

230 fold fluorescence enhancement by 3D Rhodamine B coated polystyrene photonic crystals

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ABSTRACT

We report a 230-fold fluorescence enhancement with fluorescent polystyrene based three-dimensional colloidal photonic crystals that can be readily fabricated over large areas. A simple and reproducible method was developed to synthesise novel fluorescent polystyrene spheres. Fluorescent polystyrene spheres were prepared through swelling-diffusion approach by the addition of a Rhodamine B as a fluorescent dye in dimethylformamide to an aqueous suspension of 310 nm mono-dispersed polystyrene spheres.

Keywords: colloidal particles, fluorescence, colloidal crystals

1 INTRODUCTION

Photonic crystals have been shown to have a significant effect on fluorescence [1-3]. Self-assembled colloidal photonic crystals (CPhCs) from monodisperse colloidal particles, often polystyrene (PS) or silica nanospheres, offers an easily accessible and inexpensive platform for studying and device fabrication.[4, 5] CPhCs have been developed to improve the FL intensity in many FL based chemosensors and lower the detection limit due to their special light manipulation properties [6, 7].

In many CPhC based fluorescence enhancement studies, the way that how the luminescent agents are introduced into the structure and its effect on the resulting luminescence has not been systematic investigated. This may have also made the quantitative comparisons among different studies unattainable. On the other hand, for many applications associated with liquid phase, such as sensing and imaging, it is important to immobilize the fluorophores on the surface of the CPhC porous structures.

In this study, we report a swelling-diffusion approach for effectively immobilizing Rhodamine B (RhB) on PS colloids' surface. Moreover, we illustrate the differences in fluorescence enhancement (afforded by the CPhC structure) as a result of the employment of different dye incorporation methods.

2 MATERIALS AND METHODS

Potassium persulfate (KPS) Styrene, acrylic acid (AA), dimethyl formamide (DMF), acetone, ethanol and Rhodamine B which were all purchased from Sigma Aldrich, were analytical grade and used as received without future purification. Glass was used as the substrate because it acts as an optical window allowing photons of interest to go through to be absorbed within the 3D photonic crystals layer on which it is deposited.

2.1 Preparation of PS, P-R and PSRhB spheres

Polystyrene spheres (PS) with the size of 310 ± 5 nm was synthesized by an emulsifier free emulsion polymerization method via our previous method and earlier report [8] in this research. AA-functionalized colloidal suspension, containing a PS spheres and carboxyl group on the surface were first synthesised and then purified by several cycles of centrifugation [9]. RhB coated PS (PSRhB) spheres were obtained through diffusion and entrapment method by the addition of RhB in DMF to an aqueous suspension of 150 μ L of PS spheres followed by the removal of DMF. A volume of 300 μ L of RhB in DMF was added drop-wise to the PS suspension until the DMF content reached 30 vol.%. As a comparison, we also prepared RhB coated PS spheres without DMF treatment. The sample is denoted as P-R. In detail, PS suspension (5vol %) were first purified by several cycles of centrifugation. Then, 2 ml of washed 310 nm PS was mixed with 2 ml of RhB for 24 hr. Finally, P-R particles were washed and purified by several cycles of centrifugation to remove excess RhB.

2.2 Fabrication of PSRhB photonic crystals

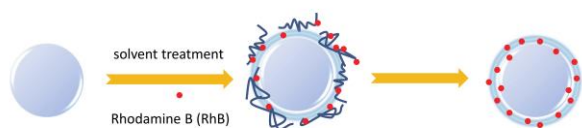
PS, PSRhB and P-R colloidal crystals were fabricated on the glass by horizontal deposition method [10]. The micro glass substrates (1.5 x 1.5 cm) were first treated with a Piranha solution to ensure super hydrophilic surfaces. As another comparison, 20 μ L of 1mM RhB ethanol solution was infiltrated into PS colloidal crystal. This sample is denoted as IPR.

2.3 Characterization

The surface morphology of PSCDs was examined by scanning electron microscope (SEM) using a JEOL 7001, operating at 15 kV. Transmission spectra were measured on Agilent 8453 UV-Vis spectrometer. FT-IR spectra were collected on a Perkin-Elmer Spectrum 100 with a resolution of 4 cm^{-1} in transmission mode. A baseline correction was applied after the measurement. Fluorescence emission spectra were recorded on a Thermo Scientific Lumina fluorescence spectrometer.

3 RESULTS AND DISCUSSION

Schematic 1 shows synthesis procedure of the PSRhB spheres. The immobilizing of RhB on the PS spheres is designed based on three main steps: firstly, swelling of the PS by DMF, secondly, diffusion and penetration of RhB and lastly, washing and removing excess DMF. The swelling agent is important for identifying the suitable swelling medium to create sufficiently big pores on sphere surface without destroying the spheres. It has been found that DMF is a suitable swelling agent for polystyrene colloids [11]. In the second step, by increasing the solubility of RhB in the DMF solution, RhB diffuses into the surface of PS. Finally, changing the solvent back to water caused deswelling of the PS spheres and ‘locking’ of RhB on the surface of PS spheres.



Schematic 1: Synthesis procedure of RhB coated polystyrene spheres (PSRhB).

Figure 1 shows the normalised FTIR spectrum of PSRhB, along with the PS, RhB and P-R as control sample for comparison. For the carboxyl-group functionalized PS, a broad shoulder between 3000 and 3700 cm^{-1} is attributed to the hydroxyl stretching vibrations of the $-\text{OH}$ stretching mode and the peaks at 1640 cm^{-1} are assigned to the $\text{C}=\text{O}$ stretching vibrations of typical carbonyl and carboxyl groups, which indicates the presence of $-\text{COOH}$ functional groups of PS nanoparticles [13]. Skeletal vibrations of 3300 cm^{-1} and 1640 cm^{-1} were both changed in intensity for PSRhB and P-R. These changes indicated that intermolecular interaction between RhB molecules and PS confirming that RhB was adsorbed on the PS surface. The successful immobilization of RhB on the PS was further supported by the appearance of a new peak at higher wave numbers of 1042 cm^{-1} for PSRhB and P-R compared to PS. The stronger intensity of this peak presented in PSRhB in comparison to P-R indicates that the amount of RhB incorporated on PS spheres is greater on PSRhB.

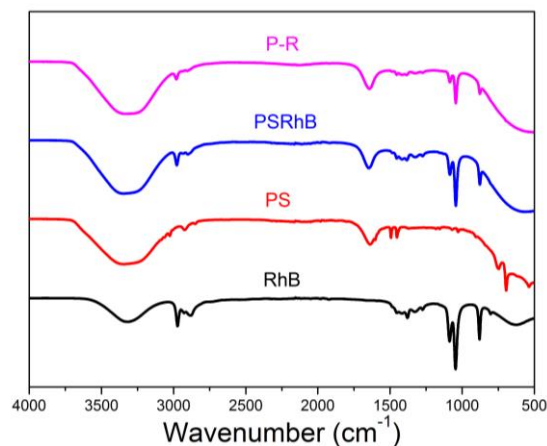


Figure 1: FTIR spectra of polystyrene spheres and Rhodamine B (RhB) as control, RhB coated on PS surface (PSRhB) and RhB coated on AA-functionalized PS spheres (P-R)

The application of fluorescence dye immobilized on colloidal particles can be limited, because the stability of fluorescence dye on PS surface may not be strong enough in different environment. To investigate how well the RhB are fixed on PS surface, we design the stability test for PSRhB and P-R samples. The PSRhB and P-R spheres were treated with ethanol, acetone and THF to demonstrate the difference in the stability of the coated RhB on the PS spheres. A series of 1ml of PSRhB and P-R were added to various concentrations of the 98 vol.% acetone, 98 vol.% ethanol and 50 vol.% THF. Solutions were mixed in ultrasonic for 5 min, followed by centrifuge for 15 min. Supernatant solutions were collected by centrifugation. The amount of RhB leached from the PS spheres in PSRhB and P-R was monitored qualitatively by fluorescence spectroscopy of the supernatant solution. Figure 2 shows the FL intensity of supernatant solution of PSRhB and P-R after treating with acetone, ethanol and THF.

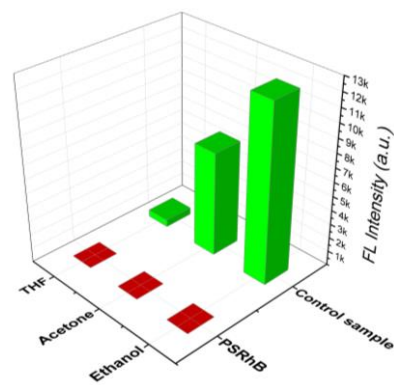


Figure 2: The fluorescence spectra of supernatant solutions of treated PSRhB and P-R by ethanol, acetone and THF

Treating the P-R with three steps solvent treatment resulted in pink-colored supernatants, indicating that some of the RhB dye leached out from the PS spheres. The fluorescence intensity of supernatant solution indicates that the leaching of RhB from PS spheres is significant in P-R. In the case of PSRhB, there is no fluorescence signal from supernatant solution after treating with solvents. The results confirm that the diffusion and penetration approach is very effective in immobilizing RhB molecules on PS surface. Figure 3 presents SEM image of PSRhB spheres and self-assembly of PSRhB spheres on glass, where large-area, high quality 3D colloidal crystal structures are revealed, showing the effectiveness of the simple horizontal method. PSRhB film exhibit face-centered cubic (FCC) crystal morphology with (1 1 1) planes oriented parallel to the surface of the substrate. The size selection of PS was based on the excitation and emission peak of RhB using Bragg's diffraction calculation [12].

$$\lambda_{\max} = 2d_{(111)}\sqrt{n_{\text{eff}}^2 - \sin^2 \theta} = 2\sqrt{\frac{2}{3}} \cdot D \cdot \sqrt{n_{\text{eff}}^2 - \sin^2 \theta} \quad (1)$$

Where λ_{\max} indicates the wavelength value of the reflection peak, $d_{(111)}$ the distance between adjacent (111) lattice planes, D the sphere diameter and n_{eff} is the effective refractive index of the structure. We therefore synthesized PS spheres of 310 nm for PSRhB to match the emission maxima of RhB, namely 610 nm.

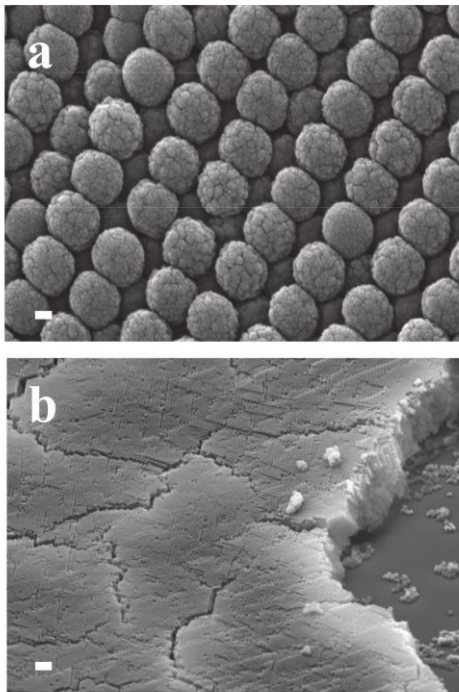


Figure 3: a) SEM images of cross section of 3D PSRhB structure, scale bar is 100 nm. b) Self-assembly of fluorescent polystyrene spheres, scale bar is 1 μm .

The fluorescence spectra of PSRhB, P-R, IPR colloidal crystals and control sample (20 μl of 1mM RhB infiltrated into the amorphous PS structure on glass slide) excited by 460 nm low power excitation light are displayed in Figure 4 for comparison. It is obvious that all the CPhC structures have augmented the fluorescence intensity of RhB considerably compared to the control. It is also apparent that the different ways of introducing RhB on PS has a considerable impact on the magnitude of enhancement.

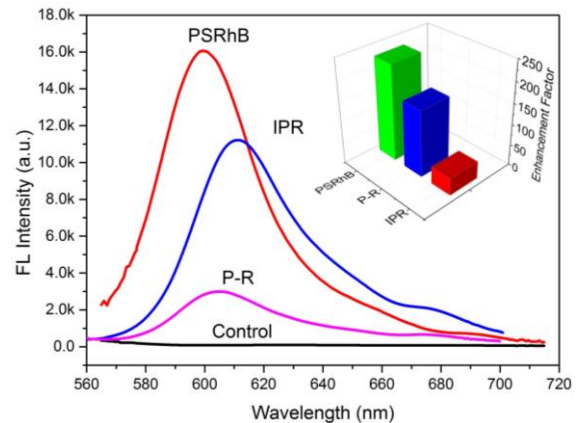


Figure 4: The fluorescence spectra of 3D PSRhB photonic crystals, RhB infiltrated into the 3D PS photonic crystal structure (IPR), RhB coated on AA-functionalized PS spheres (P-R) and RhB dye dropcast on amorphous PS film as a control sample. Inset: enhancement factor for PSRhB, P-R and IPR.

There is enormous enhancement of 230-fold at maximum emission wavelength in 3D PSRhB colloidal crystal compared with IPR of 160-fold and P-R of 42-fold. The difference in enhancement magnitude between PSRhB and P-R can be attributed to the greater amount of RhB molecules are immobilized on PS surface in PSRhB. Infiltration of dyes in colloidal crystals is the most commonly used method in the field, here the results are showing that by uniformly incorporating the dyes on the building blocks' surface of the colloidal photonic crystals, better fluorescence enhancement effect can be obtained. Particularly, with the perspective of further device development, such a dye immobilization method is very useful; it achieves not only the immobilization, but also a better FL enhancement performance.

4 CONCLUSIONS

In summary, we demonstrated that the swelling-diffusion method is an effective approach to incorporate RhB on the surface of PS colloids. The stability test by

washing with various solvents has further proved the firm immobilization owing to the strong physical binding. We further illustrated that the different ways of introducing RhB into colloidal crystal structure can result in significant difference in fluorescence enhancement magnitude. With the perspective of further device development, the swelling-diffusion method is very useful; it achieves not only a firm dye immobilization, but also a better FL enhancement performance.

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