, leading to a phase with a relatively sustained release at a relatively low concentration. At the end of the cycle, the PLGA polymer matrix starts to degrade, leading to a second phase of burst release. The proposed underlying mechanism is illustrated in Figure 5(b). Indeed, the release results from PLGA microparticle suspensions stirred at 800 rpm for 15 and 46 h have the characteristic release profiles. They differ mainly in the initial burst release rate, and overall rate of release, as shown in Figure 5(a). During day 1, microparticle suspensions stirred at 800 rpm for 15 h have about 2% of the drug released; while microparticle suspensions stirred at 800 rpm for 46 h have released $14.1\% \pm 0.4\%$ of the total drug content. Moreover, for microparticle suspensions stirred at 800 rpm for 46 h, 25% of the drugs are released within the first 3 days; for microparticle suspensions stirred at 800 rpm for 15 h, it takes 15 days to release the same fraction of drug. This indicates that the extent of stirring influences the release kinetics of PLGA microparticles.

As in the case of the microparticle morphology, changing the stirring speed has a very weak effect on the release kinetics of the microparticles. Suspensions of microparticles stirred

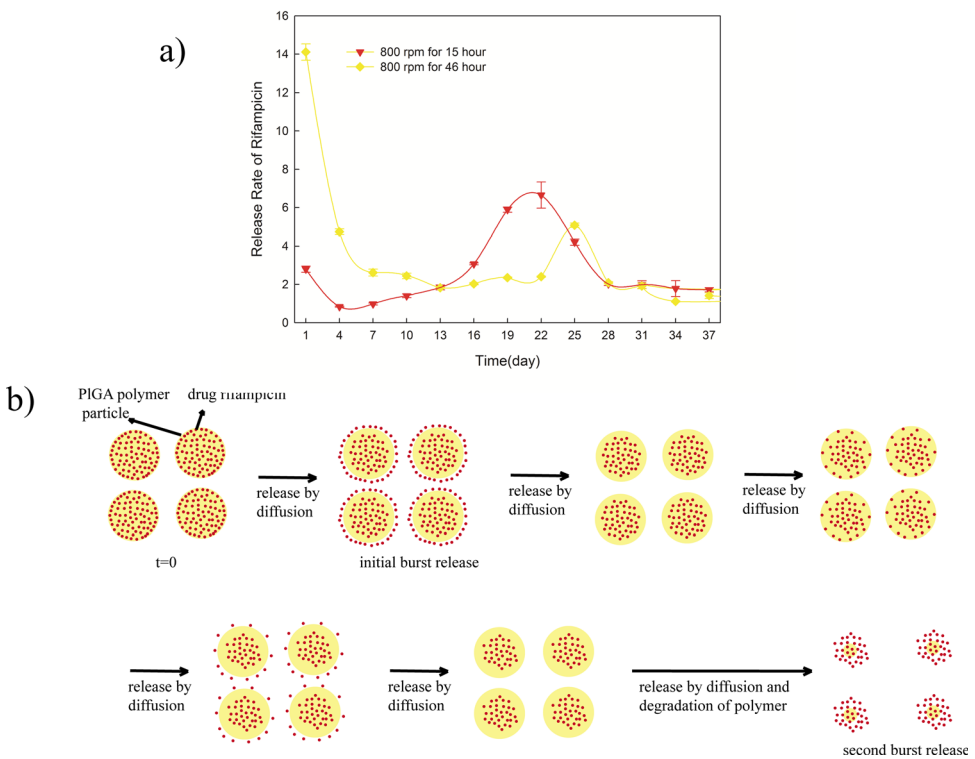

FIG. 5. (a) A plot of rifampicin release rate as a function of time; (b) schematic illustration of a proposed release mechanism of drugs from PLGA microparticles as drug carriers.

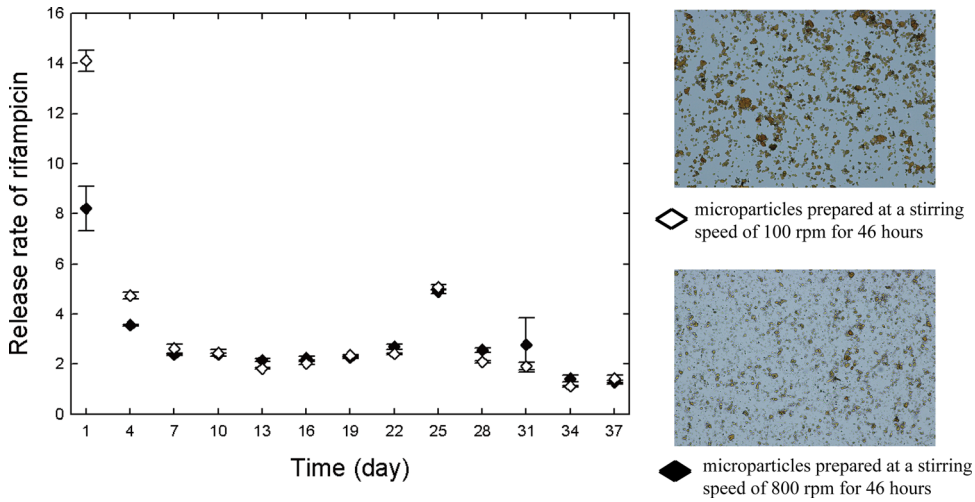

FIG. 6. Release rate of rifampicin from microparticles fabricated at different stirring speeds for the same period of time.

at different speeds for the same period of time show similar release profiles, as shown by the drug release curves in Figure 6.

However, as the morphology of the microparticles depends strongly on the stirring time, the drug encapsulation efficiency and release kinetics of rifampicin-loaded PLGA microparticles also depend critically on the stirring time. As the stirring time increases, the microparticles breaks up into fragments, drugs that are encapsulated inside the microparticles are gradually released from the broken microparticles to the surroundings. Thus, the efficiency of the resultant PLGA microparticles decreases, as shown in Figure 7(a). The drug encapsulation efficiency for suspensions of microparticles that have been stirred for 46 h decreases by 50%, when compared to that of microparticles stirred for 2.5 h. Although prolonged stirring time leads to low

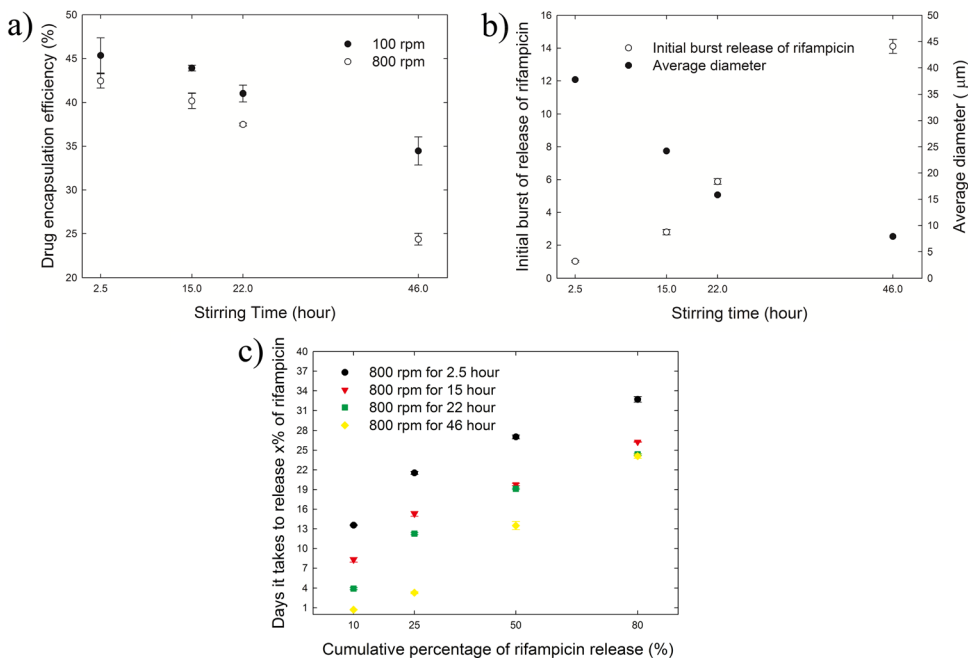

FIG. 7. (a) A plot of drug encapsulation efficiency as a function of stirring time; (b) initial drug release rate of rifampicin from microparticles fabricated using the same stirring speed for different periods of time; (c) cumulative percentage of rifampicin released from microparticles fabricated by stirring for different periods of time.

drug encapsulation efficiency, it generates microparticles with small average size and thus distinct release kinetics.

Polydisperse microparticles with a small average size have a very high initial drug release rate, as shown in Figure 7(b); consequently, most of the drug encapsulated is released initially. For microparticle suspensions stirred for 46 h, almost 50% of rifampicin is released within the first 13 days. As the average size of the microparticles increases, the initial drug release is reduced and most of the drug encapsulated is released at a later stage, as shown in Figure 7(c). For microparticle suspensions stirred for 2.5 h, only 10% of rifampicin is released within the first 13 days, and another 55% of rifampicin is released in the following 11 days. The result indicates that size of microparticles has a significant influence on their release kinetics. Generally, for microparticles with a smaller diameter, the initial burst release is high and thus a large amount of the drugs are released at an early stage; for microparticles with larger diameter, drug release rate is relatively low at the beginning and becomes faster later on. This behavior can be understood as the relative contributions of release through diffusion from the surface of the microparticles and through the degradation of the polymer microparticles, as illustrated in Figure 5(b). For larger microparticles, the surface area-to-volume ratio is lower; thus, the release through diffusion from the particle surface takes place at a relatively low rate. Therefore, a large fraction of the drug remains encapsulated inside the microparticles. However, as the polymer microparticles degrade, the diffusion of the drugs through the particle is sped up, thus raising the subsequent release rate. Our results provide important guidelines for fabricating appropriate microparticles for the target applications. The characteristics of microparticles, such as size, size distribution, and morphology is strongly related to their release kinetics, when they are used as carriers of active ingredients. By understanding this relationship, we can tailor-design delivery vehicles with a specified release rate, and program the release of active ingredients to meet the needs of applications that require a complex release profile.

CONCLUSION

Solvent evaporation is critical in the fabrication of microspheres for encapsulation applications. Solvent evaporation conditions significantly affect the size, size distribution, morphology, as well as release kinetics of the fabricated particles. We show that magnetic stirring produces polydisperse microparticles from monodisperse droplet templates; with an increase in stirring time, microparticles become more irregular in shape and smaller in average size. In comparison to the stirring time, stirring speed has a weak effect on the attributes of the microparticles. The morphology of the microparticles has an important effect on their release kinetics. Polydisperse rifampicin-loaded PLGA microparticles with a small average size have a high initial drug release rate, while, for microparticles with a larger average size, most of the rifampicin is released at a later stage. Our results provide important guidelines on how to tune fabrication conditions to fabricate appropriate microparticles for applications. Due to the limitation of current approach of fabricating microparticles, initial burst release is hard to avoid. A new class of drug carriers with a different structure should be designed and fabricated to achieve zero-order release for applications that require a long and sustained therapeutic effect. Nevertheless, our results show that the release kinetics of drug carriers is strongly related to their preparation conditions and morphologies. By understanding the relationship between the drug carrier characteristics and their release kinetics, tailor-designed drug carriers with pre-determined release rates that meet the needs of specific applications can be fabricated.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the Seed Funding Programme for Basic Research from the University of Hong Kong (201101159009), and the Research Grants Council of Hong Kong (GRF718009 and GRF718111).

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