Synthesis of Small Polymeric Nanoparticles Sized below 10 nm via Polymerization of a Cross-linker in a Glassy Polymer Matrix

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S1. Materials and instruments:

4-vinyl pyridine (95%, Aldrich) and divinylbenzene (technical, mixture of isomers, ~50% (GC), Fluka) were dried by CaH_2 for 24 hours and distilled before use. Azobisisobutyronitrile (CP, Shanghai No.4 Reagent & H.V. Chemical Co., Ltd) was purified by recrystallization in ethanol. Other chemicals are of analytical grade and used without further purification.

Preparation of the P4VP:

Poly (4-vinyl pyridine) was synthesis via free radical polymerization. The mixture of 4-vinyl pyridine (60 mL), AIBN (0.3 g) and ethanol (15 mL) was degassed by bubbling argon for 30 min, then heated to 70℃ and kept for 1 hour under mechanical stirring in the atmosphere of argon. The products were purified by re-precipitation in ethyl ether (45 g, 77%). The number average molecular weight (M_n) and polydispersity of P4VP (M_w/M_n) is 60.0 kDa and 2.27, respectively, as determined by GPC (DMF as eluent, polystyrene as standard). The glassy transition temperature (T_g) is 150℃ determined by DSC.

Fourier Transform Infrared (FT-IR):

The FT-IR spectra were recorded on a Nicolet Magna 550 spectrometer. All the samples were prepared by placing drops of solutions on CaF_2 substrates and drying under infrared lamp.

Dynamic Light Scattering (DLS) and Static Light Scattering (SLS):

A modified commercial light scattering spectrometer (ALV/SP-125) equipped with an ALV-5000 multi-digital time correlator and ADLAS DPY425Ⅱ solid-state laser (output power = 22 mW at λ_0 = 632.8 nm) was used. All the measurements were performed at 25℃. Prior to the light scattering measurements the sample solutions were filtered using filters (Millex, Nylon, 0.2 μm). All samples were analyzed at diluted aqueous (pH=4). The data evaluation of the dynamic light scattering measurements was performed with the CONTIN algorithm.

Transmission Electron Microscopy (TEM):

TEM observations were conducted on a Philips CM120 electron microscope at an acceleration voltage of 80 kV. The ultrathin polymerized films with a thickness of ca. 50 nm were obtained by microtoming. These microtomed films were deposited on carbon-coated copper grids and then stained with OsO_4 vapor (evaporating from a 1 wt% aqueous) for 24 hours before TEM observations. Samples of pure PDVB nanoparticles for TEM observations were prepared by depositing a drop of suspension of the hydrolyzed nanoparticles onto carbon-coated copper grids and drying in ambient conditions. RuO_4 vapor (fresh prepared from the reaction between RuO_2 and $NaIO_4$ in aqueous) was used to stain the samples to increase the contrast.

S2. DVB evaporation during the drying and polymerization processes:

The amount of DVB remained in each of the mixture films after the polymerization was determined and presented in Table S1. The compositions of the materials in the solutions were simply the weight ratios of DVB to P4VP (which were weighed, respectively, before the mixing) mixed in methanol to form the mixture solutions. As is mentioned in the text, the mixture films were prepared by film casting of the mixture solutions in methanol on to Teflon dishes. After having been dried at room temperature for 4 days, the films were polymerized at 65℃ for 24 hours. The polymerized films were peeled off from the Teflon dishes and weighed. The weight of a polymerized film minus the weight of the P4VP (the amount of the P4VP used in each of the films was fixed at one gram and the weight ratio of AIBN/P4VP was fixed at 0.05%) is the weight of PDVB. The difference between the weight of DVB in a feed solution and the weight of PDVB in the corresponding polymerized film is the DVB loss. As indicated in Table S1, the amount of DVB loss due to evaporation in a mixture system depends on the weight ratio of DVB/P4VP in the mixture solution in methanol; the higher the weight ratio, the larger the amount of the DVB loss. However, even when the DVB/P4VP weight ratio in the mixture solution is as high as 7.28% (sample P5, Table 1), there is 40% of DVB remained and transferred into PDVB after the processes of the drying and the polymerization.

Table S1. Compositions of DVB/AIBN/P4VP mixtures.

Samples	Weight ratio of DVB/P4VP in methanol (%)	Weight ratio of PDVB/P4VP in polymerized films (%)	Weight ratio of DVB that transfer into PDVB after polymerization (%)
P1	0.91	0.91	100
P2	1.82	1.16	64
P3	3.64	1.67	46
P4	5.46	2.49	44
P5	7.28	2.92	40

S3. The mixture films with P4VP as the matrix:

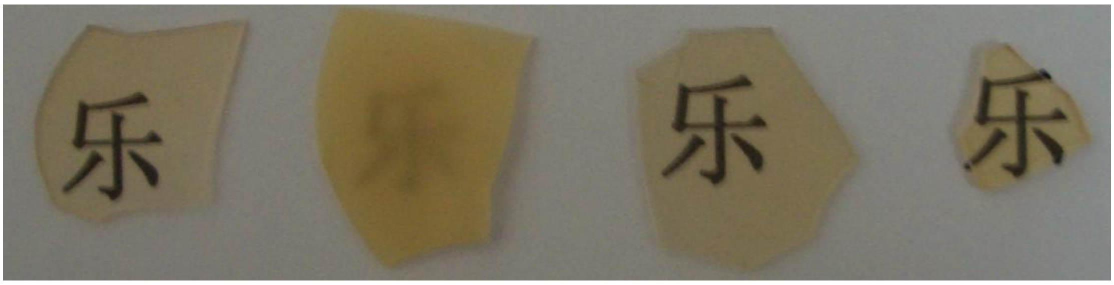

Fig. S1 Digital photographs of the films with P4VP as the matrix. From left to right is pure P4VP, liquid paraffin/P4VP mixture, DVB/AIBN/P4VP before polymerization and DVB/AIBN/P4VP after polymerization, respectively. The weight ratios of the solute/P4VP in the mixture films are 3.64%. In the photographs, behind each film is a Chinese character pronounced “le” which means happy.

S4. Size distributions of PDVB nanoparticles:

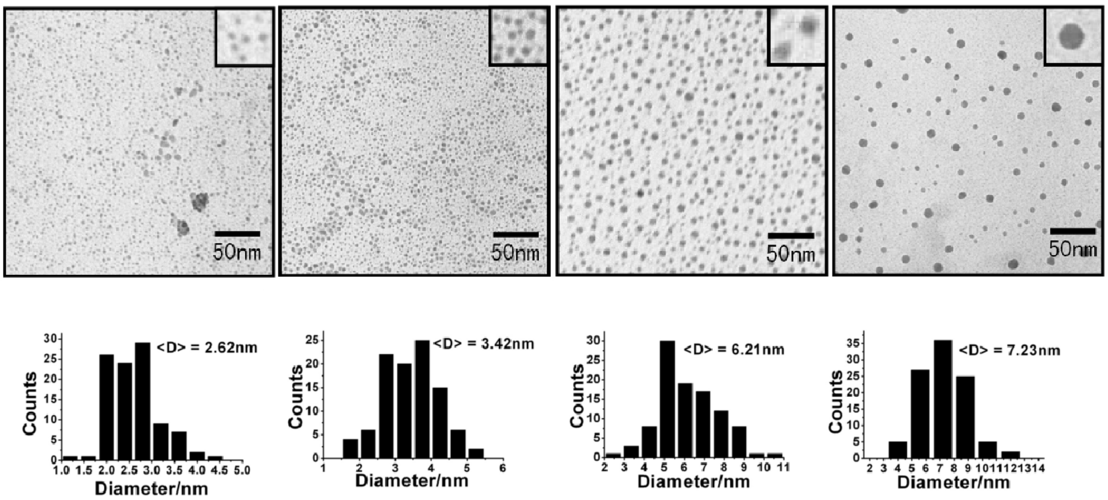

Fig. S2 TEM images of small PDVB nanoparticles (RuO_4 staining). The TEM images from left to right present nanoparticles produced from the samples P1, P2, P3 and P4, respectively. Insets are the images with larger magnifications. The size profile of nanoparticles in a TEM image was calculated by software and is given below the image.

S5. TEM images of microtomed polymerized mixture films:

To reveal how the large nanoparticles formed, the polymerized mixture films were microtomed, stained with osmium tetraoxide and then observed by TEM. In the TEM images of the microtomed films, the PDVB nanoparticles sized several nm cannot be clearly distinguished even they were stained since the thickness of surrounding P4VP films were about 50 nm. However, if there had been such large nanoparticles sized tens of nm (which should contain considerable amount of carbon-carbon double bonds, as indicated by the strong signal at 1632 cm^{-1} in Fig. 1, and be stained by osmium tetraoxide) in the microtomed films, they should have been observed. In other words, the fact that no large nanoparticles sized about tens of nm were found in the microtomed films by the TEM observations demonstrated that there were no such large nanoparticles.

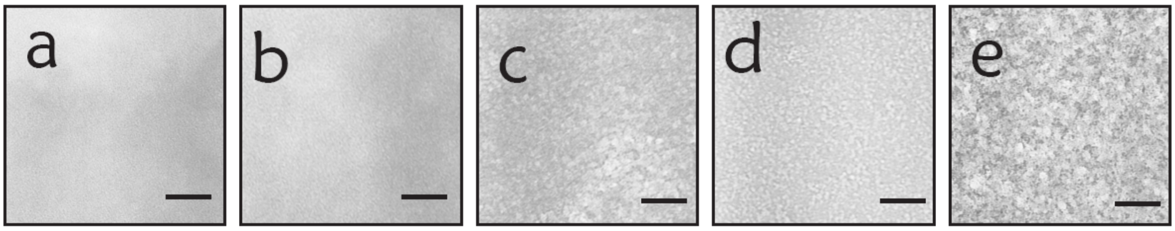

Fig. S3 TEM images of microtomed PDVB/P4VP films stained by OsO_4: a, P1; b, P2; c, P3; d, P4; e, P5. The scale bar represents 50 nm.

S6. Reason for using number (rather than intensity) averaged DLS curves to demonstrate the size profiles of the PDVB nanoparticles:

The intensity averaged DLS curves of the hydrolyzed PDVB nanoparticles show bimodal size distribution of the nanoparticles, indicating the coexistence of the small nanoparticles of several nm with large ones sized tens of nm. In principle, the scattering light intensity is proportional to both the number of scatterers and the square of the scatterer's mass, and the scatterer's mass is proportional to the cube of its radius (*Macromolecules*, 2000, 33, 2119.). In other words, the light scattering intensity by a nanoparticle sized 100 nm is equal to that by a million nanoparticles sized 10 nm with a similar density. Considering the high size ratio of the large nanoparticles to the small nanoparticles in each of the suspensions, it is reasonable to use number averaged DLS curves to show the size distributions of the as-prepared PDVB nanoparticles.

S7. Ratio of <Rg>/<Rh> determined by DLS and SLS:

We used light scattering to characterize the structure of the nanoparticles. It is known that ratio of <Rg>/<Rh> (<Rg> and <Rh> are average gyration radius and average hydrodynamic radius, respectively.) is related to the structure of the nanoparticles (*Colloid & Polymer Sci* 265:897-902 (1987); *Macromolecules* 1993, 26, 3821-3825). When the nanoparticles have a hyperbranched structure, the value of <Rg>/<Rh> is around 1.5 (*Journal of Colloid and Interface Science* 323 (2008) 242−246). We noted that, different from <Rh> values, <Rg> values measured for nanoparticles are with a certain instrument error (according to handbook of the LS instrument we used in the present study, the instrument error is 2%. This may lead to an instrument error in Rg of several nm), which affects smaller nanoparticles remarkably. The size of the PDVB nanoparticles prepared from P4 is larger than the sizes of the nanoparticles from P1, P2 and P3. Therefore, the <Rg>/<Rh> ratio of the PDVB nanoparticles prepared from P4 was measured to be 1.4. According to this <Rg>/<Rh> value and also the consideration on the large difference in the sizes of the same PDVB nanoparticles by TEM and DLS, we suggest that the PDVB nanoparticles may have a loose structure due to the diffusion limited mechanism for the particle formation. (*Colloid & Polymer Sci*. 261,635-644 (1983)).

S8. Polymerization of PDVB nanoparticles:

$(NH_4)_2S_2O_8$ (0.4 μg mL^{-1}) was used to initiate polymerization of the nanoparticles in the aqueous suspension (the nanoparticles were produced from the sample P3 according to the abovementioned processes. The concentration of the nanoparticles is 0.2 mg mL^{-1}) at 65℃ for 12 hours. The polymerization led to formation of precipitates within the suspensions. TEM and AFM observations demonstrated that the precipitates were nanoparticle films show porous structure, in which the outline of the primary PDVB nanoparticles is discernable.

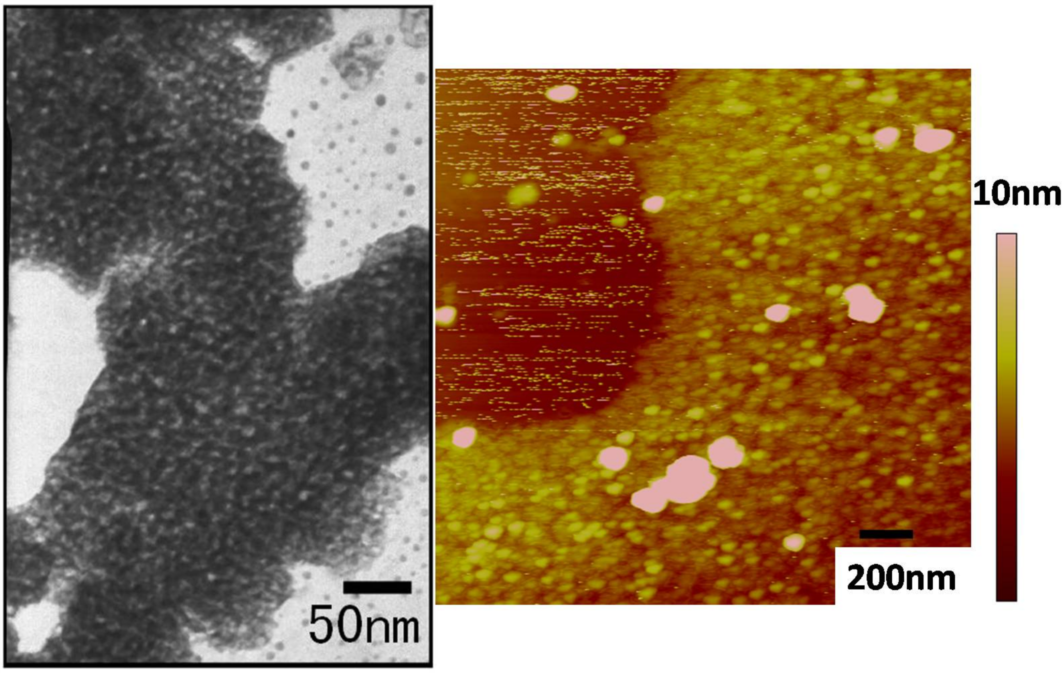

Fig. S4 TEM image (left) and AFM image (right) of nanoparticle films generated by polymerizing the PDVB nanoparticles (originated from sample P3) in the aqueous suspension.

S9. Hydrophobic domains within PDVB nanoparticles probed by pyrene:

Fluorescent spectrum of pyrene in the aqueous suspension of the PDVB nanoparticles was compared with that in pure water. The concentrations of pyrene in the aqueous suspension and the blank system (pure water) was 1.0×10^{-7} M. The concentration of PDVB in the aqueous suspension was ca. 0.5 mg mL^{-1}. The aqueous suspension and the blank system were degassed by bubbling argon gas through the solution for 1h before fluorescence measurements. The wavelength for excitation of pyrene is 335 nm and the spectra were recorded in the range of 350 ~ 550 nm. In the spectrum of the sample, as compared with the spectrum of the blank system, we can see the enhancement of the monomer peak and the appearence of a broad peak ranging from 390 ~ 550 nm. This demonstrates that there are hydrophobic domains within PDVB nanoparticles. (*Langmuir*, 1990, 6, 1437.)

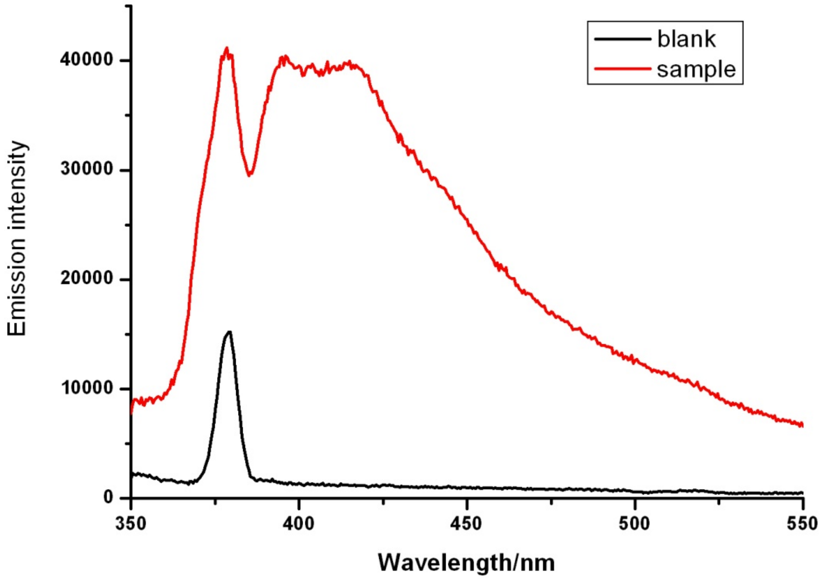

Fig. S5 Fluorescent spectra of pyrene in the aqueous suspension of PDVB nanoparticles and pure water.

S10. Nanoparticles smaller than 10 nm as drug carriers:

Nanoparticles smaller than 10 nm will be cleared rapidly by a biosystem. To overcome this disadvantage, we are trying to form superparticles using these small nanoparticles as the building blocks. It is expected that, when used as drug carrier the superparticles can dissociate into individual small nanoparticles under certain conditions and should behave very differently from the drug carriers based on molecular superstructures.