Video Article

Fabrication of Polymer Microspheres for Optical Resonator and Laser Applications

Yohei Yamamoto[1,2,3], Daichi Okada[1], Soh Kushida[1], Zakarias Seba Ngara[1], Osamu Oki[1]

[1]Faculty of Pure and Applied Sciences, University of Tsukuba

[2]Tsukuba Research Center for Interdisciplinary Materials Science (TIMS), University of Tsukuba

[3]Center for Integrated Research in Fundamental Science and Technology (CiRfSE), University of Tsukuba

Correspondence to: Yohei Yamamoto at yamamoto@ims.tsukuba.ac.jp

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Abstract

This paper describes three methods of preparing fluorescent microspheres comprising π-conjugated or non-conjugated polymers: vapor diffusion, interface precipitation, and mini-emulsion. In all methods, well-defined, micrometer-sized spheres are obtained from a self-assembling process in solution. The vapor diffusion method can result in spheres with the highest sphericity and surface smoothness, yet the types of the polymers able to form these spheres are limited. On the other hand, in the mini-emulsion method, microspheres can be made from various types of polymers, even from highly crystalline polymers with coplanar, π-conjugated backbones. The photoluminescent (PL) properties from single isolated microspheres are unusual: the PL is confined inside the spheres, propagates at the circumference of the spheres via the total internal reflection at the polymer/air interface, and self-interferes to show sharp and periodic resonant PL lines. These resonating modes are so-called "whispering gallery modes" (WGMs). This work demonstrates how to measure WGM PL from single isolated spheres using the micro-photoluminescence (μ-PL) technique. In this technique, a focused laser beam irradiates a single microsphere, and the luminescence is detected by a spectrometer. A micromanipulation technique is then used to connect the microspheres one by one and to demonstrate the intersphere PL propagation and color conversion from coupled microspheres upon excitation at the perimeter of one sphere and detection of PL from the other microsphere. These techniques, μ-PL and micromanipulation, are useful for experiments on micro-optic application using polymer materials.

Video Link

The video component of this article can be found at https://www.jove.com/video/55934/

Introduction

Polymer nano/micro-sized particles are widely used for a variety of applications, including as catalyst support, column chromatography fillers, drug delivery agents, fluorescent probes for cell tracking, optical media, and so forth[1,2,3,4,5,6,7,8,9]. In particular, π-conjugated polymers have inherent luminescent and charge conducting properties that are beneficial to optical, electronic, and optoelectronic applications using polymer spheres[10,11,12,13,14], especially laser applications using soft organic materials[15,16,17]. For example, the three-dimensional integration of spheres with several hundred nanometer diameters forms colloidal crystals, which show photonic band gaps at a certain wavelength[18,19]. When light is confined in the intersphere periodic structure, lasing action appears at the middle of the stop band. On the other hand, when the size of the spheres increases to the several-micrometer scale, light is confined inside a single microsphere via total internal reflection at the polymer/air interface[20]. Propagation of the light wave at the maximum circumference results in interference, leading to the appearance of a resonant mode with sharp and periodic emission lines. These optical modes are so-called "whispering gallery modes" (WGMs). The term "whispering gallery" originated from St. Paul's Cathedral in London, where sound waves propagate along the circumference of the wall, allowing whispers to be heard by a person on the other side of the gallery. Because the wavelength of light is on the sub-micrometer scale, which is far smaller than sound waves, such a large dome is not necessary for the WGM of light: tiny, micrometer-scale, well-defined vessels, such as microspheres, microdiscs, and microcrystals, fulfill the WGM conditions.

Equation 1 is a simple form of the WGM resonating condition[21]:

$$n\pi d = l\lambda \quad (1)$$

where n is the refractive index of the resonator, d is the diameter, l is the integer number, and λ is the wavelength of the light. The left part of (1) is the optical path length through one circle propagation. When the optical path coincides with the integer multiple of the wavelength, resonance occurs, while at the other wavelength, the light wave is diminished upon rounding.

This paper introduces several experimental methods to prepare microspheres for WGM resonators from conjugated polymers in solution: vapor diffusion[22,23,24,25,26,27,28,29,30], mini-emulsion[31], and interface precipitation[32]. Each method has unique characteristics; for example, the vapor

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diffusion method affords well-defined microspheres with very high sphericity and smooth surfaces, but only low-crystallinity polymers can form these microspheres. On the other hand, for the mini-emulsion method, various kinds of conjugated polymers, including high-crystalline polymers, can form spheres, but the surface morphology is inferior to that obtained from the vapor diffusion method. The interface precipitation method is preferable for creating microspheres from dye-doped, non-conjugated polymers. In all cases, the selection of the solvent and the non-solvent plays an important role in the formation of spherical morphology.

In the second half of this paper, μ-PL and micro-manipulation techniques are presented. For the μ-PL technique, microspheres are dispersed on a substrate, and a focused laser beam, through a microscope lens, is used to irradiate a single isolated microsphere[24]. The generated PL from a sphere is detected by a spectrometer through the microscope lens. Moving the sample stage can vary the position of the excitation spot. The detection point is also variable by tilting the collimator optics of the excitation laser beam with respect to the optical axis of the detection path[28,32]. To investigate intersphere light propagation and wavelength conversion, the micro-manipulation technique can be used[32]. To connect several microspheres with different optical properties, it is possible to pick up one sphere using a micro-needle and put it on another sphere. In conjunction with the micromanipulation techniques and the μ-PL method, various optical measurements can be carried out using conjugated polymer spheres, which are prepared by a simple self-assembly method. This video paper will be useful to readers who wish to use soft polymer materials for optical applications.

Protocol

1. Fabrication Protocols of Polymer Microspheres

1. **Vapor Diffusion Method**
    1. Dissolve 2 mg of conjugated polymers, such as P1 (poly[(9,9-dioctylfluorene-2,7-diyl)-*alt*-(5-octylthieno[3,4-*c*]pyrrole-4,6-dione-1,3-diyl)])[28] and P2 (poly[(N-(2-heptylundecyl)carbazole-2,7-diyl)-*alt*-(4,8-bis[(dodecyl)carbonyl]benzo[1,2-*b*:4,5-*b'*]dithiophene-2,6-diyl)])[28], in 2 mL of chloroform (a good solvent) in a 5-mL vial.
    2. Put 5 mL of methanol (a poor solvent) in a 50 mL vial.
    3. Put the 5 mL vial containing the chloroform solution of the polymer into the 50 mL vial containing methanol.
    4. Cap the 50 mL vial and keep it for 3 days at 25 °C to allow for the precipitation of the polymer microspheres.

2. **Mini-Emulsion Method**
    1. Dissolve 5 mg of conjugated polymers, such as poly[9,9-di-n-octylfluorenyl-2,7-diyl] (PFO) and poly[2-methoxy-5-(3',7'-dimethyloctyloxy)-1,4-phenylenevinylene] (MDMOPPV), in 1 mL of chloroform.
    2. Dissolve 30 mg (~50 mM) of sodium dodecyl sulfate (SDS) in 2 mL of deionized water.
    3. Add 100 μL of the chloroform solution of the polymer to 2 mL of water containing SDS.
    4. Stir the chloroform/water mixture vigorously using an ultra-high-speed homogenizer at 30,000 rpm for 2 min to emulsify the solution.
    5. Keep it for 1 day without capping the vial to evaporate the chloroform.
    6. Centrifuge the dispersion in a 1.5 mL microcentrifuge tube for 5 min at 2,200 x g. Remove the supernatant aqueous solution containing SDS.
    7. Add 2 mL of deionized water and shake vigorously.
    8. Repeat step 1.2.6 and 1.2.7 thrice to wash out the residual SDS.

3. **Interface Precipitation Method**
4. Dissolve 200 μg of polystyrene (PS) and 10 μg of fluorescent dye (boron dipyrrin, BODIPY) to 0.2 mL of tetrahydrofuran (THF).
5. Gently pour the THF solution onto 1 mL of the water layer.
6. Keep the two-layer separated THF/water for 6 h without capping the vial to allow for the precipitation of the polymer microspheres.

2. Micro-photoluminescence (μ-PL) Measurement

1. **Sample Preparation**
    1. Dilute a suspension of the microspheres prepared in section 1 in a non-solvent (*i.e.,* methanol or deionized water).
    2. Spin-cast one drop (20-30 μL) of the diluted suspension of the microspheres onto a quartz substrate using a spin-coater (typically, 2,000 rpm for 50 s).
    3. Air-dry the resultant casted film until the solvents have evaporated completely (~5 min).

2. **Experimental Setup**
    1. Put the quartz substrate (15 x 15 x 0.5 mm^3) on the sample stage of an optical microscope.
    2. Find well-defined microspheres that are isolated from other spheres and appropriate for the μ-PL measurement.
    3. Select a laser (*i.e.,* wavelength, continuous wave or pulse, irradiation time, integration, *etc.*).
    4. Select the magnification of the lens.

3. **Measurements**
    1. Use a focused laser beam to irradiate the microsphere. Use the following laser condition: cw or pulsed laser with excitation wavelengths (λ_{ex}) of 405 nm (cw), 450 nm (cw), 355 nm (pulse laser; frequency, 1 kHz; pulse duration, 7 ns), and 470 nm (pulse laser; frequency, 2.5 MHz; pulse duration, 70 ps).
    2. Record the PL spectrum at the excited spot using a spectrometer with a grating of 300 or 1,200 grooves mm^{-1}.
    3. Take a fluorescent image.
    4. Change the excitation spot by moving the sample stage.
    5. Change the detection spot by tilting the collimator (if necessary).

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3. Micromanipulation Technique

1. **Manipulation of Microspheres**
    1. Set a quartz substrate upon which the microspheres are immobilized on the sample stage of an optical microscope.
    2. Find a well-defined microsphere appropriate for the μ-PL measurement.
    3. Set a plastic micro-needle on a micro-manipulation apparatus.
    4. Move the micro-needle using a computer-controlled joystick to pick up a microsphere.
    5. Move the microsphere and connect it to another microsphere.
    6. Measure the μ-PL from the connected microsphere.

Representative Results

Figure 1 shows schematic representations of the vapor diffusion method (a), mini-emulsion method (b), and interface precipitation method (c). For the vapor diffusion method (**Figure 1a**), a 5 mL vial containing a $CHCl_3$ solution of polymers (0.5 mg mL^{-1}, 2 mL) was placed in a 50 mL vial containing 5 mL of a non-solvent, such as MeOH. The outside vial was capped and then allowed to stand for 3 days at 25 °C. The vapor of the non-solvent slowly diffused into the solution, resulting in the precipitation of the polymers through the supersaturated state. For the mini-emulsion method (**Figure 1b**), a $CHCl_3$ solution of polymers (5 mg mL^{-1}, 200 μL) was added to an aqueous solution of sodium *n*-dodecyl sulfate (SDS, 1 mM, 2 mL). The water/$CHCl_3$ two-phase-separated solution was emulsified by vigorously stirring with a homogenizer (30,000 rpm, 5 min). The resultant emulsion was allowed to stand for 24 h at 25 °C and 1 atm to naturally evaporate the $CHCl_3$. The excess SDS was removed by exchanging the supernatant water through centrifugation (3 times) to obtain a precipitate of conjugated polymers. For the interface precipitation method (**Figure 1c**), a THF solution of a mixture of polystyrene (PS, [PS] = 1.0 mg mL^{-1}) and fluorescent dye ([dye] = 0.002-1.0 mg mL^{-1} = 6.4-3,200 μM) was carefully added to the non-solvent layer of a water/EtOH mixture (6/1 v/v, 1 mL). The slow diffusion of the solvents, along with the simultaneous evaporation of THF to air, resulted in precipitation after 6 h of aging.

Scanning electron microscopy (SEM) micrographs of the resultant microspheres prepared by each method are displayed in **Figure 1**. For the vapor diffusion and interface precipitation methods, well-defined microspheres with high sphericity and smooth surfaces were obtained. On the other hand, for the mini-emulsion method, well-defined microspheres were obtained, but the surface morphology was not so smooth in comparison to those produced by the other methods. This was because the surfactant covers the whole surface of the microspheres. However, the merit of the mini-emulsion method is that microspheres can be made from various kinds of conjugated polymers. This is quite advantageous because, with the vapor diffusion method, polymers with high crystallinity hardly form spherical geometry. In the interface precipitation method, water is often used as a non-solvent on the bottom layer. However, π-conjugated polymers are usually very hydrophobic, so heavy agglomeration of the resultant microspheres occurs. This is disadvantageous for isolating each single microsphere on a substrate for μ-PL measurements.

Figure 2 shows a schematic representation of the μ-PL experimental setup. An optical microscope with a 50X or 100X objective was used to identify suitable particles and to determine their diameters (*d*). For measurements, a μ-PL system was used with a microscope combined with a monochromator (grating: 300 or 1,200 grooves mm^{-1}) and a CCD camera. The perimeter of a single microsphere was photoexcited at 25 °C under ambient conditions by a cw or pulsed laser with an excitation wavelength (λ_{ex}) of 405 nm (cw), 532 nm (cw), 355 nm (pulse laser; frequency, 1 kHz; pulse duration, 7 ns), or 470 nm (pulse laser; frequency, 2.5 MHz; pulse duration, 70 ps).

For μ-PL measurements with different excitation and detection positions, the spheres were excited by a 405-nm laser, and the light was collected in a confocal setup by a 50X objective and detected by a spectrometer with a 300-grooves mm^{-1} grating. Spot size, laser power, and integration time were 0.5 μm, 0.5 μW, and 1 s, respectively. To separate the detection spot from the excitation, the collimator optics of the excitation laser beam was tilted with respect to the optical axis of the detection path.

Figure 3 displays the WGM PL of a single microsphere of π-conjugated polymers: **P1**$_{16k}$, **P2**, and their blends.[28] Clear WGM PL spectra were observed from all the single microspheres. The Q-factor, defined by a peak wavelength divided by the half-width of the peak, reached as high as 2,200 for microspheres of **P1**$_{16k}$, whereas microspheres of **P2** showed a Q-factor of only 300, possibly because of the rough surface morphology[28]. For the polymer blend microspheres, efficient intrasphere energy transfer occurred, resulting in a significant shift of the WGM PL from a yellow- to a red-colored region. A high Q-factor (1,500) was maintained due to the smooth surface.

Furthermore, the intersphere energy transfer cascade was investigated by a combination of micro-manipulation and μ-PL techniques. Thus, polymorphic boron-dipyrrin (BODIPY) dye-doped PS microspheres with PL colors of green, yellow, orange, and red were connected one by one to form tetraspheres with linear and T-shaped configurations (**Figure 4**)[32]. Detailed analysis of the energy transfer efficiency indicated that the light energy transfer from green to yellow and from yellow to orange took place efficiently, while the energy transfer from orange to red hardly occurred because of the small overlap between the PL band of the energy donor and the absorption band of the energy acceptor. Needless to say, the up-converted energy transfers, such as from red to orange, yellow, and green, hardly occurred.

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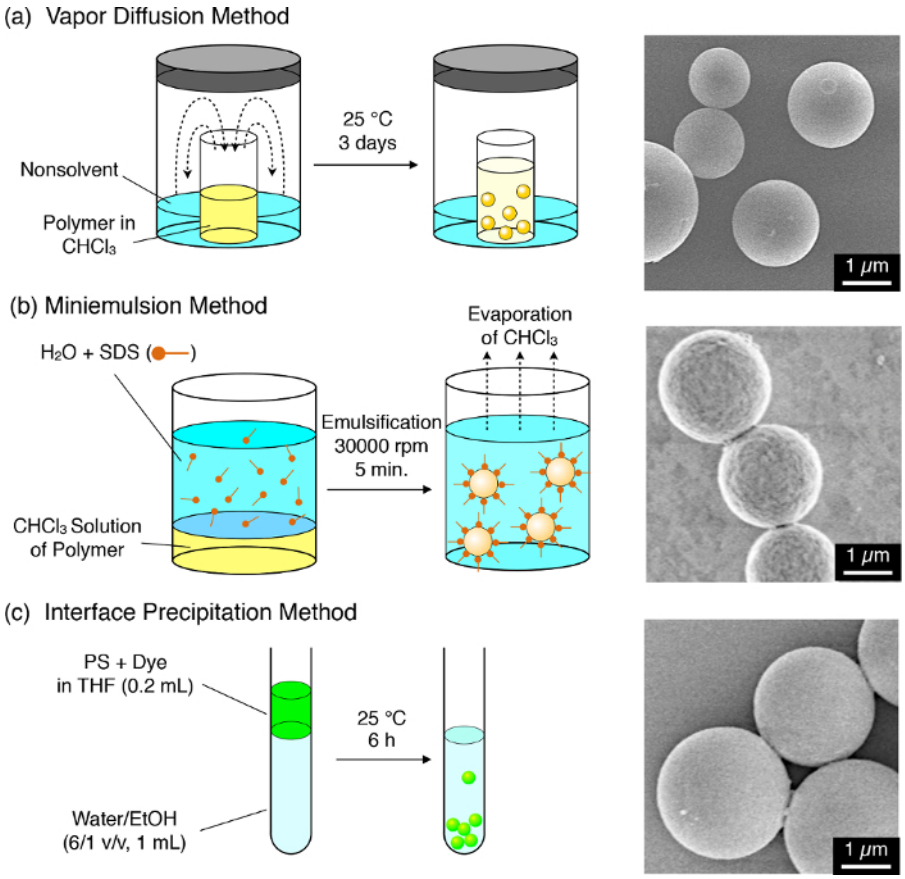

Figure 1: Preparation method of polymer microspheres. Schematic representations of the vapor diffusion method (**a**), mini-emulsion method (**b**), and interface precipitation method (**c**) and SEM micrographs of the resultant polymer microspheres from each preparation method. Please click here to view a larger version of this figure.

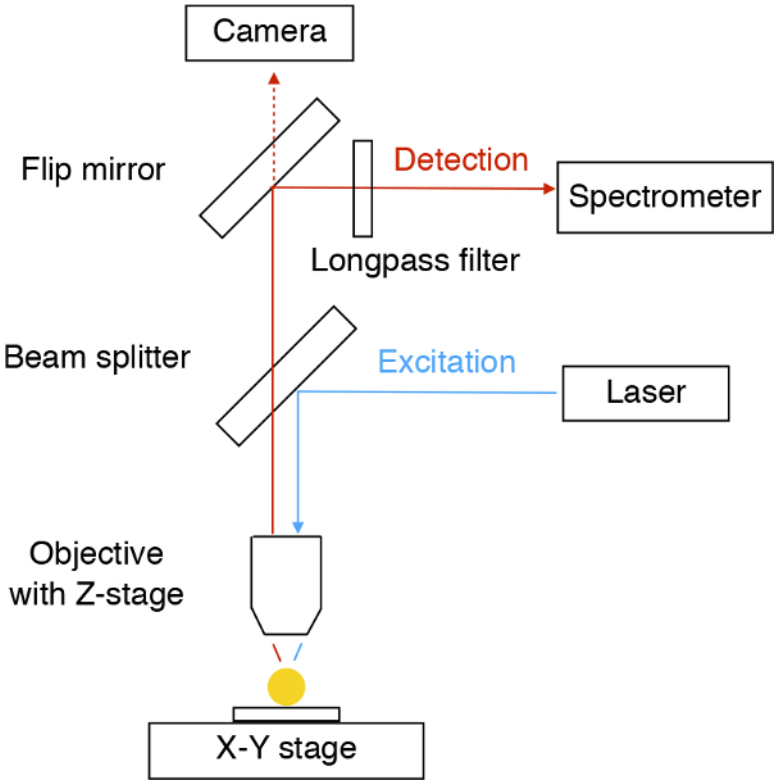

Figure 2: Schematic representation of the experimental setup for μ-PL measurements. Please click here to view a larger version of this figure.

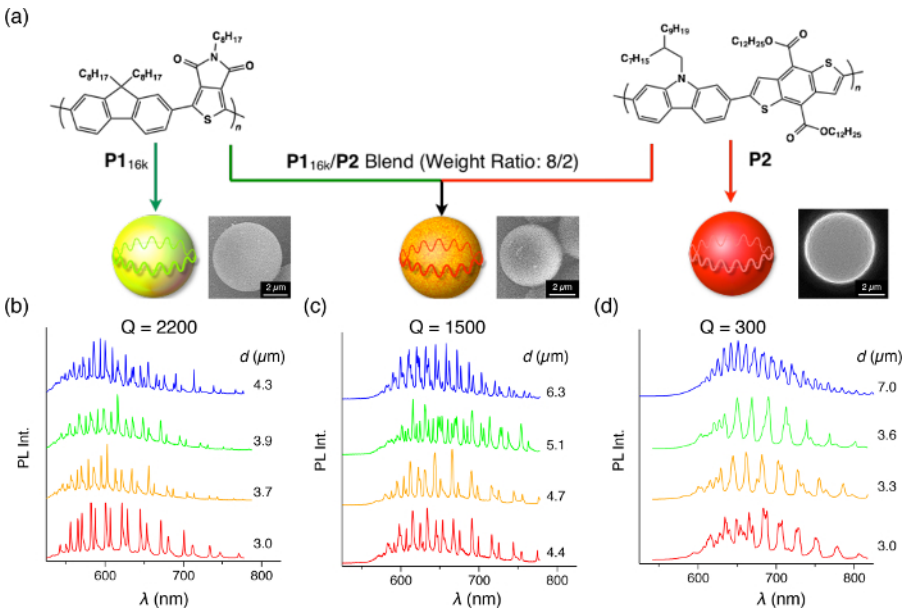

Figure 3: PL spectra from a single microsphere. (**a**) Molecular structures of **P1**$_{16k}$ and **P2** and schematic representations of the self-assembled microspheres from **P1**$_{16k}$, **P2**, and their blend (**P1**$_{16k}$/**P2** = 8/2 w/w), along with their SEM micrographs. (**b**-**d**) PL spectra from a single microsphere formed from **P1**$_{16k}$ (**b**), **P1**$_{16k}$/**P2** blend (**c**), and **P2** (**d**). Please click here to view a larger version of this figure.

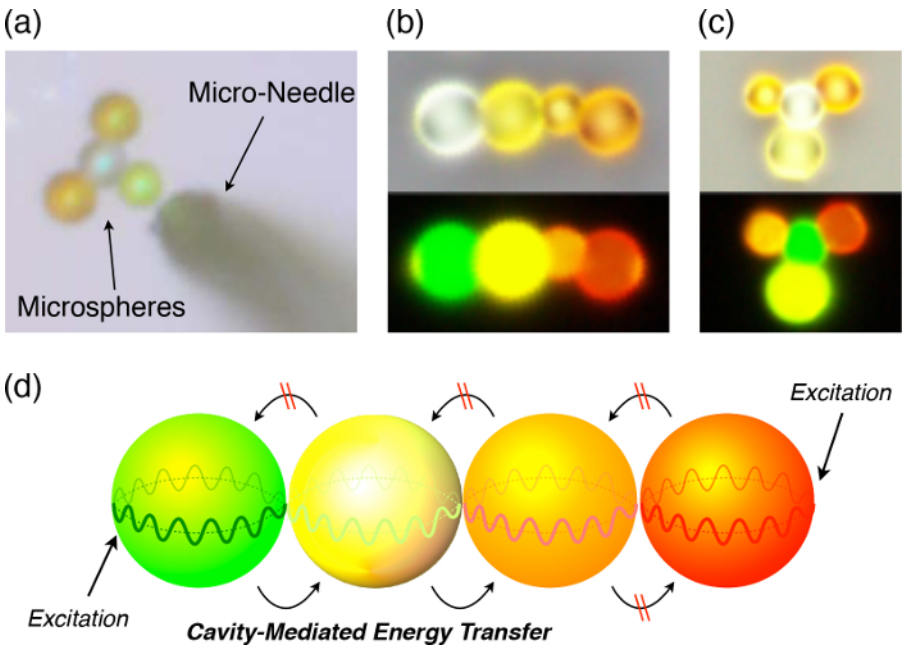

Figure 4: Micromanipulation technique to arrange the microspheres. (**a**) Optical micrograph of polymorphic BODIPY-doped PS microspheres, manipulated by a thin micro-needle. (**b and c**) Optical (top) and fluorescent (bottom) micrographs of the connected microspheres with linear (**b**) and T-shaped (**c**) configurations. (**d**) Schematic representation of cavity-mediated, long-range intersphere energy transfer. Please click here to view a larger version of this figure.

Discussion

The selection of a good solvent and non-solvent is very important for the self-assembly of well-defined microspheres. If the solubility of a polymer is too high, precipitation will not occur. Also, in general, π-conjugated polymers are hydrophobic, so polar non-solvents, such as MeOH, acetonitrile, and acetone, are often used in the vapor diffusion method to minimize the surface energy required to form a spherical shape. The interface precipitation method is often adopted for the preparation of dye-doped polymer microspheres. Non-conjugated polymers, such as polystyrene, poly(methylmethacrylate), and poly(dimethylsiloxane), are often used as the sphere medium. These polymers are less hydrophobic than typical π-conjugated polymers, so heavy agglomeration does not occur after precipitation in water. If microspheres are not obtained using these methods, other methods, such as post-polymerization dispersion and emulsion polymerization, can be adopted[33].

The crystallinity of polymers is the important factor. Generally, π-conjugated polymers have a flat, π-electronic plane. Thus, π-stacking results in the crystallization of the polymers, which prevents the isotropic growth of spheres. Hence, polymers with highly twisted π-electronic planes are favorable for the formation of well-defined microspheres, which prevents interchain π-stacking and amorphous aggregation. To prepare microspheres from polymers with high crystallinity, the mini-emulsion method is appropriate. However, for this method, the surface morphology of the resultant spheres is not very smooth because of the coverage of the surfactant.

The preferred method of observing WGM PL is to use a focused laser beam to irradiate the rim of a microsphere. The contact point with the substrate causes a defect in the microsphere where the confined light leaks. Therefore, when a focused laser is used to irradiate a microsphere, all the confined light passes through the contact point with the substrate. On the other hand, when the rim of the microsphere is excited, most routes of the confined light do not pass through the contact point with the substrate, leading to better confinement. Thus, well-defined, high-Q WGM PL lines appear.

To conduct WGM lasing from conjugated polymer microspheres, several conditions are required: [1] For the population inversion, high-density optical pumping is needed by using, for example, a femto-second laser pulse. [2] Polymers must hardly be photodamaged upon strong photoexcitation. [3] Highly dispersed microspheres with smooth surfaces are preferred. If these conditions are satisfied, WGM lasing properties can be observed from conjugated polymer microsphere resonators. Fluorescent dye-doped polymer microspheres are also applicable to chemical and biological sensing tools and can be used for laser and optical circuit applications[34,35].

Disclosures

The authors declare no competing financial interests.

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