

Synthesis of Quantum Dot-Tagged Submicrometer Polystyrene Particles by Miniemulsion Polymerization

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Introduction

Submicrometer fluorescent particles exhibiting narrow particle size distribution as well as good photostability are offering useful and suitable tools in a large number of medical and biotechnological applications, such as diagnosis, imaging or optical tracking. Recently, luminescent semiconductor quantum dots (QDs) have attracted the interest of many research groups as fluorescent probes because of their unique properties¹. Some of the most studied and documented QDs are composed of sole CdSe, or of a CdSe core passivated with a ZnS-shell. A few attempts have already been made to incorporate these outstanding fluorescent labels onto the surface or inside colloidal polymer particles via polymerization in dispersed medium. Using suspension polymerization^{2,3} or emulsion polymerization^{4,5}, QDs have been incorporated into polystyrene (PS) particles. More recently, two groups took advantage of miniemulsion polymerization^{6,7}, which allows the direct encapsulation of hydrophobic species into polymeric particles.

With the aim of synthesizing submicrometer and monodisperse fluorescent particles, we have carried out an extensive study of the incorporation of hydrophobic TOPO-coated CdSe/ZnS QDs into polystyrene particles via miniemulsion polymerization⁸. QDs concentration as well as the surfactant concentration have been varied in order to investigate the influence of these parameters on the miniemulsion polymerization kinetics and photoluminescence properties of the final particles. Finally, functionalized fluorescent particles have been obtained via incorporation of carboxylic groups onto the surface of the particles.

Experimental

Styrene (with acrylic acid (AA), if used) was first mixed with hexadecane and QDs nanocrystals (3.5-4 nm TOPO-coated CdSe/ZnS QDs either purchased from Evident Technologies or home-made). This organic phase was then added to the aqueous phase (deionized water, sodium docetyl sulfate (SDS) and sodium hydrogencarbonate) under vigorous stirring. After 10 minutes, the resulting mixture was ultrasonified (HD 2200 Bandelin Sonoplus ultrasonic homogenizer) for several minutes. The obtained stable miniemulsion was then transferred to the reactor and was deoxygenated by purging with nitrogen for 45 minutes, while the temperature was raised to 75°C. Finally, the addition of potassium persulfate (KPS) or 4,4'-azobis(4-cyanopentanoic acid) (ACPA) gave the zero time of the polymerization. Monomer consumption was followed by gravimetry. The particle size and particle size distribution were obtained by dynamic light scattering (Zetasizer 3000HS, Malvern Instruments). The particles morphologies and particle size distribution were further examined by (high resolution) transmission electron microscopy (Philips CM 120 and Jeol 2010F). Fluorescence emission spectra were recorded using a fluorescence spectrophotometer (LS 50 system, Perkin Elmer). Fluorescence imaging was performed with a Zeiss AxioPlan 2 Imaging microscope, equipped with a camera and a 100X infinity-corrected 1.3 numerical aperture oil objective. Surface charge density (σ) was determined using a 4330 Jenway conductimeter - pHmeter.

Results and discussion

Influence of initial QDs concentration. First experiments in miniemulsion were carried out with various initial amounts of TOPO-coated QDs. In contrast with what was observed in conventional emulsion polymerization, stable and slightly pinkish latexes were obtained, which became fluorescent under UV light. The addition of QDs actually had no marked effect on the polymerization kinetics, and complete conversion was reached in ca. 4 h. Final particle diameters were in each case close to 250 nm, indicating that the initial QD

concentration had no significant influence on the final particle size. All of the latexes exhibited a narrow particle size distribution.

Fluorescence analysis of the washed and dried particles dissolved in chloroform followed the expected trend, i.e. the fluorescence signal increased with the number of incorporated QDs. All of the latexes exhibited narrow emission peaks (fwhm ca. 40 nm) with an emission wavelength of ca. 589 nm, a slightly red-shifted value compared with that of the QDs alone (583 nm), which indicated an alteration of the optical properties of the QDs. This may be correlated with changes induced by the polymerization in the vicinity of the QDs surface. To explain the slight red shift of the emission maximum, the latexes were further analyzed by electron microscopy and EDX analysis, and QDs were found to be essentially located in the outer shell of the particles. A phase separation between PS and QDs occurred during the polymerization, driving the QDs toward the particle/water interface. It was, however, difficult to determine if the phase separation induced (partial) aggregation of the QDs or if the QDs were just excluded from the PS phase without aggregation. In any case, these observations may explain the slight red shift of the emission observed for all of the tagged particles.

The number average of QDs per polystyrene particle N_{QP} was found to be between 20 and 130, depending on the initial QD concentration. N_{QP} stands in the range of values recently reported for emulsion polymerization⁵ or miniemulsion⁷. Moreover, linear evolution of the fluorescence intensity with the number of QDs introduced is observed. Finally, a further indication of effective incorporation of the QDs was given by optical microscopy which suggest by comparison of transmitted light signal and fluorescence that the whole of the particles is effectively fluorescent.

Influence of surfactant concentration. To assess the influence of the number of QDs per particle, a series of experiments was performed with varying amounts of SDS. As expected, increasing SDS concentration induced a higher polymerization rate, which was directly correlated with an increase in the final number of particles N_p . Complete conversion was achieved in each case, and all of the latexes were isodispersed in size with a final particle diameter of between 168 and 320 nm. The number of QDs per particle N_{QP} logically decreased when N_p increased, and N_{QP} could be correlated with the initial amount of SDS for a given QD concentration.

Functionalization of the particles. To be successfully used in bio-related fields, QDs-tagged particles need to interact with biomolecules, which implies functionalization of the particle surface with reactive groups such as carboxylic acids. We tried to incorporate carboxylic groups via two different procedures. Acrylic acid was first copolymerized with styrene but this led to non fluorescent latex. This was explained by a possible interaction of acrylic acid and the surface of the QDs. In the second case, ACPA was used instead of KPS. The final particle diameter was lower (140 nm) but the particle size distribution remained narrow. Surface charge titration of the dialysed latex indicated an effective incorporation of the carboxylic groups onto the surface of the particles ($\sigma = 21 \mu C/cm^2$).

Conclusions

In conclusion, submicrometer and isodispersed fluorescent latexes functionalized with carboxylic groups can be easily synthesized using miniemulsion polymerization. The results reported herein open up new opportunities for the development of biofunctionalized submicrometer particles suitable for a variety of applications in biotechnology.

References

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