Facile fabrication of PDMS microspheres with and without fluorescent dye doping

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Abstract*—Poly(dimethyl siloxane) (PDMS) microspheres are increasingly gaining importance for a wide range of applications due to their flexibility and inertness. In this paper, we have fabricated PDMS microspheres using facile emulsion formation in water by stirring with and without additional ultrasonic excitation. It was found that the particle size distribution, which can be attributed to the formation rate of cross-linked PDMS networks in the hydrophobic microspheres, depends on the temperature of the aqueous medium. Swelling of the microspheres in acetone was suggested by permeabilization as evidenced by diffusion and encapsulation of fluorescent dyes within the PDMS. Using scanning electron microscopy, the surface morphology of the spheres was confirmed to have no surface roughness or irregularity. Using fluorescence microscopy, we found that the encapsulated dyes randomly and thus uniformly distributed themselves within the cross-linked PDMS networks and retained the fluorescent properties and characteristic emission color, implying their potential for drug carrier.*

***Keywords*—poly(dimethyl siloxane) (PDMS), microspheres, swelling, encapsulation, fluorescent dyes.**

I. INTRODUCTION

Among the many important polymeric microparticles, those comprised of poly(dimethylsiloxane) (PDMS) have attracted much attention due to their biocompatibility, thermal stability, optical transparency in the UV-visible region, flexibility, non-toxicity, low polarity, low electrical conductivity, and chemical inertness [1-6]. In addition to industrial and medical applications, PDMS microspheres are also utilized in a number of new scientific disciplines, including sensors, actuators, bioanalysis and additives for polymer resins [7]. For these applications, functional compounds can be incorporated into the PDMS microspheres upon mixing them in less-polar organic solvents. With all of these properties, PDMS microspheres can be used as a matrix to deliver the functional compounds to a desired location through microcirculation. Moreover, PDMS microspheres can be suspended in solution to achieve a fluid with controllable rheological properties [8]. Such a wide range of applications has stimulated great interest in the fabrication of PDMS microspheres and in their microfluidic systems for bioanalyses and beyond. For instance, amphiphilic PDMS microspheres within a few tens to a few hundreds of microns in size have been successfully fabricated by flow-focusing and co-flowing methods [9-10], whereas for smaller PDMS spheres, with dimensions within 2.5 to 25 μm, an aqueous emulsion technique has been employed [10-11]. In this technique, the dispersed cross-linked PDMS which is immiscible in water can naturally form micro spheres by virtue of their need to reduce their hydrophobic interactions. By using this technique, the cost of fabrication can be greatly reduced and the process is environmentally friendly.

Driven by the aforementioned potential applications of PDMS microspheres, the objective of this work was focused on low cost and efficient PDMS microsphere fabrication methods. Given the hydrophobic nature of PDMS, in this work we fabricate PDMS microspheres from PDMS elastomer in an aqueous medium by creating a stirred emulsion during crosslinking, with and without sonication. The stirring and the ultrasonic waves are expected to mediate separations of agglomerates of PDMS microdroplets during and after their injection into water, keeping the particles divided and spherical as they cross link and effectively freeze out further coalescence into larger domains. Prior to the cross-linking induced freezing out of droplet fusion and coalescence processes, the small particle size is maintained due to the response of the microspheres to the mechanical or acoustic radiation force exerted on them [12]. With this method, we evaluated the effect of the temperature of the medium on the size distribution of the PDMS microspheres. This is because the rate of cross linking is expected to be strongly temperature dependent. We characterized and examined the PDMS microspheres using bright field microscopy, Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Subsequently, we also evaluated the incorporation of ionic fluorescent dyes within the PDMS microspheres. The fluorescent dyes which are used broadly as coloring and staining biological analytes can be considered as model photo-functional and ionic compounds incorporated in

the PDMS microspheres. This study would improve our understanding about dye incorporation in the cross-linked PDMS networks in the microspheres.

II. EXPERIMENTAL

PDMS (SYLGARD® 184 silicone elastomer base) and its curing agent, produced by Dow Corning Co., were supplied by Panasonic Singapore. The elastomer base and curing agent were thoroughly mixed in a Teflon dish in a 10:1 ratio by weight. This ratio was selected because it produces an optimum curing profile based on the low viscosity of cross-linker mixture and rapid rise in elasticity with time [13].Newly prepared mixed elastomer (~500 µL in volume) was immediately dispensed dropwise into 25 mL of aqueous medium using a small nozzle pipette. The reaction vessel was immersed in a temperature-controlled water bath and the reaction mixture was vigorously stirred or sonicated at frequency of 37 kHz. The solution rapidly turned cloudy, indicating the formation of microparticles. The suspension was continuously stirred or sonicated for 2 h. The dry product microspheres were collected by filtration and then dried. The effect of temperature on the size distribution of the PDMS microspheres was evaluated by varying the temperature of the water medium from 30 to 80 °C during the crosslinking reaction.

The chemical structure of the PDMS microspheres before and after dye encapsulation was evaluated by using an FTIR spectrometer (Cary 630, Agilent Technologies, USA). Approximately 0.1 g of the dried PDMS microspheres was directly subjected to FTIR measurement using attenuated total reflection (ATR) method. The surface morphology of the PDMS microspheres was evaluated from their scanning electron microscopic (SEM) images. The SEM image of dried PDMS microspheres, pasted onto a carbon tape, was recorded on a SEM microscope (JEOL, JSM-7610F).

The size distribution of the PDMS microspheres was evaluated from their bright-field microscopic images. For this purpose, the microspheres were cast onto a glass slide. The glass slide was then mounted on the sample stage of a microscope (Nikon, Eclipse 50iPOL) equipped with a conventional condenser lens (Nikon Achr, NA 0.40) and an objective lens (Nikon; 40×; NA 0.60). The microspheres were irradiated with white light from halogen lamp (λ=380–750 nm) through the condenser lens. The bright-field images of the microspheres in the forward direction were collected and transmitted by the objective lens into a CCD camera (Nikon; DS-Fi1C). From the bright field images, we were able to estimate the sizes of the microspheres using ImageJ software.

Ionic fluorescent dyes, including methylene blue, methyl violet 2B, crystal violet, congo red, rhodamine B, and malachite green were each individually dissolved in acetone. The concentration of the dyes was adjusted to be 5 ppm, as determined from their respective absorption spectrum which was measured using a UV-visible spectrophotometer (Shimadzu UV-1601 PC). Incorporation of the fluorescent dyes was performed by adding 0.5 mg of the dried PDMS microspheres into 5 mL of the solutions of fluorescent dyes. The mixture was shaken for 20 min and kept overnight followed by filtration to end the process of dye percolation. The filtrate was washed three times with distilled water, and then dried overnight in oven at 45 °C.

Incorporation of the fluorescent dyes in the PDMS microparticles was evidenced by fluorescence microscopic imaging using a microscope (Nikon Eclipse 50iPOL, Japan). In this measurement, a beam from a high pressure mercury lamp was utilized as an excitation light source. The excitation wavelength at 365 nm was selected by passing the beam through a band pass filter (360–390 nm). The beam then was focused by the objective lens into the microspheres mounted on the sample stage. The backward (Epi) fluorescent light from the microspheres was collected by the same objective lens, and passed through a dichroic mirror (λ>415 nm) and a long pass filter (λ>435 nm). Elastic light scattering from the excitation light was completely cut by a long pass filter. Finally, the fluorescence image was captured by a color CCD camera. By comparing the fluorescence images with that of a control measurement which was performed for bare PDMS microspheres, we can monitor the existence of the fluorescent dyes incorporated in the PDMS microspheres.

III. RESULTS AND DISCUSSION

3.1 Size Distribution of PDMS Microspheres

The particle size and surface morphology of PDMS microspheres fabricated using the two methods described above were evaluated by bright-field and SEM micrographs, respectively. Fig. 1A shows representative bright-field micrographs of the dry product retrieval of PDMS microspheres fabricated by precipitation and sonication at 30°C. This revealed that both stirring and ultrasonic wave radiation are versatile, simple, and effective methods to generate PDMS microspheres. In particular, the formation of PDMS microspheres under ultrasonic wave radiation has also been investigated by Rankin *et al*.

[14] and O'Donnell and McGinity [15]. The micrographs indicate that the microspheres show a wide particle size distribution and less agglomeration, similar to those generated by a co-flowing method [16]. With the two methods, we found that the smallest PDMS microspheres are roughly 0.8 μm, proving that smaller sized microspheres were not favored. This could be due to the high viscosity and high surface energy of PDMS oligomers in water. High viscosity would tend to hold the particles together and is connected with the internal volume free energy of the particle. Thus, the high viscosity of PDMS implies that there is a large amount of overall binding interaction between the oligomer strands. This is probably driven by entropy considerations related to chain entanglement rather than the Van der Walls forces that are dominant in such non-polar systems. A viscous particle is harder to mechanically shear and subdivide. The surface energy of the particles is expected to be high for a hydrophobic sphere in an aqueous medium. Hence surface energy considerations would also tend to drive the particle size higher. The counter to these energetic considerations are the shear forces exerted by the stirring and the acoustic waves. In other words, energy has to be provided to overcome the coalescence to form larger spheres.

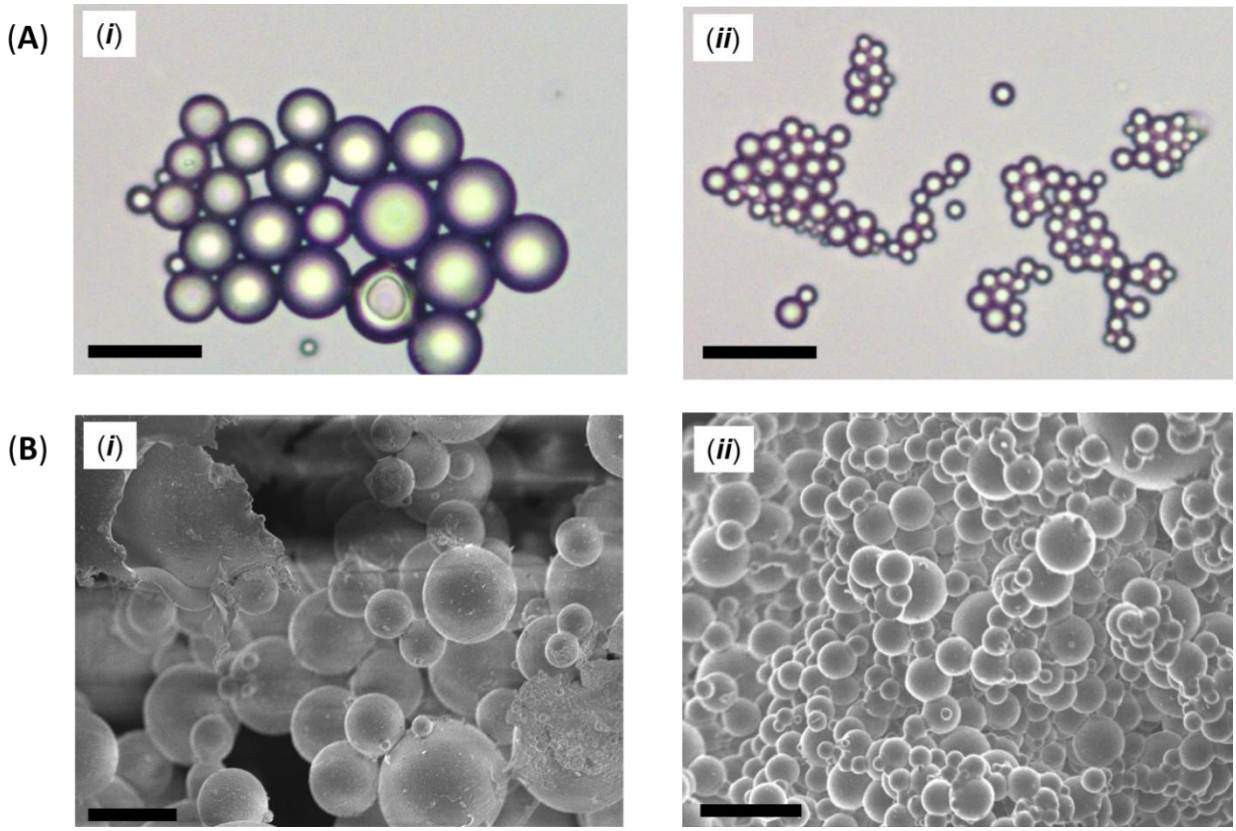

FIGURE 1. (A) The bright-field images and (B) SEM images of the dry product retrieval of PDMS microparticles fabricated by (*i*) stirring-shear and (*ii*) sonication method. Scale bars represent 10 μm.

The surface morphology evidenced by SEM micrographs of the PDMS microspheres produced by stirring shear and sonication methods are shown in Fig. 1B. The SEM micrographs of the PDMS microspheres indeed revealed that the particles were ultra-smooth and free of surface defects, although the microspheres were agglomerated with a very broad particle size distribution. The agglomeration is expected in the preparation of the dry product for microscopy analysis. It is noteworthy that, in this work, with the ratio between the elastomer base and curing agent being 10:1, the surface of the PDMS microspheres should have no significant reactive handles related to an excess of the vinyl functionalized polymers [13]. Therefore, surface impacts and imperfections should be suppressed during the microsphere formation.

By evaluating at least twenty bright-field micrographs with more than 500 microspheres, we quantified the size distribution of the multiphase microspheres to be in the range of 0.8 to 20 μm. From Fig. 2(A), it is clearly seen that the particle size distribution of the PDMS microspheres produced by the stirring-shear method is unimodal with the peak of the distribution at 1.2 μm with coefficient of variant being 152%. The peak distribution is slightly narrower with the peak distribution at 2.2 μm with coefficient of variant being 118% when the PDMS microspheres were produced by sonication method. Considering that the average particle size generally decreases with the strength of mechanical force which provides the initial shearing force to break-up the spheres, these results suggest that the mechanical force of ultrasonic radiation is stronger than for the

mechanical stirring. We may note that the smallest size of the microspheres was comparable to those fabricated in the presence of surfactant, though the size distribution of the latter case was trimodal with the peaks at 1, 10, and 100 μm [13]. These findings indicate that the PDMS microspheres fabricated with the two methods has a multidispersion similar to those usually observed for polymer microspheres produced by agitation, potentiometric dispersion, or sonication [13]. The unimodal distribution of the PDMS microspheres with the maximum distribution at a few tens of μm [14,15] can be attributed to low possibility of the existence of reactive functional groups on the surface to form inter-particle covalent bonds, as discussed above [13].

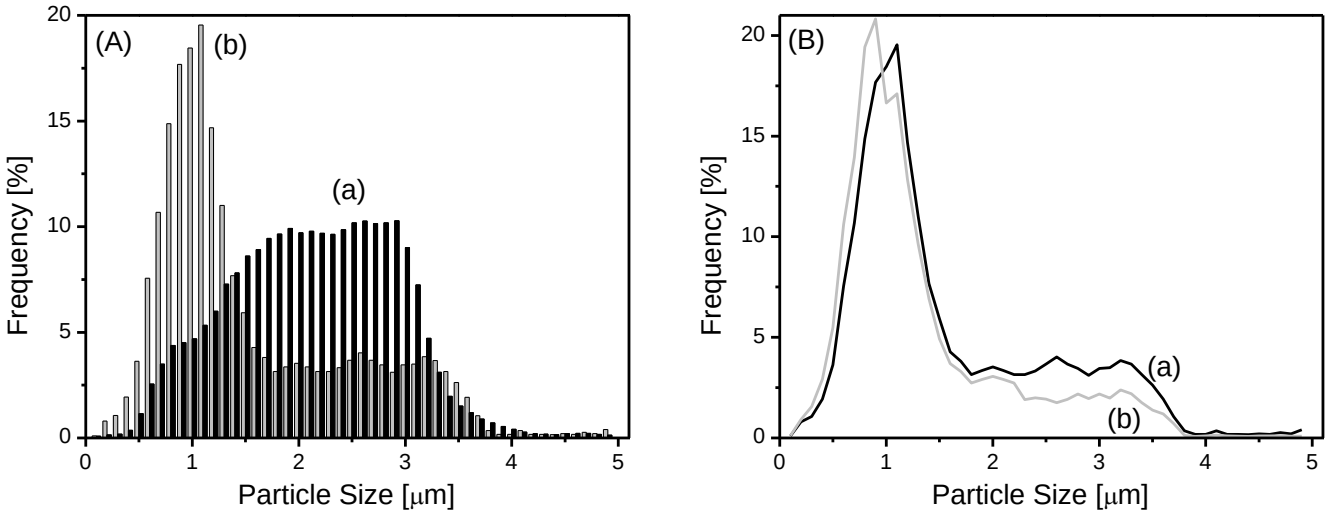

FIGURE 2. (A) The radius size distribution of PDMS microparticles fabricated by (a) stirring-shear and (b) sonication method, and (B) the representative size distributions of the PDMS microspheres fabricated using sonication method at different temperatures; (a) 30 °C and (b) 50 °C.

It is known that the PDMS takes a long time to cure, and hence aggregates with inter-sphere covalent bonds leading to larger size microspheres would probably be more favorable at low temperatures. Therefore, we systematically evaluated the effect of the water medium temperature. We found that the size distribution of the microspheres becomes much narrower and the peak shifts downward as temperature increases, as shown in Fig. 2(B). This suggests that the sizes of PDMS microspheres reduces with the curing temperature most probably due to an increase in the cross-linked PDMS networks resulting in less dangling surface chains at higher temperatures [17]. The size reduction of PDMS microspheres with curing temperature has also been observed by Lee and Lee [18]. The different cross-linked PDMS networks should be evidenced by defect content, equilibrium swelling, and dye encapsulation capacity. The cross-linking of PDMS is increasingly important with recent focus on surface stiffness of the PDMS microspheres [19]. The difference in crosslinking networks, however, is not reflected in the surface morphology of the PDMS microspheres, as SEM micrographs of the PDMS microspheres fabricated at different temperatures show similar surface morphology (Fig. 1B).

3.2 Dye Encapsulation

In order to prepare fluorescent PDMS microspheres, various ionic fluorescence dyes were incorporated in the microspheres with different cross-linking networks. Incorporation of the dyes depends strongly on the swelling of PDMS microspheres in organic solvents, and we have selected acetone as the organic solvent, since this organic solvent swells PDMS microspheres to a small extent and it is miscible with water [20]. After the solvent was removed, incorporation of the dyes into the PDMS microspheres was evaluated by fluorescence microscopy. In Fig. 3, we show the fluorescence and bright-field images of PDMS microspheres incorporating methylene blue, methyl violet, crystal violet, congo red, rhodamine B, and malachite green-incorporated PDMS microparticles under irradiation with light at 365 nm. The bright field and fluorescence images of bare PDMS microspheres are also presented in Fig. 3 for comparison. It is clearly seen that, in contrast to bare PDMS microspheres, those containing the dyes show obvious fluorescence. This provides an interpretation that the chemical structures of the fluorescent dyes as well as the cross-linked PDMS networks remain intact after encapsulation. It is also proposed that the dyes are incorporated in the polymer matrix, but they do not change the cross-linked PDMS networks in the microspheres. This is because we obtained the same size distribution for bare and dye incorporated PDMS microspheres. Such an unchanged PDMS network was also demonstrated by FTIR spectrum, as discussed below. Additionally, we can

suggest that the dyes are incorporated as monomers and not as aggregates. Aggregated dyes, specifically rhodamine dyes, are well known to have greatly diminished, even zero fluorescence yields. Clearly the dyes maintain their emission properties as if they are unaggregated.

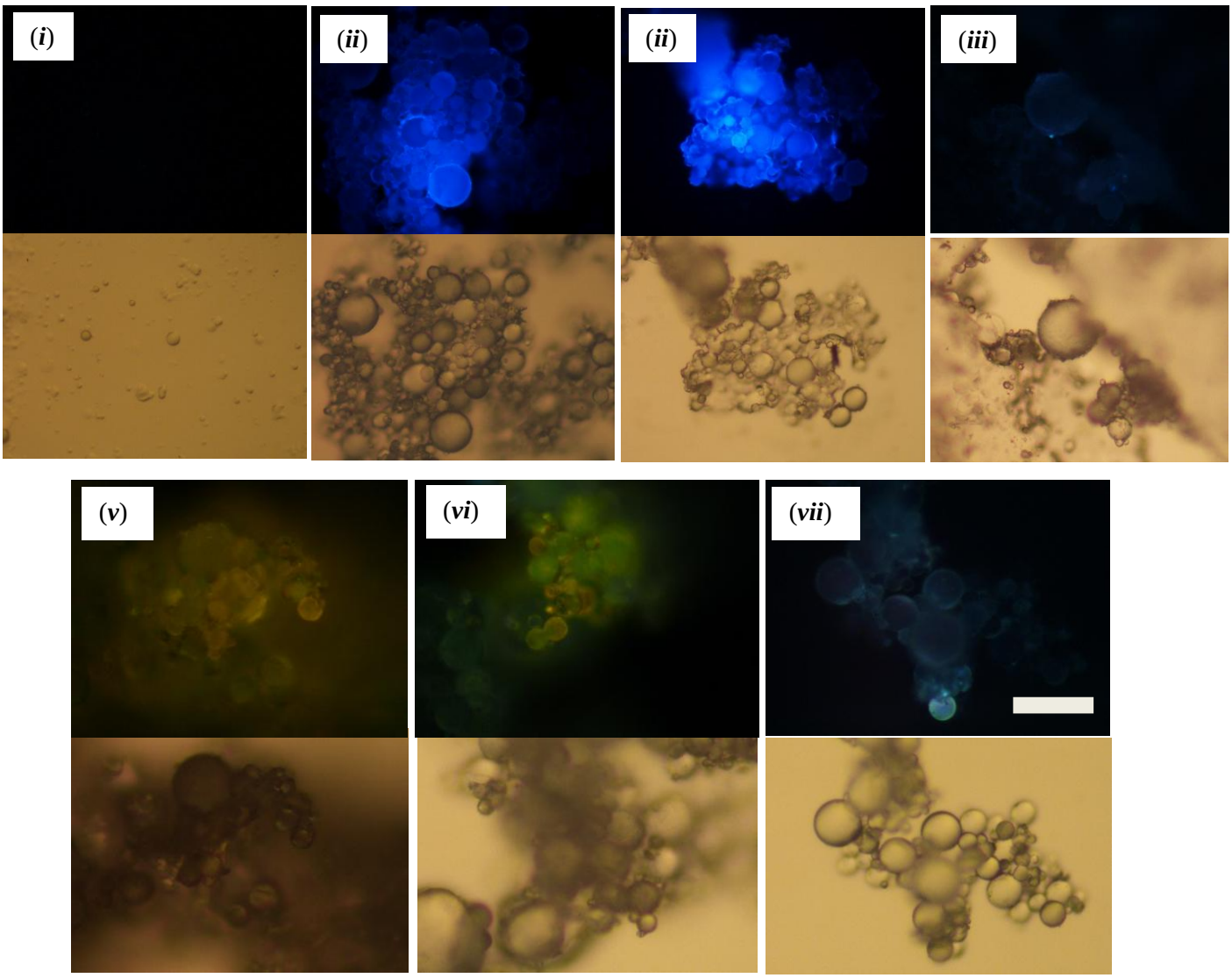

FIGURE 3. Fluorescence images of (*i*) bare PDMS microparticles, and (*ii*) methylene blue, (*iii*) methyl violet, (*iv*) crystal violet, (*v*) congo red, (*vi*) rhodamine B, and (*vii*) malachite green-incorporated PDMS microparticles. Their respective bright-field images are shown in the bottom raw. The white scale bar represents 10 μm.

In principle, the changes in cross-linked PDMS networks should incorporate different concentrations of dyes. However, the different cross-linked PDMS networks in the microspheres upon changing the curing temperature is very tiny, thus the different concentrations of dyes is not obviously observable. Nevertheless, based on the fluorescence microscopy the fluorescent dyes are incorporated in the microspheres during the swelling-deswelling process of PDMS microspheres in acetone. It is seen that acetone is a good solvent for swelling of the PDMS microspheres. Though the degree of swelling of bulk PDMS in non-polar solvent such as cyclohexane has been found to be around eight-fold [21], the swelling kinetics of the PDMS microspheres in different organic solvents are still an open question. With the swelling-deswelling process, we consider that the fluorescent dyes are randomly contained within the microspheres because in the solution the dyes are distributed randomly and the microspheres swell isotropically. A similar feature has been observed even for encapsulation of magnetic particles by PDMS spheres [22]. The fluorescence intensity remains unchanged when measured after two weeks, revealing the stability of the fluorescence stability of the PDMS microspheres, which can be attributed to stable formation of the dyes incorporated in the PDMS structure.

3.3 ATR-FTIR of Bare and Dye Incorporated PDMS Microspheres

We conducted FTIR analyses on bare and dye incorporated PDMS microspheres to evaluate whether there were significant changes in PDMS network upon dye encapsulation. This was done to confirm the above discussion on fluorescence. As displayed in Fig. 4(a), the FTIR spectrum of bare PDMS microspheres shows vibrational peaks at 1258 and 788 cm^{-1}, attributed to the deformation and rocking vibrations of CH_3 in $Si–CH_3$, and at 1065 and 1011 cm^{-1} due to Si–O–Si asymmetric deformation. In addition, the peaks observed at 2962 and 2906 $cm^{\wedge-1}$ in the spectrum are assigned to the symmetric and asymmetric stretching vibration of CH_3. The Si–H vibration of the cross-linking agent at 2156 cm^{-1} was not observed in the spectrum, indicating that the concentration of the curing agent in the PDMS microspheres with the ratio of silicone elastomer base and curing agent being 10:1 is not excessive [13], as this particular band would appear when either the curing agent is excessive or cross-linking of siloxane oligomer is not efficient. The peak positions of the PDMS were invariant with and without the dyes and every spectrum was identical within the resolution limit of the FTIR spectra (1–4 cm^{-1}). Furthermore there was no evidence of the dye peaks, indicating that the dye concentration was too low to register in the absorption measurement. This is not unexpected as it is well known that fluorescence is far more sensitive than absorption, since the former is dark-field and the latter is a bright-field technique.

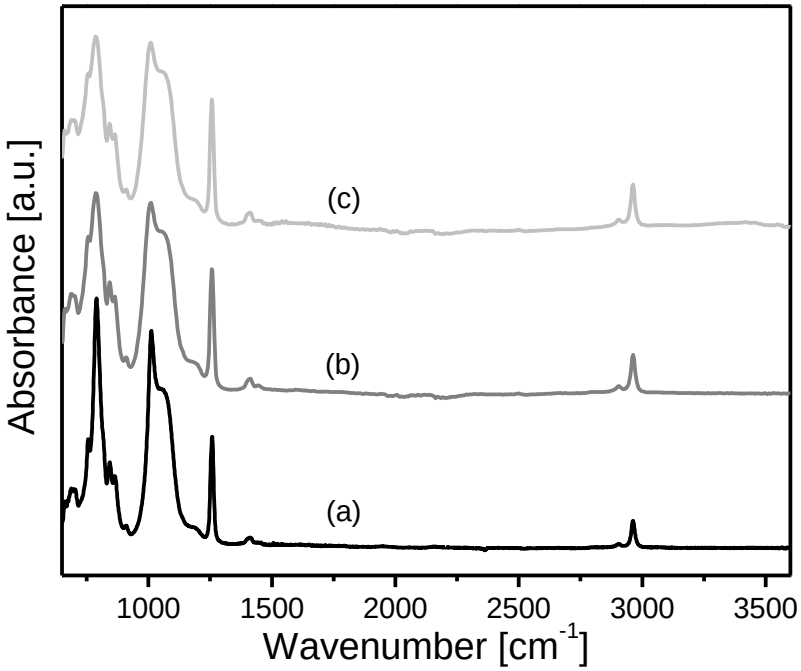

FIGURE 4. FTIR spectra of (a) bare PDMS microparticles, (b) methylene blue-incorporated PDMS microparticles, and (c) methyl violet-incorporated PDMS microparticles.

As shown in Fig. 4 (b), the dyes incorporated PDMS microspheres show similar spectral pattern to that of the bare ones, although there are slight modifications in the intensities of the peaks, which are attributable to differences in the resolution between the measurements. These changes were made to optimize the signal to noise levels. This confirms that the functional groups of PDMS microspheres remain intact upon dye incorporation, in contrast to significant changes observed after UV/ozone treatment which readily changes the chemistry of the PDMS [22-23]. Our ATR-FTIR results show that there are no new bands observable in the spectrum upon incorporation of the fluorescent dyes and no spectral shifts (see Fig. 4(c)). This implies that the dyes are weakly interacting with the PDMS matrix, or that there is simply not enough dye to be observed by any interaction effects on the matrix.

The SEM images also indicate that the dyes incorporated PDMS microspheres did not agglomerate forming their own distinct phase domains or crystal habits. Since the dyes incorporated PDMS microspheres have been washed with water, we should also consider that the dyes are encapsulated inside rather than on the surface of the microspheres. Therefore, we may expect that the cross-linking PDMS network acts as an inert matrix, instead of one in which there is chemical adsorption incorporating the dyes tightly and rigidly to the PDMS chains. This is expected because the ionic portions of the dyes should not strongly interact with the non-polar PDMS chains.

IV. CONCLUSION

In summary, we have demonstrated that PDMS microspheres can be fabricated simply by stirring induced shear and sonication methods using water as a medium. These simple methods are able to fabricate and to control the inter-sphere cross-linking of PDMS microspheres by adjusting the temperature of the water medium. We found that the particle size tends to be smaller and the size distribution is narrower with temperature, indicating that the crosslinking rate for PDMS networks in the hydrophobic microspheres is slightly increased. We also showed that the PDMS microspheres undergo swelling process, incorporating fluorescent dyes, upon replacing the medium from water to an organic solvent with dissolved dyes. Upon incorporation of the dyes, the cross-linked PDMS network in the microspheres remain intact. Thus, the PDMS microspheres can act as a matrix to encapsulate fluorescence dyes, making them fluorescent microspheres. Using scanning electron and fluorescence microscopy, we found that the fluorescent dyes are randomly distributed in the cross-linked PDMS networks and the dyes do not change either their own chemical structures or the crosslinking PDMS networks in the microspheres. This work provides and paves the way for utilization of PDMS microspheres as matrices for medicinal, surfactants, electron donor, or electron acceptor compounds for various applications.

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