Microreactors

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Synthesis of Carbohydrate-Functionalized Quantum Dots in Microreactors**

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Large quantities of monodisperse semiconductor nanocrystals,[1] quantum dots (QDs), are needed for applications in electronics and the life sciences.[2] For biological applications, the surface of QDs is often functionalized with carboxylic acids for the attachment of proteins[3] or directly with carbohydrates.[4] Traditional batch processes are of limited utility for the production of QDs on a larger scale owing to limited temperature control and lack of homogeneous mixing.[5] Continuous-flow microreactors provide precise control over reaction conditions, including temperature, and the production time is independent of the process scale.[2,6] The high surface-to-volume ratio[5] of the microreactor channels enables precise temperature control as well as efficient mixing, allowing for the preparation of QDs with narrow size distribution.[7] QDs have been prepared using microfabricated gas–liquid and liquid–liquid flow reactors.[8] The preparation of surface-functionalized QDs under mild reaction conditions in the liquid phase remains challenging. Ideally, a continuous process would serve to both produce the quantum dots and to functionalize them.

Herein we present a single-phase microfluidic system for the synthesis of highly luminescent, surface-functionalized CdSe and CdTe nanoparticles. In contrast to batch processes, which require temperatures of 250–300 °C, temperatures of 160 °C are sufficient in the flow process.[8] Both the formation of the zinc sulfide shell and the functionalization of the nanoparticles with carboxy groups and carbohydrates were perfomed in a continuous-flow system (Figure 1). Different-sized quantum dots were obtained by simply varying the reaction time in the flow reactor.[9] High reaction temperatures usually result in fast nucleation, and large nanocrystals are quickly obtained. At low temperatures, the size of the nanocrystals and the concentration of the unreacted precursors in the mixture can be balanced. Thus, continuous nucleation is suppressed, the residence time distribution (RTD) is narrowed, and homogeneous QD fractions are obtained by varying the reaction time. The homogenous reaction mixture and slow nucleation results in a mild process for the production of QDs using microreactors.

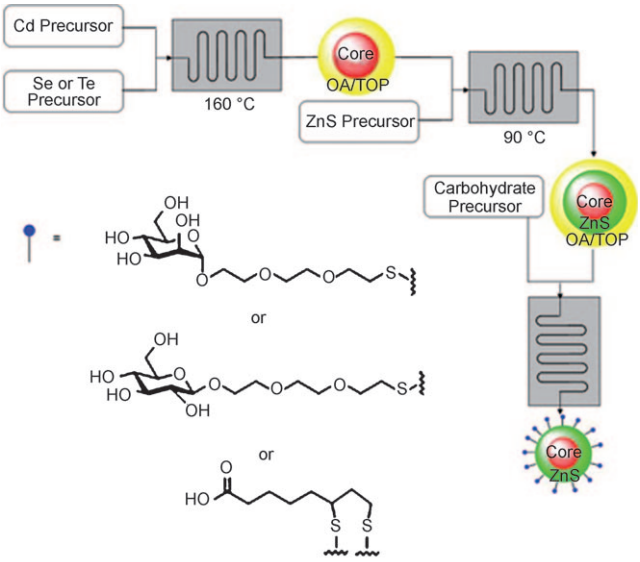

Figure 1. Microreactor setup for the continuous-flow synthesis of functionalized QDs (OA: oleic acid; TOP: tri-*n*-octylphosphine).

CdSe and CdTe nanoparticles with different emission maxima were prepared by injection of a 1:1 mixture of Cd precursor[8] and Se or Te precursor. The Cd precursor was prepared by the addition of oleic acid and oleylamine to a solution of cadmium oxide dissolved in lauric acid at 150 °C. The Se and Te precursors were prepared by dissolving elemental selenium or tellerium powder in tri-*n*-octylphosphine (TOP) in a Syrris microreactor. Reaction times ranged from 3 to 30 minutes. The CdSe and CdTe cores were purified by precipitation from methanol/chloroform/*n*-hexane and dried under vacuum. The average size distribution of each sample was calculated from the absorbance spectra (see Figure 1 in the Supporting Information).[12]

The optical properties of the QDs show a time-dependent bathochromic shift in the band-edge emission and enhanced intensity. The photoluminescence peaks of CdSe QDs are sharp, with fwhm (full width at half maximum) values of the band-edge luminescence between 40 and 50 nm (Figure 2), which indicates the narrow size distribution of the QDs. However, after 30 minutes of reaction time the fwhm increased from 40 to 90 nm, and a decrease in quantum yield indicated that saturated nucleation occurred after 20–

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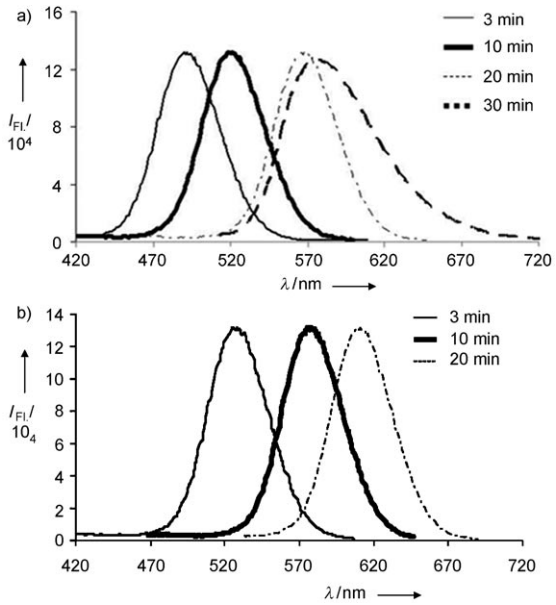

Figure 2. Normalized luminescence spectra of a) CdSe nanoparticles in chloroform after 3, 10, 20, 30 min, and b) CdTe nanoparticles in chloroform after 3, 10, 20 min. For more information see Table 1.

30 minutes at 160 °C. Longer reaction times decreased the quantum yield of the nanoparticles and altered the dispersity (Table 1). A slow reaction between unreacted precursor and saturated nanocrystals is likely responsible for this result.

Table 1: Photophysical properties of CdSe (entries 1–4) and CdTe (entries 5–7) nanoparticles.

Entry	*t* [min]	λ_{max} [a,b] [nm]	*d*(particle) [b] [nm]	QY [c] [%]
1	3	489 ± 4	1.45 ± 0.22	8 ± 1
2	10	514 ± 6	1.83 ± 0.26	11 ± 1
3	20	558 ± 4	2.64 ± 0.43	19 ± 1
4	30	562 ± 5	3.06 ± 0.34	15 ± 1
5	3	521 ± 3	1.67 ± 0.27	14 ± 1
6	10	565 ± 4	3.01 ± 0.36	21 ± 1
7	20	598 ± 4	3.24 ± 0.49	23 ± 1

[a] Excitation at λ_{max} 350 nm; sample was prepared in toluene. [b] Error represents standard deviation from the mean of three experiments. [c] Quantum yield was determined relative to that of fluorescein at 470 nm (0.93).

After assessing the optical properties of the QDs, we selected $CdTe_{598}$, which was produced in 20 minutes at 160 °C, for further modifications by continuous and discontinuous processes. A freshly prepared mixture of hexamethyl disilathiane, TOP, diethylzinc in toluene, and zinc sulfide was injected separately into the microreactor from the QD solution. The temperature was maintained at 90 °C and the residence time was 30 minutes.[10] The resulting ZnS-coated $CdTe_{598}$ particles were purified by precipitation from methanol/chloroform followed by drying under vacuum. Transmission electron microscopy (TEM) images reveal highly crystalline, monodisperse, cubic nanoparticles (Figure 3).

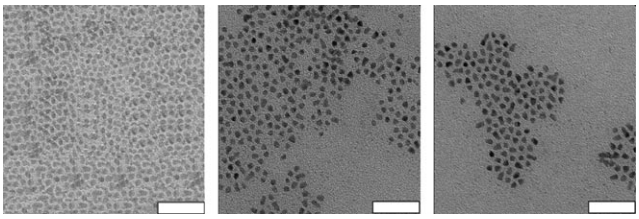

Figure 3. TEM images of a) CdTe core nanoparticles; b) CdTe/ZnS nanoparticles; c) CdTe/ZnS/mannose nanoparticles. Scale bars = 50 nm.

Photoluminescence measurements of these QDs demonstrate that the quantum yield increased from 23 % to 31 % because the ZnS shell stabilizes the $CdTe_{598}$ core.

The ZnS-coated $CdTe_{598}$ quantum dots were functionalized by ligand exchange with pyridine in continuous flow. Freshly prepared oleic acid coated $CdTe_{598}$/ZnS QDs were dissolved in pyridine and injected into the microreactor, where they resided at 60 °C for 30 minutes. The resulting pyridine-coated $CdTe_{598}$/ZnS QDs were surface-modified with carbohydrates. A mixture of freshly prepared dihydrolipoic acid and mercapto-polyethylene glycol (PEG) α-mannose or mercapto-PEG β-galactose in dichloroethane/ethanol (1:1) and QD solutions were simultaneously injected into the microreacter; the reaction mixture had a residence time of 30 minutes at 50 °C. The sugar-coated quantum dots were purified by precipitation from a mixture of *n*-hexane/chloroform/methanol (9:1:1) and dissolved in water for characterization. The UV/Vis and fluorescent spectra of the quantum dots did not change following sugar coating. Dihydrolipoic acid functionalized quantum dots were purified by treatment with tetramethylammonium hydroxide solution and served as a basis for further surface functionalization.[2f,11] The mannose-modified quantum dots are monodisperse particles of the same crystalline form as the precursor quantum dots. The TEM images indicate that the QD surface was not altered by the sugar coating.

Interactions between the sugar-coated QDs and proteins were studied by turbidity measurements using the prototypical lectin concanavalin A (ConA) and α-mannose-functionalized QDs. The binding of ConA to the mannose QDs resulted in immediate turbidity, whereas reaction of the β-galactose QDs showed little turbidity (Figure 4). ConA binds

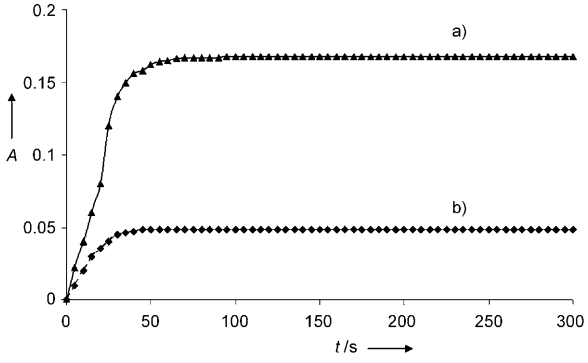

Figure 4. Kinetics of turbidity by a) α-mannose and b) β-galactose-coated QDs upon addition of concanavalin A.

to mannose but not to galactose. Addition of excess mannose inhibited binding, which demonstrated that the turbidity is caused by specific carbohydrate–protein interactions (see Figure 2 in the Supporting Information). Similar results were also observed in fluorescent mode and proved that agglutination results from the specific interaction between the ConA and the carbohydrates on the QD surface (see the fluorescence measurements in Figure 3 in the Supporting Information).

In conclusion, several continuous-flow microreactor processes were utilized to synthesize carbohydrate and carboxylic acid functionalized quantum dots with emission maxima ranging from 480 to 598 nm. The carboxylic acid groups on the surface of the QDs serve as convenient handles for the attachment of molecules of interest. Commercially available starting materials were used, and lower reaction temperatures resulted in narrow size distribution. Nanocrystals of defined size can be prepared reproducibility and efficiently on a large scale. The microreactor system was not only used to synthesize quantum dots, but also to modify the surface of the QDs with biologically relevant molecules. Specific carbohydrate–lectin interactions were observed between carbohydrate-coated QDs and ConA. The continuous-flow synthesis of PEGylated quantum dots and the surface modifications of these nanoparticles with other biological molecules are currently under investigation.

Experimental Section

Preparation of CdSe and CdTe QDs: The cadmium precursor was prepared by heating cadmium oxide (100 mg, 0.75 mmol) with lauric acid (600 mg, 3.1 mmol) at 150 °C until a clear solution was obtained. This solution was cooled to room temperature and oleic acid and oleylamine (1.5 mL each) were added to the flask. A solution containing either selenium (80 mg, 1.0 mmol) selenium in trioctylphosphine (2 mL, 6.76 mmol) or tellurium (120 mg, 0.97 mmol) tellurium in trioctylphosphine (2 mL, 6.76 mmol) was prepared. A solution of the cadmium precursor (0.097 mmol) in squalene (0.5 mL) and a solution of the Se or Te precursor (0.097 mmol) in squalene (0.5 mL) were pushed into the microreactor using two syringe pumps.[6] CdSe or CdTe QDs were prepared at reaction times of 3, 10, 20, and 30 min at flow rates of 333, 100, 50, and 33.33 μL min^{-1}. The QDs were purified by precipitation from anhydrous $CHCl_3$/MeOH/*n*-hexane/isopropanol (1:3:4:2–1:4:4:1) to yield 13 mg (72%, after 3 min), 15 mg (71%, after 10 min),18 mg (67%, after 20 min), and 17 mg (66%, after 30 min) of product.[13]

Preparation of CdTe/ZnS QDs: A solution of the CdTe QDs (20 mg) in toluene (1 mL) and trioctylphosphine (2 mL) was used as the nanocrystal precursor. Trioctylphosphine (2 mL), hexamethyldisilathiane (50 μL, 0.28 mmol) and 10% diethylzinc in toluene (400 μL) were mixed. The two solutions were injected into the microreactor at 90–110 °C at a flow rate of 33.33 μL min^{-1} (residence time of 30 min). The resulting QDs were purified by precipitation from chloroform/methanol (3:7–1:9) to yield 22 mg (68%)[13] of product. The oleic acid coating on the CdTe/ZnS QDs was exchanged for pyridine by dissolving the QDs (20 mg) in pyridine and passing the solution through the microreactor at 60 °C at a flow rate of 33.33 μL min^{-1} (30 min residence time). Precipitation from *n*-hexane followed by centrifugation yielded 12 mg (74%)[13] of the pyridine-coated QDs.

Preparation of mannose- or galactose-coated QDs: A solution of the pyridine-coated CdTe/ZnS QDs (10 mg) in dichloroethane (1 mL) and a solution of either 2-(2-(2-thioethoxy)ethoxy)ethoxy-α-D-mannopyranoside (35 mg, 0.11 mmol) or 2-(2-(2-thioethoxy)ethoxy)ethoxy-β-D-galactopyranoside (35 mg, 0.11 mmol) in 1 mL dichloroethane/ethanol (1:1) were prepared. The solutions (0.5 mL each) were simultaneously injected into the microreactor, which was preheated at 50 °C, at a flow rate of 33 μL min^{-1} (30 min residence time). The solvent was evaporated and the carbohydrate-coupled QDs were precipitated from *n*-hexane/chloroform/methanol (9:1:1). The final concentration of the sample was estimated by using a published procedure.[14]

Preparation of dihydrolipoic acid coated QDs: A solution of pyridine-coated CdTe/ZnS QDs (10 mg) in dichloroethane (1 mL) and a solution dihydrolipoic acid (20 mg, 0.10 mmol) in 1 mL of dichloroethane /ethanol (1:1) were prepared. The solutions (0.5 mL each) were simultaneously injected into the microreactor at 50 °C at a flow rate of 33.33 μL min^{-1} (residence time 30 min). The solvent was evaporated and dihydrolipoic acid coated QDs were precipitated by addition of tetramethylammonium hydroxide. The final sample concentration was estimated by using a published procedure.[14]

Preparation of mannose- or galactose-coated CdSe/ZnS QDs: The cadmium precursor was prepared by heating cadmium oxide (100 mg, 0.75 mmol) with lauric acid (600 mg, 3.1 mmol) at 150 °C until a clear solution was obtained. This solution was cooled to room temperature, and oleic acid and oleylamine (1.5 mL each) were added to the flask. A solution containing selenium (80 mg, 1.0 mmol) in trioctylphosphine (2 mL, 6.76 mmol) was prepared. A solution of the cadmium precursor (0.097 mmol) in squalene (0.5 mL) and a solution of the the Se precursor (0.097 mmol) in squalene (0.5 mL) were introduced to the microreactor using two syringe pumps (residence time 15 min, flow rate 66.66 μL min^{-1}). The CdSe QDs were then flushed directly into another microreactor at 90–110 °C. A solution of trioctylphosphine (2 mL), hexamethyldisilathiane (50 μL, 0.28 mmol) and 10% diethylzinc in squalene (400 μL) was prepared and injected separately. Finally a solution of the CdSe/ZnS QDs in dichloroethane (1 mL) and a freshly prepared solution of 2-(2-(2-thioethoxy)ethoxy)ethoxy-α-D-mannopyranoside (35 mg 0.11 mmol) were flushed into a third microreactor at 60 °C to give 12 mg (52%) of the final compound.

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