Green Synthesis and Potential Application of Low-toxic Mn: ZnSe/ZnS Core/Shell Luminescent Nanocrystals

Experimental

Chemicals. The human IgG (Ag), goat anti-human IgG (Ab1) and Au nanocrystals(NPs) linked with rat anti-human IgG (Ab2) were purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China). N-hydroxysuccinimide (NHS) and bovine serum albumin (BSA, 96–99%) were obtained from Sigma (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Pierce (Rockford,IL). 3-Mercaptopropionic acid (MPA, 99%) was a product of Fluka. Na_2S(99%), $Zn(CH_3COO)_2$, $Zn(NO_3)_2$ (99%), $MnCl_2$(99%), $NaBH_4$ (96%), Sodium oleate(99%) and selenium powder (99.999%, about 200 mesh) were obtained from Shanghai Reagent Company. Ethanol (CH_3CH_2OH, anhydrous) was of analytical grade and used without further purification. Other chemicals were of analytical grade. Phosphate buffer solution (PBS, 25 mM, pH=7.4) was prepared by mixing the solutions of K_2HPO_4 and NaH_2PO_4. The ultrapure water with 18.2MΩ (Millipore Simplicity, USA) was used in all procedure.

Apparatus. A microwave synthesis system (CEM Discover) made by CEM Instruments (USA) was used for the preparation of Mn-doped ZnSe nanocrystals, which was equipped with controllable temperature and pressure units. The system can operate at 2450 MHz frequency and work at 0-300 W power. The reaction temperature, pressure and time can be programmed by users. The synthesis of nanocrystals was preformed in a cylindrical digestion vessel that was high-strength vessel consisting of a special kind of glass. The volume of vessel used in the reaction was 80 mL. X-ray diffraction(XRD) measurements were performed on a Japan Shimadzu XRD-6000 powder X-ray diffractometer, using Cu Kα (λ = 1.5405 Å) as the incident radiation. High resolution transmission electron microscopy (HRTEM) images were recorded on a JEM-2010F with an accelerating voltage of 200 kV. HRTEM samples were prepared by dropping the samples dispersed in water onto carboncoated copper grids with excess solvent evaporated. UV-Vis absorption spectra were obtained using a UV-3600 spectrophotometer (Shimadzu). Fluorescence measurements were performed using a Bruker RF-5301PC fluorescence spectrometer. The room-temperature PL QY of the nanocrystals was estimated following the procedure of ref S1 using Rhodamine 6G as a reference standard. Inductively coupled plasma atomic emission spectroscopy(ICP-AES) was performed on a Perkin-Elmer Optima 3000DV after dissolving the aqueous nanocrystals in 5% hydrochloric acid. The X-ray photoelectron spectra (XPS) were taken on a Thermo Scientific K-alpha electron energy spectrometer using Al Kα (1486.6eV) as the X-ray excitation source. XPS sample was purified and washed carefully in order to remove any impurity phase.

Preparation of the Mn:ZnSe/ZnS Core/shell Nanocrystals. Using sodium oleate as capping reagents was based on the recently published procedure[S2]. Se^{2-} was generated by the reaction of

selenium powder and $NaBH_4$ according to the method described in ref S3. In a typical experiment, 3.0 g sodium oleate was added to the mixture of 15mL water and 5mL ethanol, and the pellucid solution was obtained. Then 10ml of aqueous solution containing 0.25g of zinc nitrate and 0.02g of manganese chloride, and 5 ml freshly NaHSe solution was added sequently to the sodium oleate solution. The typical molar ratio of Zn^{2+}:Se^{2-} was 3:1 in our experiments. The mixture was transferred to an 80ml cylindrical digestion vessel under agitation. The reaction was maintained at 170 °C and 175 psi for 40 min under microwave irradiation (260W). After purification using the standard precipitation-dissolution procedure, the as-prepared Mn-doped ZnSe nanocrystals about 80mg coated with the original alkyl ligands were dissolved in 20 ml chloroform and treated with 200μL MPA. The mixture was shaken for 30 min with sonication. The chloroform solution gradually became turbid due to the original ligands with a long hydrophobic alkyl chain were replaced by hydrophilic carboxyl chain of MPA. The MPA-coated Mn-doped ZnSe nanocrystals precipitate was isolated by centrifugation and decantation. Excess MPA was further removed by washing the precipitate three times with chloroform. The final precipitate was dried in vacuum and the flaxen Mn-doped ZnSe nanocrystals powders were obtained. Then the Mn-doped ZnSe nanocrystals were dissolved in water, adjusting pH 8.0 with $NaHCO_3$. The solution was deaerated with N_2 bubbling for 30 min. For the growth of ZnS shell, 1 .0mL of 2.4mM Na_2S solution and 1.0mL of 2.4mM $Zn(CH_3COO)_2$ solution was dropped alternately with strong stir. Reaction temperature was maintained 70℃ for 40mins. The size of the nanocrystals could be controlled by changing the heating time, which was monitored by UV–Vis absorption.

Bioconjugation of the Mn:ZnSe/ZnS Nanocrystals with Antibody. The synthesized aqueous solution of MPA-capped Mn:ZnSe/ZnS core/shell nanocrystals were purified with Milipore filtration tube(CMW= 3000) and dispersed in phosphate buffer solution (PBS, 10mM, pH 7.4). Then, the MPA-capped Mn:ZnSe/ZnS core/shell nanocrystals could conveniently conjugate to goat anti-human IgG (Mn:ZnSe/ZnS-Ab1). Then 250μL of freshly prepared 10 mg/mL EDC and 5 mg/mL NHS stock solution was respectively added to 5mL of the Mn:ZnSe/ZnS core/shell nanocrystals solution, and stirred for 30 min to activate the carboxylate groups. Then 200μL of the goat anti-human IgG solution containing 1.0mg of antibody in 200μL of PBS(pH 7.4) was added to the activated solution. To reduce nonspecific interaction, the rest active sites were blocked with 1% BSA solution. After reaction overnight, the free nonconjugated Mn:ZnSe/ZnS core/shell nanocrystals as well as the isourea byproduct of the conjugation reaction were removed by ultrafiltration. A 5.7-mL aliquot of the above mixture was subjected to ultrafiltration using a 50 000 MW filter; after the lower phase was removed, the upper phase containing Mn:ZnSe/ZnSe-Ab1 conjugations was decanted, diluted to 6 mL with PBS, and the solution was stored at 4 ℃.

Fluorescence Measurement. The fluorescence measurements were carried out at room temperature.

For every series of measurements, the mixture of Mn:ZnSe/ZnS-Ab1 and AuNPs-Ab2 was first treated as blank sample, and fluorescence spectrum was recorded. Various concentrations of the human IgG were then added to the blank and incubated for 1 h at 37 ℃ in darkness, and quenching spectra were taken.

S1 J. N. Demas and G. A. Crosby, *J. Phys. Chem*., 1971, **75**, 991.
S2 X. Wang, J. Zhuang, Q. Peng and Y. D. Li, *Nature*., 2005, **437**, 121-124.
S3 D. L. Klayman and T. S. Griffin, *J. Am. Chem. Soc*., 1973, **95**, 197-199.