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Facile Preparation of Polydiacetylene-Based Uniform Porous Fluorescent Microspheres for Potential Immunoassay Application

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Fluorescent microspheres are prepared by attaching self-assembled polydiacetylene (PDA) vesicles with carboxyl side groups onto the substrate amino-modified poly(glycidylmethacrylate) (APGMA) microspheres. The characterizations by SEM, confocal microscopy and flow cytometry demonstrated that the final resulting microspheres are highly uniform both in size (with a diameter of 5 μm) and in fluorescence emission (coefficient of variance < 3%).The Brunauer-Emmett-Teller (BET) surface area for these spheres is 114 m^2/g. In addition, there are evenly distributed pores with an average size of 20.6 nm on the spheres. These spheres are found to have good thermal stability and photostability, and do not suffer from fluorophore leaching. Fluorescein isothiocyanate (FITC) label bovine serum albumin (BSA) as representative biomolecules, can be easily attached onto the fluorescent microspheres. All these characteristics possessed by the APGMA-PDA spheres allow them to be directly used as carriers of biomolecules in the lab-on-a-chip immunoassay systems.

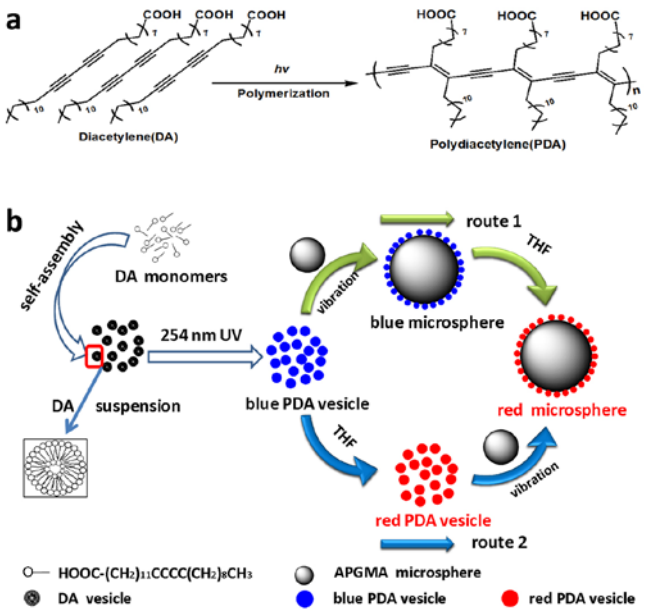
1 Introduction

The preparation of fluorescent microspheres has received increasing interest due to the great demand in both academic and industrial communities for applications in pollutant sensing, disease diagnose, immune assay and so on.[1-4] Noticeable efforts have recently been devoted to the fast, accurate and sensitive immunoassay based on fluorescent microspheres, using well-established flow cytometry technique at the lab, or newly emerging microfluidic device for point-of-care clinical diagnosis.[5-9] However, such modern techniques cannot benefit most ordinary people with acceptable cost due to the lack of sufficient fluorescent microspheres with desirable properties required by these applications. To produce fluorescent microspheres to meet the special demand of immunoassay, many characteristics have to be considered, such as the size (usually several micrometers in diameter) and narrow distribution, steady and uniform emission, large surface-to-volume ratio, and surface functionalization (carboxyl group or amino group preferred for interaction with biomolecules) .[4, 10]

Most fluorescent microspheres have been prepared using organic dyes or quantum dots as the fluorophores, while very few reports employed conjugated polymers.[11-13] Conjugated polymers own many distinct merits, such as easiness in the structure tuning, resistance to the photobleaching and high thermal/mechanical stability. However, only very few reports employed conjugated polymers in preparing fluorescent microspheres, which may due to the complicated and strict synthetic/preparative process.[14, 15] Recently, our group have advanced a strategy for facile preparation of fluorescent microspheres by coating poly(p-phenylenevinylene) (PPV) onto a highly crosslinked polymer core, which has been demonstrated very successful in realizing the steady emission, uniformity in size and fluorescence as well as the thermal/mechanical stability.[16] Continuous seeking new materials/strategies for preparing the fluorescent microspheres are still ongoing in our lab, to meet different requirements from diverse applications, or further simplify the preparative process. Polydiacetylene (PDA) has received tremendous attention due to its outstanding optical and electrical properties.[17-22] The most

distinct merit for PDA is the convenient polymerization, which can be accomplished directly from the commercial monomer under the irradiation of ultraviolet or γ-ray at room temperature.[23] PDA rapidly changes its colour (usually from blue to red) when exposed to external stimuli. [24-30] Blue PDA is nonfluorescent but red PDA has fluorescence emission. [17, 19, 26] Using red-phase PDA as the fluorophore to prepare fluorescent spheres with a commercially available core might be advantageous for avoiding the tedious synthetic process.

To find an appropriate way for immobilizing PDA onto the core spheres, learning from some existing methods for immobilizing PDA onto the solid supports seems necessary. Many literature are about fixing PDA onto the surface of planar substrates for various applications,[31-34] while only two reports are about preparing PDA-based beads,[35, 36] to our knowledge. Two general ways for immobilizing PDA onto the substrates have been developed. The first method is to directly attach the diacetylene (DA) monomers from solution onto the substrate surfaces followed by polymerization under UV irradiation. The second one is to prepare DA monomer vesicles through self-assembly at first, then attach the vesicles onto the substrate surfaces; The photopolymerization can be before/after the attachment. More preference was given to the second method, since the possible low local concentration and uneven distribution of DA monomers on the surface in the first method may make polymerization inefficient.



Scheme 1. (a) Polymerization of DA under UV irradiation; (b) Schematic illustration for the strategy of preparing fluorescent APGMA-PDA microspheres. APGMA refers to the amino-modified PGMA spheres.

Aiming to develop a method for facile preparation of fluorescent microspheres specifically for immunoassay, we proposed our detailed strategy as illustrated in Scheme 1. The whole preparative procedure can be divided into four steps. The DA monomers were self-assembled into DA vesicles, which turned into blue PDA vesicles after 254 nm UV irradiation. Afterwards the PDA vesicles were immobilized onto the substrate spheres to give the blue spheres suspension in water and the fluorescent red spheres were obtained after adding THF into the suspension (route 1); Or, the blue-red transition was accomplished before immobilizing PDA vesicles onto the substrate sphere (route 2). We selected 10,12-pentacosadiynoic acid as the DA monomer, which is based on the consideration of introducing carboxyl functionality and fluorophore simultaneously. Polymerization is to be carried out before introducing the material onto the spheres, which is to rule out the unevenness in the polymerization since the polymer substrate spheres might block the UV light in some directions. Different from literature, here we used commercially available, monodispersed, amino-modified porous poly(glycidylmethacrylate) (APGMA) microspheres with a diameter of 5 μm as the substrate sphere. We hope the porosity will further increase the surface-to-volume ratio and thus enhance the interaction between the microspheres and the analytes. The size of the spheres was selected according to the application requirement. In addition, no additional reagents were added except the solvents, during the whole preparative process in our strategy, which is favorable for purification as well as maintaining the chemical stability of the spheres. The strong interaction between the spheres and biomolecules, was confirmed by using fluorescein isothiocyanate (FITC) modified bovine serum albumin (BSA) as the representative. Thus, all the characterizations demonstrated the resulting PDA fluorescent spheres possess many merits, meeting the requirements from practical application in immunoassay.

2 Experimental

Materials

10, 12-pentacosadiynoic acid (PCDA, also abbreviated as DA in this study) were purchased from Alfa Aesar Chemical Co. DA was purified according to the following procedure. After dissolving DA in chloroform, the solution was filtered using a nylon membrane with a pore size of 0.22 μm to remove any polymerized species; the purified DA was obtained after removing of chloroform at 40 ^{o}C under reduced pressure. The amino-modified poly(glycidylmethacrylate) (APGMA) microspheres (NH_2 content: 1.01 mmol/g) were kindly supplied by Suzhou Nano-Micro Bio-Tech Co. Ltd. and washed with deionized water for three times before use. The amino groups were obtained by the controlled surface modification of the epoxy groups on poly(glycidylmethacrylate) spheres through ring-opening reaction with ethylenediamine. Thus hydroxyl groups coexist with the amino groups. Fluorescein isothiocyanate (FITC) modified bovine serum albumin (BSA) was supplied by prof. Hong Chen's Group in Soochow University. Other commercially available reagents of analytical grade were from Sinopharm Chemical Reagent Co., Ltd and were used without further purification.

Preparation of poly (10, 12-pentacosadiynoicacid) (PDA) vesicles

Typical procedure: DA (3.74 mg) was dissolved in 0.4 mL of dimethyl sulfoxide (DMSO). The solution was injected dropwise into 10 mL of deionized water, followed by ultrasonic vibration for 30 min to give suspension, and the temperature for the solution/suspension was controlled around 80 ^{o}C during the process. The suspension was filtered through a cotton filter (with a diameter of 0.22 μm) to remove possible solid aggregates. The filtrate was kept at 4 ^{o}C overnight to give evenly dispersed DA aqueous suspension, which was irradiated under 254 nm UV light (1 W/cm^2) for 20 min to produce blue PDA vesicles suspension. To remove undesired small molecules, the PDA suspension was dialyzed against deionized water using the dialysis tubing cellulose membrane with a molecular mass cutoff of 3500 Da. During the dialysis, the total volume of the dialysis system was kept as 200 mL. The water was changed every two hours for six times, and the last time (the seventh time) was dialyzed overnight. Then the PDA vesicle suspension inside the membrane (about 11 mL) was quickly transferred into a glass container and stored in refrigerator at 4 ^{o}C for the next step.

Preparation of APGMA-PDA microspheres

Typical procedures for Route 1: 15 mg of APGMA microspheres (in white colour) were dispersed in 1 mL of water and then was dropped into 3 mL of blue PDA vesicle suspension. The mixture was shaken for 5 minutes to give blue APGMA-PDA microspheres suspension. Adding 400 μL of THF into the above suspension gave red microsphere suspension. The water and THF in the suspension was removed by centrifugation. The resulting red microspheres were washed with 6 mL of deionized water for three times and further dried in a freeze dryer overnight (Fig. 2).

Typical procedures for Route 2: 400 μL of THF were added into 3 mL of PDA vesicles suspension to induce blue-red transition of PDA. Afterwards, the suspension was dialyzed in deionized water using the dialysis tubing cellulose membrane with a molecular mass cutoff of 3500 Da to remove THF. During the dialysis, the total volume of the dialysis system was kept as 100 mL. The water was changed every two hours for six times, and the last time (the seventh time) was dialyzed overnight. 15 mg of APGMA microspheres were dispersed in 1 mL of water and drop the suspension into 3 mL of red PDA vesicles solution obtained above. After being shaken for 5 minutes, red APGMA-PPCDA microspheres were obtained by centrifugation and then washed with 6 mL of deionized water for three times, followed by being dried in a freeze dryer overnight.

Loading FITC labeled BSA (BSA-FITC) onto the fluorescent microspheres

Fluorescent microspheres (prepared by mixing 15 mg of APGMA microspheres and 3 mL of red PDA vesicles solution) and 1.76 mg of BSA-FITC were mixed in 2 mL of phosphate buffer solution (PBS, pH=7.4) at 25 ^{o}C in dark and stirred for 2h. Afterwards, the resulting microspheres (APGMA-PDA-BSA-FITC) were washed with 2 mL of PBS for three times. In comparison, we prepared FITC solution in buffer solution which has the same emission intensity as that of FITC-BSA solution. 2 mL of FITC solution was mixed with fluorescent microspheres (prepared by mixing 15 mg of APGMA microspheres and 3 mL of red PDA vesicles solution) at 25 ^{o}C in dark and stirred for 2h. Afterwards, the resulting microspheres (APGMA-PDA-FITC) were washed with 2 mL of PBS for three times.

Instruments and methods

UV-vis absorption spectra were measured on a Shimazu UV-3150 spectrophotometer. Fluorescence spectra were obtained on an Edinburgh FLS920 spectro fluorometer. An Olympus invented microscope model IX71 was used to obtain fluorescent images. The size and morphology of PDA vesicles were obtained on transmission electron microcopy (TEM TecnaiG220, FEI company, US). To avoid the obvious aggregation of PDA vesicles, the PDA vesicle suspension was diluted into 10^{-6} M (with respect to the initial monomer concentration). 10 μL of the diluted vesicle suspension was placed onto a copper grid followed by being dried in a freeze dryer. Scanning electron microscope (SEM) images of microspheres were obtained with Hitachi S-4700. The microspheres were immobilized onto a layer of conductive adhesive and sputter-coated with a layer of gold before measurement. The average size of PDA vesicles was measured using a Zetasizer Nano-ZS (Malvern Instruments). Confocal fluorescent images were recorded using a Leica TCS SP5 laser confocal microscopy (with excitation at 488 nm) by dispersing the microspheres on the glass slides with water. The emission intensity of fluorescent microspheres was recorded on a Beckman Coulter Cytomics FC500 Flow Cytometer. The measurements were carried out by dispersing the APGMA-PDA microspheres in water with a concentration of 10.0 g/L in a 10 mL plastic tube. An EXSTAR TG/DTA 6300 instrument was used to study the thermal stability of microspheres. 1.0 gram of fluorescent microspheres were used for measuring the surface area and porosity using the Micromeritics TriStar Ⅱ Surface Area and Porosity Analyzer.

3 Results and discussion

The preparation and characterization of DA/PDA vesicles

The amphipathic 10,12-pentacosa-diynoicacid was selected for our system among many diacetylene (DA) monomers with different substituents. This type of DA monomers readily form vesicles in water[37] and give typical properties of PDA after polymerization.[38] In addition, having carboxyl side groups is

also very desirable for using this material in immunoassay applications. However, the vesicles tend to form aggregates and then precipitate out of water, which may make the amount of DA used for the next several steps uncontrollable. To reduce the aggregation as much as possible, dimethyl sulfoxide (DMSO) was introduced into the aqueous system [39] and the temperature was maintained around 80 ^{o}C during the preparative process of DA vesicles. PDA vesicles formed after UV irradiation. After optimization, PDA vesicles with controlled morphology were obtained using a PDA concentration about 1 mM (with respect to the initial monomer unit). The optimized preparative conditions are described in experimental section. It is to be noted that deionized water is ideal environment for the formation of PDA vesicles. No strict requirement for strict control of pH value of the system makes the whole process very simple. To remove the undesired small organic molecule in the system, PDA suspension was dialyzed against deionized water to give the final suspension for being loaded onto the substrate spheres.

The formation of DA vesicles suspension was first confirmed by the appearance of the typical Tyndall effect, that is, the trajectory of the laser beam can be clearly observed in the suspension when using laser pointer to irradiate it at one side (Fig. 1a). DA vesicles in water/DMSO mixed solvent as obtained was found to have an average size about 118 nm and a narrow distribution (PDI≈0.13) measured by a Zetasizer (see ESI, Fig. S1), which is very close to those of PDA vesicles (average size≈113 nm and PDI≈0.12) after dialysis against water (see ESI, Fig. S2). Thus the polymerization and dialysis processes had no obvious effect on the size and distribution of the vesicles. TEM image shows the PDA vesicles had a typical round shape with an average size slightly larger than 100 nm (Fig. 1b), which is consistent with above results from the suspension. We believe the dialysis against water not only removed the DMSO and other small molecules, but also made the morphology of the vesicles fixed. The resulting blue PDA vesicle suspension was stored in refrigeration for future use when needed.

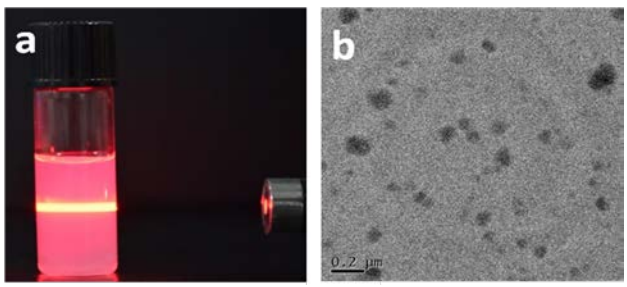


Fig. 1 (a) Photos of DA monomers vesicles with red laser irradiation; (b) TEM image of blue PDA vesicles obtained with the initial DA monomer concentration of 1mM.

Preparation of PDA microspheres

As previously shown in Scheme 1, two routes are advanced for preparing red fluorescent PDA microspheres, starting from the blue PDA vesicles. All the details are described in experimental section. These two routes gave the similar final products (Fig. 2). The discussion here will focus on the route 1. Details about route 2 are provided in ESI. There are two steps in the route 1. The first step is immobilizing PDA vesicles onto the spheres, which can be quickly accomplished by mixing the substrate spheres and PDA vesicles followed by five minutes of shaking. The second step is to realize the colour-change of PDA simply by adding sufficient THF into the sphere suspension.

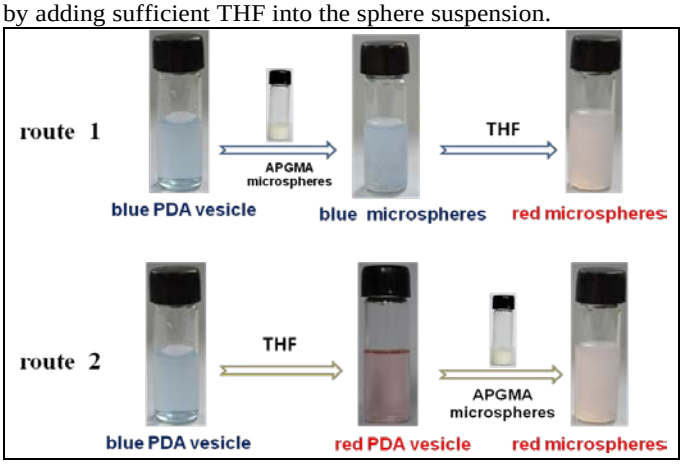


Fig. 2 Photos for the process to prepare red-phase PDA loaded microspheres, through the two routes advanced in Scheme 1.

By using a porous amino-modified polymer substrate spheres, the immobilization of PDA onto the substrate spheres went very smoothly and quickly. Previous reports about preparing PDA microspheres, usually use some specific agents (such as DIC and HOBT, DMPC) to immobilize PDA.[35, 40, 41] However, toxicity, instability or interference with analytes in future application might be a problem due to the existence of these additional agents. In our system, PDA was successfully attached onto the APGMA microspheres without the presence of any additional agent, in a much shorter time and under a much milder reaction condition than the reported methods. The high porosity and the amino-modification of the substrate spheres, might account for the easiness in the reaction. Very likely PDA was first adsorbed onto the spheres due to the high surface-to-volume ratio, then got fixed via interaction between carboxyl groups from these vesicles and amino groups from microspheres.[42] It is to be noted the coexistence of hydroxyl groups on the spheres would not weaken the interaction between PDA and the spheres, since hydroxyl group might also form H-bonding with carboxyl groups. Some fluorescence measurements were carried out during the preparative process. Comparing with the relatively strong emission of the initial red PDA vesicle suspension before being adsorbed onto the spheres, the filtrate after removing the APGMA-PDA spheres gave very low emission (about 8% of the initial one at the peak) (see ESI, Fig. S3). Such observation confirmed that the PDA can be effectively adsorbed onto the spheres, very likely due to the existence of strong interaction between PDA and the spheres.

To ensure the complete removal of the possible residual organic solvents, such as DMSO and THF, further washing and

drying step was performed on the PDA-APGMA spheres. The spheres was dispersed into 6 mL of deionized water and shaken for 10 minutes, followed by centrifugation. This procedure was repeated for three times. Afterwards, the spheres were placed in a freeze dryer overnight to give the final dry products ready for practical applications.

To investigate whether the feeding ratio between APGMA microspheres and PDA vesicles affects the emission of final APGMA-PDA spheres, different amount of APGMA microspheres were used for the preparation by fixing the amount of PDA vesicles. Excessive THF (4 mL) was applied for the second step in route 1 to ensure the complete blue-to-red colour-change of the spheres. Fig. S4 gives the fluorescent properties of resulting red PDA spheres. The coefficient of variation (CV), the ratio of the standard deviation to the mean value, is very commonly used to evaluate the uniformity of the emission the spheres. All the CVs for the microspheres are below 3%, indicating an excellent uniformity in the fluorescent emission intensity (Fig. S4b). We choose to use 15 mg APGMA microspheres vs 3 mL of PDA vesicles as the optimized feeding ratio, based on the consideration of emission properties together with the easiness of the handling in lab treatment.

THF was selected to realize the blue-red transition of PDA, since such water-miscible solvent may impose equal influence on all the microspheres in the suspension than other stimuli and it is easy to be completely removed afterwards. To find out the least but sufficient amount THF to realize the complete blue-red transition, different amounts of THF were added into the blue microspheres suspension (obtained using the optimized feeding ratio above). Colour change took place immediately after gentle shaking. Interestingly, microspheres changed their colour gradually from blue to a transitional purple, and finally red colour, with increasing the amount of THF (Fig. 3a). According to the literature, the blue-to-red colour change of polydiacetylene can be attributed to the complete conformation change of polydiacetylene backbone, while a purple colour of polydiacetylene indicates an intermediate conformation change.[43] The photophysical studies were also carried out on the APGMA-PDA microspheres (Fig. 3b, Fig. 3c). The blue spheres had an absorption peak around 650 nm and negligible emission. The red APGMA-PDA microspheres obtained with 400 μL or more of THF displayed typical red-phase PDA characteristics, having absorption peaks around 500 nm and 550 nm, and emission peaks around 575 and 625 nm. The spheres obtained with 200 μL or 300 μL of THF displayed a combination of red PDA and blue PDA in the absorption, while a more or less blue-shift in the emission comparing to the red PDA emission.

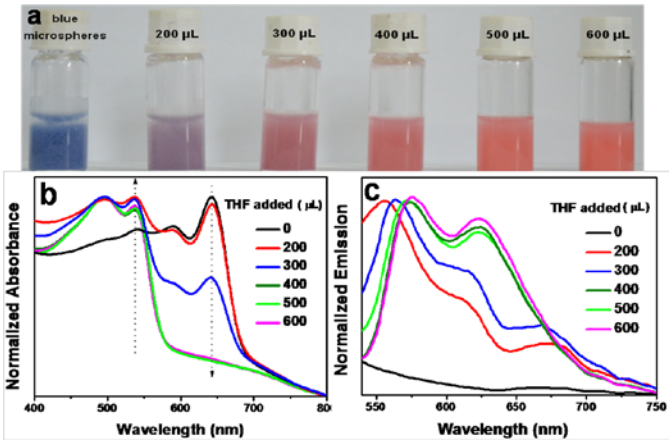


Fig. 3 (a) Photos，(b) absorption and (c) the emission spectra (excited at 500 nm) of original blue APGMA-PDA microspheres and the microspheres treated with 200, 300, 400, 500 and 600 μL of THF, respectively. The normalization has not been performed on the emission spectrum of blue APGMA-PDA microspheres since it has negligible fluorescent emission.

Microscopy studies

Fig. 4 shows the regular photograph, SEM and confocal microscopy images of the final fluorescent spheres obtained using the optimized experimental condition. The red-phase PDA spheres give an apparent appearance as pinkish powder under normal light (Fig. 4a). After coating PDA, no obvious change was observed in the general morphology appearance in the SEM (Fig. 4b), compared to the original substrate spheres (see ESI, Fig. S5). The spheres have a uniform size of 5 μm in diameter, with evenly distributed pores and uniform pore size. The confocal fluorescent images of red APGMA-PDA microspheres (Fig. 4c) suggests that the coating of PDA not only on the surface, but also into the interior of the sphere, due to the existence of the pores. The fluorescence emission is also uniform in the general. It is to be noted that, the spheres were not exactly on the same level on the slide, which might make them look like having different sizes as seen in the confocal images.

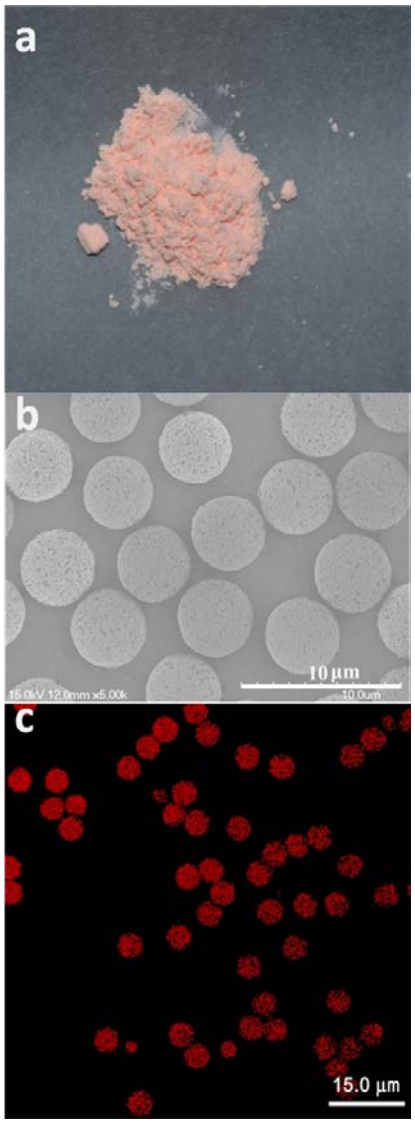


Fig. 4 (a) Photo, (b) SEM and (c)confocal fluorescence microscopic (excited at 488 nm) images of red APGMA-PDA microspheres.

Porosity and stability measurements

The porosity is preferred since the high surface-to-volume ratio is favourable for increasing the interaction between the spheres and the analytes. The Brunauer-Emmett-Teller (BET) surface area of final APGMA-PDA spheres is found to be 114 m^2/g. This surface area is higher than literature reports about spheres for immunoassay.[2] The measurement also shows that the average pore size is 20.6 nm with a deviation of ± 0.5 nm. Such high porosity properties with uniform pore size would be favorable for increasing the assay efficiency.[44, 45]

The thermal stability and the fluorescence stability of the spheres are very important for real application. The TGA curves (see ESI, Fig. S6) showed that the decomposition of the APGMA-PDA microspheres (>10% weight loss) started around 250 °C, which is very similar to the profile of APGMA spheres. The introduction of PDA onto APGMA microspheres has negligible effect on the thermal stability of APGMA microspheres. Such thermal stability is good enough to the immunoassay applications, which are usually carried out at room temperature.

The fluorescence stability of the spheres was examined from two aspects，the photostability and the solvent-resistance. The photostability, was studied by recording their emission spectra of the red APGMA-PPCDA microspheres every 10 min when exposed to continuous irradiation at 500 nm for 1 h (see ESI, Fig. S7), using a fluorometer with a regular set-up for routine fluorescence measurements for solid materials. Considering that these microspheres would not be used in some harsh environments, no strict photo bleaching experiment with a strong excitation light source was carried out. The emission intensity decreases slightly with the irradiation time prolongs. After being irradiated for an hour, the emission intensity of these spheres drop to 83% of their initial intensity. No significant changes can be found in the spectra profile, demonstrating that these spheres possess good photostability.

Another concern is that if fluorescent PDA on the spheres can be washed off by water since immunoassay usually is carried out in aqueous environment. The red APGMA-PDA microspheres were washed with deionized water twice. The filtrates from the washing step after removing the spheres displayed negligible emission (see ESI, Fig. S3). In addition, negligible changes in the emission intensities of the microspheres, before and after washing, from flow cytometer measurement (see ESI, Table S1), which gives direct proof for the fact the PDA loaded on the APGMA microspheres cannot be easily washed off. Therefore, conclusion can be made that fluorescence of the microspheres are stable against water, further confirming the existence of the strong interaction between PDA and the spheres.

Interaction between the fluorescent microspheres and biomolecules

Bovine serum albumin (BSA) is widely used as a repsentative biomolecule with amino groups, which can interact with carboxyl groups, in the bio-related research. FITC label BSAs (BSA-FITC) are selected in our system because the fluorescence emission of FITC would make it easy for us to detect if the bomolecules have been loaded onto the fluorescent microspheres. For comparison, APGMA-PDA-BSA-FITC microspheres and APGMA-PDA-FITC microspheres were prepared, by mixing the same amount of APGMA-PDA with same volume of BSA-FITC solution and FITC solutions, respectively. The concentration of the two solutions was controlled to give the same emission intensity. The emission of APGMA-PDA, APGMA-PDA-BSA-FITC and APGMA-PDA-FITC are shown in Fig.5. It is to be noted that APGMA-PDA microspheres has emission in 550 nm~ 650 nm, while FITC (or BSA-FITC) solution has emission in 500 nm~ 550 nm. For APGMA-PDA-BSA-FITC, the emission in 500 n~550 nm is

much stronger than that in 550 nm~ 650 nm, indicating BSA-FITC molecules have been successfully loaded onto APGMA-PDA microspheres. In comparison, much less FITC molecules was loaded onto the fluorescent microspheres,,since the emission of FITC is much weaker than that of PDA for e APGMA-PDA-FITC microspheres. In addition, APGMA-PDA-BSA-FTIC emitted much stronger fluorescence than APGMA-PDA-FITC when placing both of them under 365 nm UV light (insets in Fig.5). Conclusion can be drawn at this point that strong interaction existed between BSA and APGMA-PDA microsphere., while much weaker physical adsorption existed between the microsphere and FITC. It Is to be noted that, the emission profile of PDA varied slightly in different kinds of microspheres, which may be attributed to that emission of solid fluorescent materials is easily affected by the environment around fluorophores.

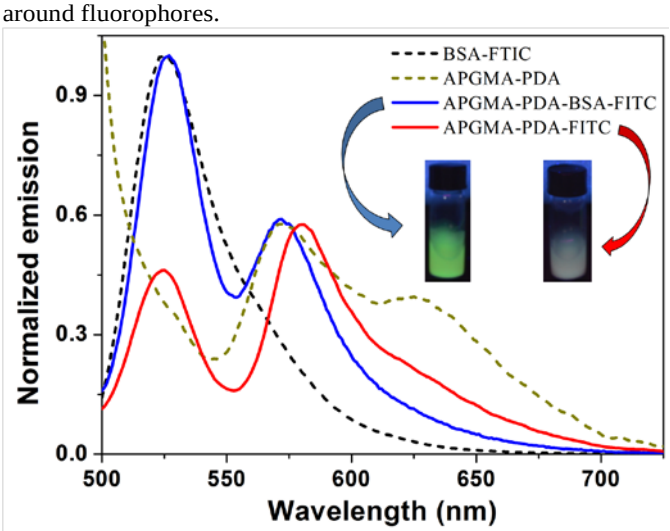


Fig.5. Normalized emission intensity of BSA-FITC in solution, APGMA-PDA microspheres , APGMA-PDA-BSA-FTIC microspheres and APGMA-PDA-FITC microspheres (in buffer solution,pH=7.4). The excitation was set at 480 nm. The insets are photos are APGMA-PDA-BSA-FTIC (left) and APGMA-PDA-FITC (right), under 365 nm UV irradiation.

4 Conclusions

In conclusion, we successfully loaded PDA onto the APGMA substrate microspheres via a self-assembled vesicle precursor pathway. The whole process for preparation and purification is very facile and controllable. The final resulting red-phase PDA-APGMA spheres were demonstrated to be very uniform in the size as well as in the fluorescence emission, indicating such fluorescent sphere is suitable for high-throughput immunoassay. In addition, these spheres have very high porosity with even pore distribution on the spheres. Such high volume-to surface area is favorable for the efficient interaction between analytes and spheres. Moreover, these spheres possess good thermal stability, photostability and do not suffer from leaching, which make them stable in real applications. The presence of carboxyl groups enable them to readily interact with many biomolecules, such as protein, antibody/antigen, oligonnucleotide and so on. Therefore, such fluorescent microspheres are very promising to be applied in the flow cytometry or microfluidic device for the fast, accurate and sensitive immunoassay.

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Notes and Reference

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1. J. V. Jokerst and J. T. McDevitt, *Nanomedicine*, 2010, **5**, 143-155.
2. H. Kawaguchi, *Prog. Polym. Sci.*, 2000, **25**, 1171-1210.
3. L. Martín-Banderas, A. Rodríguez-Gil, Á. Cebolla, S. Chávez, T. Berdún-Álvarez, J. M. Fernandez Garcia, M. Flores-Mosquera and A. M. Gañán-Calvo, *Adv. Mater.*, 2006, **18**, 559-564.
4. C. Lim and Y. Zhang, *Biosens. Bioelectron.*, 2007, **22**, 1197-1204.
5. L. Gervais and E. Delamarche, *Lab on a Chip*, 2009, **9**, 3330-3337.
6. E. Verpoorte, *Lab on a Chip*, 2003, **3**, 60N-68N.
7. L. Gervais, N. De Rooij and E. Delamarche, *Adv. Mater.*,2011,**23**, 151-176.
8. S. H. Kim, J. W. Shim and S. M. Yang, *Angew. Chem. Int. Ed.*, 2011, **123**, 1203-1206.
9. L. Riegger, M. Grumann, T. Nann, J. Riegler, O. Ehlert, W. Bessler, K. Mittenbuehler, G. Urban, L. Pastewka and T. Brenner, *Sens. Actuators, A: Physical*, 2006, **126**, 455-462.
10. L. J. Lucas, J.-H. Han and J.-Y. Yoon, *Colloids Surf., B:Biointerfaces*, 2006, **49**, 106-111.
11. A. Burns, H. Ow and U. Wiesner, *Chem. Soc. Rev.*, 2006, **35**,1028-1042.
12. H. Duan and S. Nie, *J. Am. Chem. Soc.*, 2007,**129**, 3333-3338.
13. J. Li, X.-W. Zhao, Y.-J. Zhao and Z.-Z. Gu, *Chem. Commun.*, 2009, 2329-2331.
14. J. H. Liao and T. M. Swager, *Langmuir*, 2007, **23**, 112-115.
15. K. Ogawa, S. Chemburu, G. P. Lopez, D. G. Whitten and K. S. Schanze, *Langmuir*, 2007, **23**, 4541-4548.
16. S. Wang, W. Zhao, J. Song, S. Cheng and L. J. Fan, *Macromol. Rapid Commun.*, 2013, **34**, 102-108.
17. X. Chen, G. Zhou, X. Peng and J. Yoon, *Chem. Soc. Rev.*, 2012,**41**, 4610-4630.
18. X. Sun, T. Chen, S. Huang, L. Li and H. Peng, *Chem. Soc. Rev.*,2010, **39**, 4244-4257.
19. B. Yoon, S. Lee and J.-M. Kim, *Chem. Soc. Rev.*, 2009, **38**,1958-1968.
20. D. J. Ahn and J.-M. Kim, *Acc. Chem. Res.*, 2008, **41**, 805-816.
21. M. A. Reppy and B. A. Pindzola, *Chem. Commun.*, 2007, 4317-4338.

22. A. Materny, T. Chen, A. Vierheilig and W. Kiefer, *J. Raman Spectrosc.*, 2001, **32**, 425-445.
23. G. Wegner, *Zeitschrift Fur Naturforschung Part B-Chemie Biochemie Biophysik Biologie Und Verwandten Gebiete*, 1969, **B 24**, 824-832.
24. J. Simon and A. Elizabeth, *Anal. Chem.*, 2006, **78**, 2231-2238.
25. J. Lee, O. Yarimaga, C. H. Lee, Y. K. Choi and J. M. Kim, *Adv. Funct. Mater.*, 2011, **21**, 1032-1039.
26. F. Lv, X. Feng, H. Tang, L. Liu, Q. Yang and S. Wang, *Adv. Funct.Mater.*, 2011, **21**, 845-850.
27. X. Pan, Y. Wang, H. Jiang, G. Zou and Q. Zhang, *J. Mater. Chem.*, 2011, **21**,3604-3610.
28. J. Lee, H. Jun and J. Kim, *Adv. Mater.*, 2009, **21**, 3674-3677.
29. H. Peng, J. Tang, J. Pang, D. Chen, L. Yang, H. S. Ashbaugh, C. J. Brinker, Z. Yang and Y. Lu, *J. Am. Chem. Soc.*,2005, **127**, 12782-12783.
30. W. Zhang, H. Xu, Y. Chen, S. Cheng and L.J. Fan, *ACS Appl. Mater. Interface*s, 2013.**5**,4603-4606
31. J. M. Kim, E. K. Ji, S. Woo, H. Lee and D. J. Ahn, *Adv. Mater.*,2003, **15**, 1118-1121.
32. M. Paley, D. Frazier, H. Abdeldeyem, S. Armstrong and S. McManus, *Journal of the American Chemical Society*, 1995, **117**, 4775-4780.
33. J.-M. Kim, Y. B. Lee, D. H. Yang, J.-S. Lee, G. S. Lee and D. J. Ahn, *J. Am. Chem. Soc*., 2005, **127**, 17580-17581.
34. K. Itoh, I. Kudryashov, J. Yamagata, T. Nishizawa, M. Fujii and N. Osaka, *J. Phys. Chem. B*, 2005, **109**, 271-276.
35. B.-H. Jun, J. Baek, H. Kang, Y. J. Park, D. H. Jeong and Y.-S. Lee, *J. Colloid Interface Sci*., 2011, **355**, 29-34.
36. W. Dong, J. Luo, H. He and L. Jiang, *Int. J. Nanomedicine*., 2013, **8**, 221-232.
37. S. Okada, S. Peng, W. Spevak and D. Charych, *Acc. Chem. Res.*, 1998, **31**, 229-239.
38. D. J. Ahn, E.-H. Chae, G. S. Lee, H.-Y. Shim, T.-E. Chang, K.-D. Ahn andJ.-M. Kim, *J. Am. Chem. Soc.*, 2003, **125**,8976-8977.
39. B. Yoon, D. Y. Ham, O. Yarimaga, H. An, C. W. Lee and J. M. Kim, *Adv. Mater.*, 2011, **23**, 5492-5497.
40. S. Kolusheva, T. Shahal and R. Jelinek, *Biochemistry*, 2000, **39**, 15851-15859.41. K.-W. Kim, H. Choi, G. S. Lee, D. J. Ahn and M.-K. Oh, *Colloids Surf., B: Biointerfaces*, 2008, **66**, 213-217.
42. Y.-l. Su, *J. Colloid Interface Sci.*, 2005, **292**, 271-276.
43. P. Palladino, V. Castelletto, A. Dehsorkhi, D. Stetsenko and I. W.Hamley, *Chem. Commun.*, 2012, **48**, 9774-9776.
44. J. V. Jokerst, J. Chou, J. P. Camp, J. Wong, A. Lennart, A. A. Pollard, P. N.Floriano, N. Christodoulides, G. W. Simmons and Y. Zhou, *Small*, 2011, **7**,613-624.
45. J.-h. Rong and L.-j. Ji, *Chin. J. Polym. Sci.*, 2013, **31**,1204-1217.

Graphic Abstract

Facile Preparation of Polydiacetylene-Based Uniform Porous Fluorescent Microspheres for Potential Immunoassay Application

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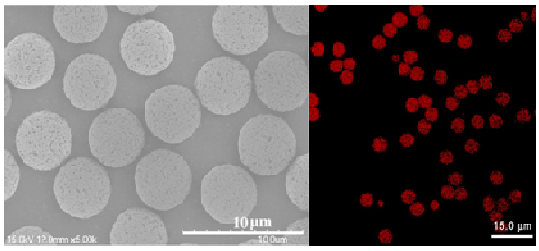

Fluorescent microspheres are prepared by loading PDA onto the substrate microspheres via a self-assembled vesicle precursor pathway.