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Realization of Fluorescence Color Tuning for Poly(p-phenylenevinylene) Coated Microspheres via a Heterogeneous Catalytic Thermal Elimination Process

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Poly(p-phenylenevinylene) (PPV) fluorescent microspheres were prepared in two steps. First, coating the positive sulfonium-salt PPV precursor (pre-PPV) onto the surface of negatively charged polymer substrate spheres; second, the pre-PPV was converted into fluorescent PPV via a heterogeneous catalytic thermal elimination process. A series of fluorescent microspheres were obtained with different apparent colors and different fluorescent emissions simply by varying the elimination temperature. The spectroscopic study showed that, comparing to the direct solid elimination, the spheres obtained via the catalyzed elimination gave much rich variation in the emission, such as larger shifting in the wavelength, more delicate spectra profiles with peaks and shoulders. Overlapping of the signals from blue channels and green channels in the confocal microscopic study, gave a direct view of the gradual changing from the blue emission to the green emission for the spheres obtained under the elimination temperature from 40 ^{o}C to 120 ^{o}C. Flow cytometry measurement found that spheres obtained from different temperatures had different combination of the intensities from four different receiving channels. These PPV spheres were also demonstrated to have smooth surface, monodispersity and clear core-shell structure, thermal stability and photostability.

Introduction

The bead-based sensing techniques have been widely used in many fields, such as pollutant detecting, disease diagnose and so on.[1-9] The beads should have detectable and differential signals and be monodispersed in micrometer size to fit for the currently employed instruments, such as flow cytometry. Fluorescent microspheres, as one type of these beads with fluorescence wavelength and intensity as the read-out signals, are greatly demanded in both academic and industrial applications.[10-18] Besides the photostability, mechanical/thermal stability and processability, conjugated polymers also have distinctive advantages comparing to the other fluorophores, with respect to the controllability/tunability in the emission due to the easy structural tuning.[19-27] Our group has prepared monodispersed microspheres by using poly(p-phenylenevinylene) (PPV)[28] or polydiacetylene (PDA)[29] as the fluorophores to be loaded onto monodispersed polymer substrate spheres. However, the emission wavelength tuning has not been studied in these systems.

Here we present a facile method to tune the emission wavelength and intensity of fluorescent PPV coated microspheres (Scheme 1). The positive sulfonium-salt polymer precursor for PPV (pre-PPV) was adsorbed onto the polystyrene-divinylbenzene spheres with negative sulfonic groups on the surface (SPSDVB). Following is the thermal elimination to give the fluorescent SPSDVB-PPV spheres. Different from our previous report with a thermal elimination in a solid phase,[28] the fluorescent microspheres were finally obtained by converting the PPV precursor to PPV via a heterogeneous catalytic thermal elimination process,[30] with spheres dispersed in organic phase. The elimination process employed here is expected to have several advantages over the previous thermal elimination in solid phase (see ESI for the mechanisms, Fig.S1).[30-36] First, the thermal elimination can be carried out at lower temperature since the presence of catalyst facilitates the reaction. Second, the elimination should be more evenly distributed among all the spheres, and whole process should be smoother than that takes place in a complete solid environment, since the reaction heat can easily dissipate into solvent and the spheres can rotate during the process when suspending in solvent. In all, the whole elimination process can be more controllable, and the delicate tuning of emission color can be achieved by varying the elimination temperature.

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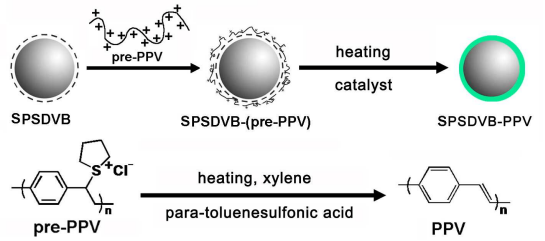
† Electronic Supplementary Information (ESI) available: Elimination emchanisms, digital photographs and emission spectra of the spheres with different elimination time while fixing the temperature, stability study and Laser scanning confocal microscopy study. See DOI: 10.1039/b000000x/



Scheme 1 The schematic diagram for the adsorption of pre-PPV to the negatively charged SPSDVB spheres followed by heterogeneous catalytic thermal elimination to give fluorescent SPSDVB-PPV(top); The reaction scheme for the thermal elimination(bottom).

The Laser scanning confocal microscopy (LSCM) and flow cytometry, together with the regular fluorometer, were employed as the major instruments for the characterization of the fluorescent spheres. Thus the emission of the spheres can be studied in various aspects, such as LSCM images to view the emission from spheres in a small range, the overall emission spectral profile of the sphere powder, to the statistical evaluation of the emission intensity from several channels based on more than 10,000 spheres. The photostability and thermal stability of these fluorescent spheres were also investigated. The results from all these characterizations demonstrated that the controllability and feasibility of our strategy in tuning the emission of the spheres. In addition, these fluorescent spheres can be directly used as encoded spheres in the flow cytometry.

Experimental

Materials

All other solvents and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. All materials were of analytical grade and were used as received unless otherwise noted. Methanol, acetone and xylene were dried previously. The polystyrene-divinylbenzene (SPSDVB) microspheres with a diameter of 35 μm, and with sulfonic groups (0.93 mmol/g) on the surface, were kindly provided by Suzhou Nano-Micro Bio-Tech Co., Ltd.. The bis(sulfonium) salt monomer of pre-PPV，p-phenylenedimethylenebis(tetrahydrothio-phenium chloride) was synthesized followed the preparation in our previous report[28].

Characterization and methods

Fluorescence spectra were measured using a Hitachi FLS920 fluorometer. The photostability experiment was carried out by recording the emission spectra every 10 min for 1 hour (excited at 405nm with a 700 mW light source), using the Hitachi FLS920 fluorometer. Flow cytometric analysis was taken by a BD FACSVerse flow cytometer. Two receiving channels, V450 (450±25 nm) and V500 (525±10 nm), were used for receiving emission signals from the spheres excited at 405 nm. Another two receiving channels, FITC (527±16 nm) and PE (586±21 nm) were used for receiving emission signals from the spheres excited at 488 nm. The measurements were carried out by dispersing the SPSDVB-PPV microspheres in water in 1.5 mL plastic tubes. Scanning electron microscopy (SEM) images were obtained on a Hitachi S-4700 microscope operating at 15.0 kV. Fluorescence microscope images were obtained using an OLYMPUS IX71 fluorescence microscope. Laser Scanning Confocal Microscopy (LSCM) images were recorded using a Leica TCS SP5 laser scanning confocal microscopy by dispersing the microspheres on the glass slides with glycerol. Thermogravimetric analysis (TGA) data were obtained with a Perkin-Elmer Pyrisl TGA. The high-temperature oscillator is from Taicang Hualida Testing Equipment Co., Ltd., which actually is an oven directly on an oscillator, and the oven and oscillator are connected together into one instrument. The digital photographs of the SPSDVB-PPV microspheres as powders were taken with a Nikon D-5100 camera.

Preparation

Precursor of PPV (pre-PPV). p-phenylenedimethylene-bis(tetrahydrothio-phenium chloride) (0.6895 g, 1.96 mmol) and deionized water (12.0 mL) were placed into a pre-dried 50 mL two-necked round bottom flask. The system was deoxygenated three times by vacuum-argon cycling, and the temperature was maintained at 0 ^{o}C. 1.7 mL of ice-cold, Ar-purged NaOH aqueous solution (1.0 mol/L) was added, in a very slow stream, into the reaction system. The mixture was stirred at 0 ^{o}C for 1 hour followed by adding 3.0 mL of HCl aqueous solution (2.0 mol/L) into the reaction system to stop the polymerization. The reaction mixture was then dialyzed against deionized water in a 500 mL beaker for 12 hours, using the dialysis tubing cellulose membrane with a molecular mass cutoff of 3500 Da. Water was changed for every 2 hours and the same volume of water was used each time. Then, the polymer precursor solution inside the membrane was quickly transferred into a container for next reaction. If the polymer precursor solution will be used later, it should be kept at a low temperature around 0 ^{o}C.

SPSDVB-(pre-PPV) microspheres. The SPSDVB microspheres (0.150 g) were dispersed in the aqueous solution of pre-PPV obtained in the previous step (5.0 mL) in a 10.0 mL centrifuge tube. The tube was oscillated using an IKA MS 3 digital oscillator with the oscillating rate of 1000 rpm for 1 hour to ensure that the electrostatic adsorption between the pre-PPV precursors and the SPSDVB spheres had reached equilibrium. The microspheres were isolated by centrifugation, and subsequently washed with 6.0 mL deionized water. The redispersion/centrifugation cycle was repeated twice to give the SPSDVB-(pre-PPV). The spheres were washed with ethanol and then were dried in a vacuum oven.

Thermal elimination. SPSDVB-(pre-PPV) (0.0300 g), xylene (3.0 mL) and para-toluenesulfonic acid (0.0080 g, 0.047 mmol) were placed into a pre-dried 30 mL ground glass tube. The tube was deoxygenated three times by vacuum-argon cycling and was generally covered by a vacuum stopper. Then the tube was placed into a high-temperature oscillator, which is a special oscillator with a closed chamber having a heating range between room temperature and 150 ^{o}C. The oscillation frequency was set at 240 rpm and the oscillator was heated to a predetermined temperature. The tube was taken out after a certain time. After centrifugation, the resulting spheres were washed by acetone, deionized water and ethanol respectively, and finally dried in vacuum to give final SPSDVB-PPV spheres. For comparison, the thermal elimination in solid state was also carried out by putting the same amount of

SPSDVB-(pre-PPV) directly into a vacuum oven over a certain time at a predetermined temperature.

Results and discussion

Preparation and spectroscopic characterization

Precursor of PPV (pre-PPV) was synthesized with a modified procedure based on our previous report[28]. The amount of NaOH used for catalyst was reduced to lower down the rate of polymerization and avoid the formation of gel. The amount of HCl as a terminator was increased to guarantee a rapid termination of the polymerization, and the residual HCl can prevent the elimination of sulfonium groups on the pre-PPV before being adsorbed onto the SPSDVB spheres. Slight modification was also made for the step of absorbing pre-PPV onto the SPSDVB compared to our previous study[28], such as the feeding ratio between pre-PPV and SPSDVB, since the diameter of the substrate spheres changed and so the specific surface area. The spheres after adsorption step and elimination step were washed with ethanol before being placed into the vacuum oven to speed up the drying. However, the elimination step was carried out in a very different way. The conversion of pre-PPV to PPV was then completed after the heterogeneous catalytic thermal elimination process. The SPSDVB-(pre-PPV) spheres were heated in the high-temperature oscillator, using para-toluenesulfonic acid as catalyst and xylene as solvent. To demonstrate the advantages advanced previously and investigate how the thermal elimination conditions affect the fluorescence of the SPSDVB-PPV spheres, a series of heterogeneous thermal elimination reactions on the SPSDVB-(pre-PPV) spheres from the same batch were carried out in parallel with different temperatures or times.

At first, different temperatures, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 100 °C and 120 °C, for heterogeneous catalytic thermal elimination were carried out on seven equal portions of SPSDVB-(pre-PPV) spheres from the same batch by fixing the elimination time as 1 hour. As shown in Fig. 1, the color-tuning effect has been realized since gradual change in the color can be observed by increasing the temperature. These spheres gave different colors from pale yellow to bright yellow under normal light, and emitted blue to green fluorescence under a 365 nm UV lamp.

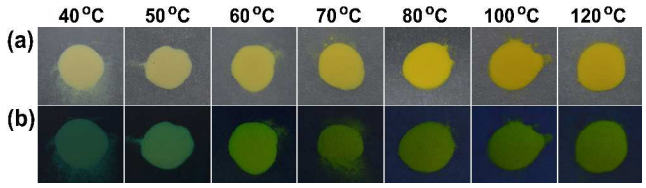


Fig. 1 Digital photographs of SPSDVB-PPV spheres as powders, obtained at different elimination temperatures, under (a) normal light; (b) a UV lamp (365 nm). The elimination time was fixed at 1 hour.

The solid state fluorescence measurement was carried out on these spheres and the results are shown in Fig. 2. The normalized spectra are shown at the left, and the derived information from the spectra is shown at the right to give a direct view of the emission wavelength or intensity ratio change. As shown in Fig.2a, for the SPSDVB-PPV spheres obtained via heterogeneous elimination, the profile for all emission spectra display more or less fine structures, having a peak and a shoulder. When the elimination temperature was low (40 °C and 50 °C), the emission spectra have a peak at shorter wavelength around 492 nm and a shoulder at longer wavelength around 506 nm. Both emissions at shorter/longer wavelength were red-shifted and all the spectra displayed clearer fine structure, with the increase of the elimination temperature. In addition, the emission at longer wavelength became stronger and stronger, which finally surpassed the one at lower wavelength in the intensity and became the dominating emission peak. The spheres obtained at 120 °C, emitted the fluorescence with a spectrum shouldered around 515 nm and peaked around 547 nm. For comparison, the thermal elimination under these temperatures was also carried out in solid. As shown in Fig. 2b, the emission spectra of spheres obtained by elimination in solid state are much simpler and display less variation with the temperature changing than those obtained with heterogeneous catalytic process. All of them have a broader profile with a single emission peak, which gradually and slightly red-shifted by increasing the temperature, from 483 nm (40 °C) to 500 nm (80 °C) and finally 506 nm (120 °C). Thus, the heterogeneous catalytic elimination provides much more delicate and greater tuning of emission properties than solid elimination. Such rich variation in the emission is favorable for further encoding spheres with different parameters according to different requirements.

The changing in the emission spectra with the elimination temperature, displayed some similarity and difference between the two series of spheres obtained with different elimination processes. Such similarity and difference are reasonable, if we take a close look at the reaction mechanisms for the heterogeneous catalytic elimination and the elimination in solid. The similarity lies in the red-shifting of the spectra with the increase of the temperature in both processes. Such phenomenon can be attributed to the larger extent of conjugation and narrower band gap since more sulfonium groups were eliminated and more C=C formed on the backbone at higher temperature. In both processes, when the temperature exceeded 80 °C, the red-shifting seems to slow down, indicating the extent of conjugation was close to the maximum in both cases.

However, there are two major differences. First, the spectra of spheres from the catalytic process located at longer wavelength region, comparing to the spectra of spheres from the non-catalytic process in solid under the same elimination temperature. Such red-shift effect, for catalytic process vs non-catalytic process, is more obvious at higher temperature. This phenomenon confirmed that the elimination process was greatly facilitated by adding the external acid, comparing to the non-catalyzed process which has to go through a five-member ring transition state, as proposed in Fig. S1. The more efficient elimination in the catalytic strategy resulted in larger extent of conversion from nonconjugated segments to conjugated segments. Second, fine spectral structure remained for spheres from heterogeneous elimination under all temperatures, especially for those obtained at 60 °C or higher, while only broad emission was observed for those from solid elimination. Such differences can be attributed to different

environments where the reaction happened. In the heterogonous elimination, the spheres can easily rotate and move around during the whole process. The elimination should be more evenly distributed among all the spheres and randomly at any place on the surface of spheres. Moreover, the conformation of pre-PPV/PPV chains can be easily adjusted with the aid of surrounding solvent molecules, In addition, the solvent can take away the reaction heat which made the process smoother. Thus, the whole process took place in a similar way as in solution. The presence of solvent facilitates the chain/segment reorganization to reach more stable conformation/vibronic structures and thus display a fine structure with peak/shoulder in the spectra. However, in a complete solid environment, the movements of the spheres/chains were more limited and the heat was also difficult to dissipate.Even some nonconjugated segments exist due to the incomplete elimination. Thus polymer segments/aggregates with different extents of conjugation exist in a statistical way. The different vibronic structures may also exist. Therefore, when the elimination takes place in a complete solid environment, the resulting spheres gave a broad emission from PPV. In all, these observations further confirmed the advantages advanced for preparing PPV coated microspheres using the heterogeneous catalytic elimination in the introduction.

The effect of the elimination time on the emission of the SPSDVB-PPV spheres was also studied. The heterogeneous catalytic elimination was carried out for 20 min, 40 min, 1 hour, 3 hour or 5 hour, respectively, with the same starting SPSDVB-(pre-PPV). The digital photographs of these spheres are shown in Fig. S2. 80 oC was selected as the elimination temperature based on the consideration that the low elimination temperature was favorable for upscale production, since 80 oC, 100 oC, 120 oC gave the similar emission profile as shown in Fig. 2a. Negligible change in the apparent color can be found under either normal light or UV light. Correspondingly, little difference can be found among the emission wavelength of the spheres obtained with different elimination time (Fig. S3). All the elimination times resulted in almost the same emission profiles with fine structure. It seems that 20 minutes is enough to produce PPV having the greatest extent of conjugation and further extending the elimination time may only increase the amount of conjugated segments. Thus the presence of catalyst should speed up the reaction. In the following studies, we take 1 hour as the reaction time, to ensure the elimination mostly completed.

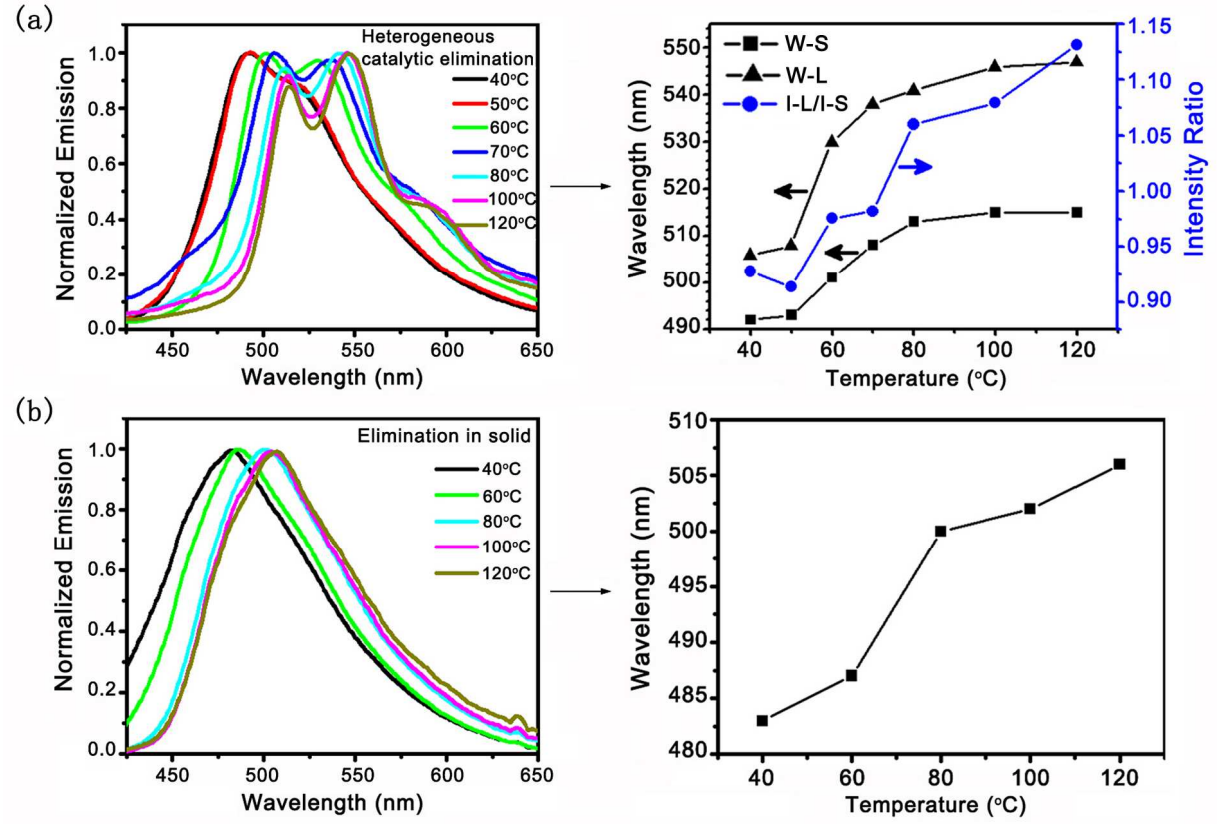


Fig. 2 Normalized solid state fluorescence emission spectra (excited at 405 nm) of the SPSDVB-PPV spheres obtained at: (a) 40 °C, 50 °C, 60°C, 70 °C, 80 °C, 100 °C and 120 °C for 1 hour through heterogeneous catalytic elimination(left), and changes of the peak/shoulder wavelengths at shorter wavelength (W-S)/ longer wavelength (W-L) or the intensity ratio between the longer/shorter peak/shoulder (I-L/I-S) with the elimination temperature(right); (b) 40 °C, 60 °C, 80 °C, 100 °C and 120 °C for 1 hour through thermal elimination in solid(left), and the changes of peak wavelengths with the elimination temperature(right).

Microscopic study

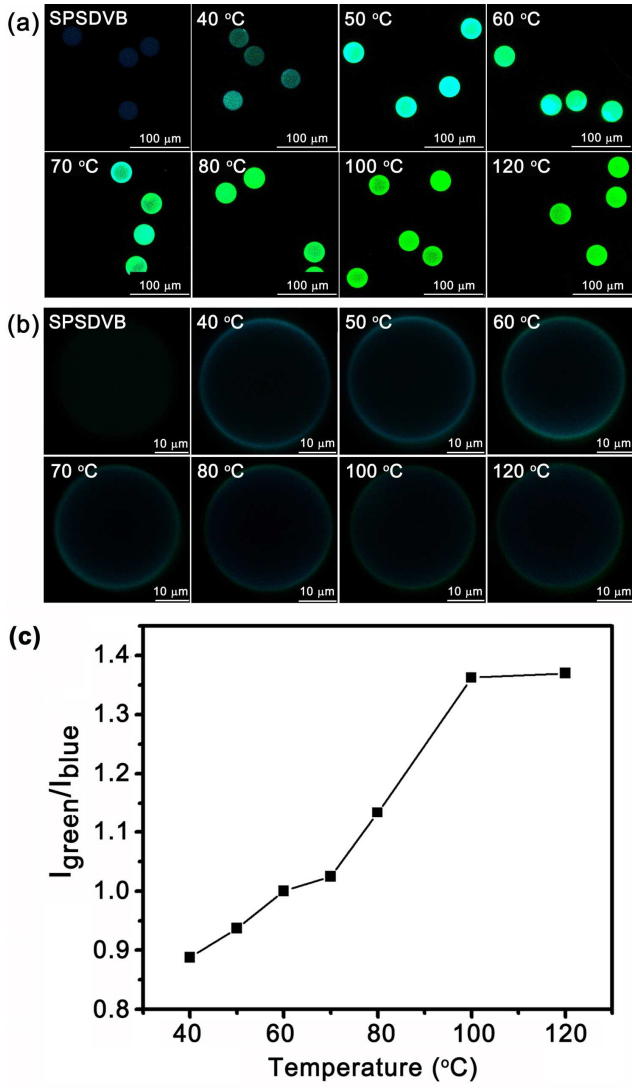


Fig. 3 (a) The LSCM images of the substrate SPSDVB microspheres and PPV coated microspheres obtained under different temperatures (excited at 405 nm) with two channels (410~492 nm, set in blue and 493~575 nm, set in green) for receiving the emission signals; (b) The core-shell images from the optical sectioning of single microspheres; (c) The emission intensity ratios between the green and blue channels, measured based on one spot on the shell of core-shell image of the spheres from different elimination temperatures.

The variation in the fluorescence emission color can be directly observed from the LSCM images (Fig. 3). With the excitation at 405 nm, we selected two channels (410~492 nm, set in blue, and 493~ 575 nm, set in green, with the same output voltage) to view the microspheres. The resulting fluorescent images from the two channels were directly superimposed with each other to give the composite images (see Fig. S4 and its caption for more explanation about the superimposition). All the parameters were set the same when measuring different microspheres. As seem from the fluorescent images for the whole spheres (Fig. 3a), the fluorescence color turn gradually from pure blue to pure green via the intermediate cyan color when the elimination temperature increased from 40 °C to 120 °C. It is to be noted that substrate spheres display weak ultramarine blue, which is due to presence of delocalized π structure in the highly crosslinked poly(divinylbenzen-co-styrene) structure. The optical sectioning of single microspheres (Fig. 3b) not only displayed the gradual color changing but also demonstrated the core-shell structure of the SPSDVB-PPV spheres and no core-shell structure was observed of the substrate SPSDVB spheres. To obtain some semi-quantitative information about the color changing, the ratios between the emission intensities from the green channel (493~575 nm) and the blue channel (410~492 nm), at one spot on the shell of each microsphere, were calculated and shown in Fig. 3c. This data suggested that the emission color kept changing with the increase of elimination temperature until 100 °C.

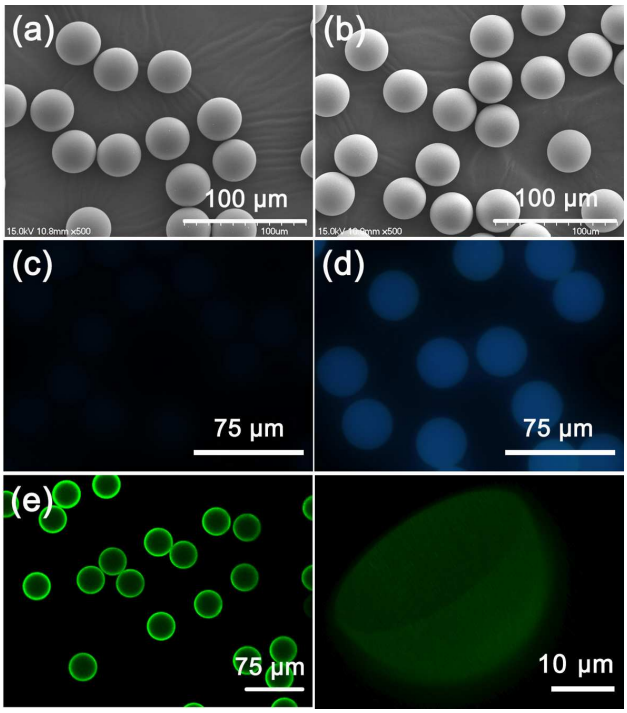


Fig. 4 SEM images of (a) SPSDVB spheres and (b) SPSDVB-PPV spheres; Fluorescence microscopy images (excited by blue light, with the same excitation) of (c) SPSDVB spheres and (d) SPSDVB-PPV spheres; (e) The LSCM image from the optical sectioning of the SPSDVB-PPV spheres around the equator plane and (f) the 3D cross-section reconstruction image of the confocal data from the optical sectioning from the apex to the equator plane of one sphere (excited at 405nm). The SPSDVB-PPV spheres were obtained at 80 °C for 1 hour.

As mentioned previously, the monodispersity of size, the surface morphology as well as some other emission properties are also very important for the final application of these spheres. More detailed microscopic studies were carried out with SPSDVB-PPV spheres obtained under 80 °C for 1 hour as the

representative. As seen from Fig. 4b, the SPSDVB-PPV spheres remained as smooth and perfectly spherical as the substrate SPSDVB microspheres (Fig. 4a). Negligible change in the size and the surface morphology of the spheres were observed. The fluorescence microscope images (Fig. 4d) showed that the fluorescent microspheres emitted evenly distributed fluorescence while little emission was observed for the SPSDVB spheres (Fig. 4c) with the excitation at the blue channel. The SEM images and fluorescence microscopic images indicated that PPV was uniformly coated on the substrate spheres due to the well-controlled preparative process, including purification of pre-PPV, electrostatic adsorption, washing, thermal elimination and etc... The LCSM optical sectioning technique was applied to get clearer image of the core-shell structure by using only one (green) receiving channel (Fig. 4e). Fig. 4f displays the 3D cross-section reconstruction of the optical sectioning images from the apex to the equator plane of one fluorescent sphere. The observation further confirmed that PPV molecules have been uniformly coating onto the substrate spheres. The uniformity not only existed among different spheres, but also on the surface of a single sphere.

Flow cytometry study

The flow cytometry measurement was carried out to evaluate the fluorescence tuning effect by varying the temperature with the heterogeneous catalytic elimination process. The flow of spheres was simultaneously excited at 405 nm and 488 nm, and the signals were received in V450 and V500 channels (for 405 nm excitation), FITC and PE channels (for 488 excitation), respectively. As shown in Fig. 5, the spheres obtained from different temperatures display very different combinations of the emission intensity received from the four channels. Such rich combinations can be attributed to the great variation in emission wavelength and intensity, which is very favorable for using these spheres as encoded spheres. It is to be noted that the intensity from different excitation are not comparable since the powers for different excitation sources are different.

The outcome from flow cytometry measurement is mostly consistent with the emission spectra in Fig. 2a. For all spheres, most emission located in the region between 475 nm~575nm. Thus smaller intensity was detected from channel V450 (the blue one) compared to channel V500 (the green one), and larger intensity was detected from channel FITC (the green one), compared to the channel PE (the orange one). The spheres from lower elimination temperatures have higher intensity at blue channel since their spectra were blue shifted compared to those from higher elimination temperature. *Vice versa*, the red-shifted spectra of spheres obtained under higher elimination temperature account for the higher intensity at the orange channel. Interestingly, the spheres obtained at 60 ^{o}C displayed the highest intensity at V500, FITC and PE channels. Such high emission may be explained that the high level of elimination took place with the help of catalyst and thus resulted in more conjugated segments, while self-quenching among these segments was rare since the reorganization of chains into appropriate distance and alignment with each other for quenching might be difficult at such relatively low temperature.

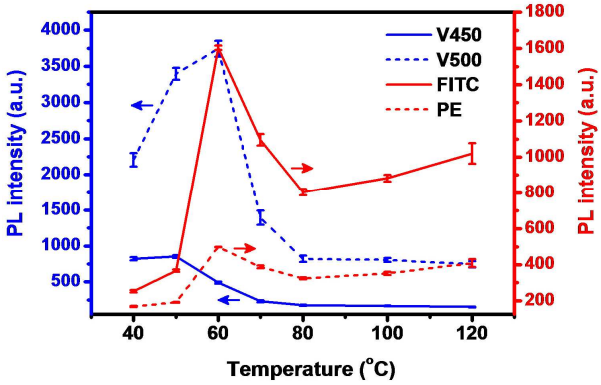


Fig. 5 The fluorescence intensity from flow cytometry measurement (V450 and V500 for excitation at 405 nm shown at left Y axis; FITC and PE for excitation at 488 nm shown at right Y axis) of the SPSDVB-PPV spheres obtained at different temperatures respectively for 1 hour. The error bars are the standard deviations for each sample, calculated according to values from the measurements of four different batches. At least 10,000 spheres were used for each measurement.

Stability studies

The thermal stability and photostability of fluorescent spheres were studied with using the SPSDVB-PPV spheres obtained under 80 ^{o}C for 1 hour as the representative. The TGA curves showed that the decomposition of the SPSDVB-PPV microspheres started around 400 ^{o}C, which actually is slightly higher than original SPSDVB spheres (Fig. S5). Such thermal stability is good enough for the general applications of these fluorescent spheres, which usually are carried out at room temperature or slightly higher. The photostabilty study was performed on SPSDVB-PPV spheres by measuring the emission spectra from the regular fluorometer. As shown in Fig. S6, almost no changes in the spectra can be observed, after being irradiated for 10 minutes. Even after 1 hour, only very slight changes can be observed, such as 3% reduction in the intensity and 4 nm of blue shifting of the emission maximum. Therefore, the spheres have been demostrated to have very good thermal stablity and photostability.

Conclusions

A series of SPSDVB-PPV fluorescent spheres were successfully prepared through an approach involving PPV precursor adsorbed onto the SPSDVB substrate spheres followed by a heterogeneous catalytic thermal elimination process. Comparing to the regular solid elimination without catalyst, the catalyzed elimination in a heterogenous system were found to be effective with a much larger temperature range and in a much shorter time. The realization of fluorescence tuning via controlling the temperature has been demonstrated in many aspects, such as apparent direct view, emission spectra, confocal images as well as the flow cytometry measurement. The smooth surface and perfect core-shell structure suggest that the whole process is well controlled. The thermal stability and photostability of the spheres provide the possibility for real application. Our method has provided a platform for preparation of fluorescent microspheres with tunable

emission using variety of PPV derivatives as the fluorophores and different substrate materials. The uniform size, bright fluorescence and tunability of the emission color allow these microspheres to be used for a variety of applications. In addition, the simply but well-controlled preparative process as well as the facile fluorescence tuning is also a good demonstration for the advantages possessed by polymer materials in processing and structural/properties tuning, compared to small molecules.

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TOC

Realization of Fluorescence Color Tuning for Poly(p-phenylenevinylene) Coated Microspheres via a Heterogeneous Catalytic Thermal Elimination Process

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Fluorescent microspheres with clear core-shell structure and various emission colors were successfully prepared via a catalytic elimination process.