Water-soluble CdSe nanoparticles stabilised by dense-shell glycodendrimers†

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A simple and rapid method has been developed to prepare water-soluble CdSe nanoparticles at room temperature, with average particle diameters around 2 nm, stabilised by maltose-modified 2nd–5th generation poly(propylene imine) (PPI) dendrimers.

Over the past decade, semiconductor nanoparticles have attracted significant attention due to their unique physical properties in different applications, such as nanomedicine and sensors.[1–4] One of the main characteristics of these materials is the ability to govern their optical properties as a function of their nanoparticle size.[1] For medical applications, the key aspect is not only the design of semiconductor nanoparticles as chromophoric tools with appropriate optical properties, but also to achieve a synergy with the biological environment, aiming for the water-solubility, biocompatibility and non-toxicity of nanoparticles.

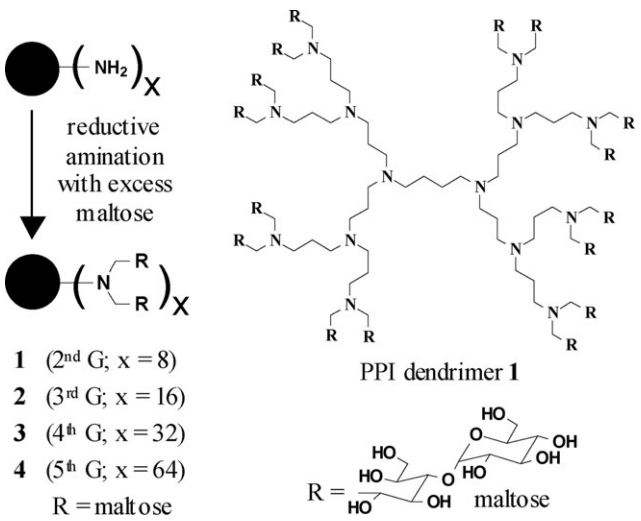
In recent years, high temperature ($\leq$300 °C) TOPO[5] or TOPO/TOP[6,7] (TOPO = trioctylphosphine oxide and TOP = trioctylphosphine) approaches have been established for the synthesis of hydrophobic CdSe nanoparticles capped with phosphine ligands. Such particles were converted with different low molecular weight ligands to achieve various surface functionalisations,[5,8,9] including (a) dendronisation[10] of the particle surface and (b) encapsulation in a dendrimer shell.[11,12] The outcome of these modification steps are stable, water-soluble nanoparticles that can be applied in bioprobes for protein interactions with biotin-labeled nanoparticles,[13] live-cell imaging[11] and protein transfection agents.[14] However, simpler methods for the preparation of semiconductor nanoparticles have been developed to realize the direct formation of water-soluble nanoparticles in an aqueous environments below 40 °C.[15–21] This process enables the preparation of water-soluble, highly fluorescent nanoparticles[22] (*e.g.* CdSe), which also includes the formation of dendronized CdSe/CdS nanoparticles[23] for use as bioprobes.

Recent developments have also outlined the formation and stabilization of nanoparticles in the presence of glycodendrimers and hyperbranched polymers with various (oligo-)saccharide units.[24–26]

Herein, we explore an alternative, simple approach to prepare CdSe particles based on the direct application of water-soluble and readily available ligands. Maltose-modified 2nd–5th generation poly(propylene imine) (PPI) dendrimers[27] **1–4** (Scheme 1) have been used for the direct *in situ* formation and stabilization of CdSe nanoparticles at room temperature in aqueous environments. These glycodendrimers are characterised by a dense-shell maltose architecture[27] that does not allow structural rearrangement, as compared to amphiphilic dendrimers[28,29] decorated with aliphatic surface groups.

Oligosaccharide-modified PPI dendrimers can serve as multi-functional and highly biocompatible macromolecules for potential diagnostic and therapeutic[27] applications because they combine apparently contrary properties (*e.g.* water-solubility, non-ionic surface groups, neutral surface charge, non-specific hydrogen bond-forming surface groups, high biocompatibility and potential drug encapsulation/release) more effectively than previously described glycodendrimers.[30] The application of these glycodendrimers to the formation and stabilisation of uniform nanoparticles with defined optical properties will further enhance their range of potential applications.

Colloidal **1–4**-stabilised CdSe particles were generated using the following method under an N_2 atmosphere. A 0.9 mL



Scheme 1 Simplified reaction scheme and the structure of **1–4** (G = generation).

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aqueous $Cd(OAc)_2{\cdot}2H_2O$ solution (10 mM) was taken up in a 10 mL aqueous solution of maltose-modified dendrimer (0.3 mM) at about pH 6.8. A second solution (0.9 mL) of NaSeH (5 mM) in ethanol was slowly added to the Cd(II) dendrimer solution at room temperature to yield different colloidal solutions of CdSe particles (Cd : Se ratio 2 : 1) stabilised by the respective dendrimer **1–4**. Additional dialysis with distilled water was carried out over 24 h to separate the undesired inorganic impurities from the solution. Subsequently, a yellow solid could be isolated by freeze drying. Additional control experiments confirmed that the neat oligosaccharide maltose was not able to stabilise the CdSe particles, while the unmodified PPI dendrimers (DAB-Am8, DAB-Am16, DAB-Am32 and DAB-Am64) could stabilize the particles to a certain extent. In either case, exceeding the limiting particle concentration lead to immediate precipitation of CdSe particles after the addition of NaSeH to the aqueous solutions. Stable colloidal solutions of CdSe particles (in a dark environment up to several weeks in a closed glass flask) were obtained only when the oligosaccharide units were chemically bound to the outer sphere of the PPI dendrimers.

Fig. 1 shows typical UV-vis absorption and photoluminescence (PL) spectra of maltose-modified dendrimer-stabilised CdSe particles with a Cd : Se ratio of 2 : 1. Colloidal solutions of CdSe particles stabilised by **1–4** revealed comparable UV-vis absorptions. The UV-vis absorption typically shows a maximum at around 450 nm, which corresponds to the generation of excitons in the semiconductor CdSe nanoparticles (gap energy E_g = 2.75 eV). This absorption behaviour below 500 nm has been reported before for very small colloidal CdSe particles ($\leq$2 nm) stabilised by n-butylamine.[31] At excitation wavelengths of 430 nm, the CdSe nanoparticles showed strong photoluminescence, with emission bands at 530 ± 10 nm. The PL intensity was, however, weak compared to CdSe quantum dots (QDs) stabilized with TOPO or TOPO/TOP.[5–7] The lower quantum yield can be attributed to the thickness of the dendrimer shell adsorbed to the nanoparticle surface. This organic shell is itself in the range of the particle diameter, and to a certain degree leads to quenching of the fluorescence.

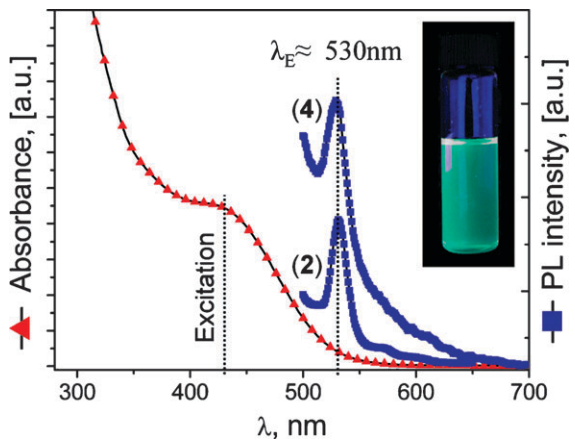

Fig. 1 Typical UV-vis absorbance and PL emission spectra of **2**- and **4**-stabilised CdSe nanoparticles in aqueous solution. The inset shows a photograph of **2**-stabilised CdSe nanoparticles under UV illumination (365 nm).

However, as typical for semiconductor QDs,[7,32] the emission band of the dendrimer-stabilized CdSe nanoparticles is relatively narrow (FWHM = 22 nm). Additionally, the photoluminescence spectrum of CdSe nanoparticles stabilized with 3rd generation PPI dendrimer, **2**, showed a weak secondary emission band at 573 nm, which may have been caused by incomplete surface passivation at low generations.[33]

The emission wavelength of around 530 nm corresponds to a photon energy of E = 2.33 eV; similar values have been reported by Alivisatos and co-workers for CdSe quantum rods with different lengths and diameters below 5 nm.[34] It was demonstrated that precise control over the nanorod dimensions and aspect ratio could significantly affect the optical properties of the nanoparticles. In contrast to that, no significant influence of dendrimers **1–4** on both the UV-vis absorption and photoluminescence (PL) of glycodendrimer-stabilized CdSe nanoparticles (Cd : Se ratio 2 : 1) was observed. The particle size of the dendrimer-stabilised CdSe nanoparticles could be estimated from the UV-vis and PL spectra,[35] giving an approximate particle diameter of 2–3 nm. Contrary to these results, the formation and stabilisation of CdSe particles by **1–4** based on a Cd : Se ratio of 1 : 1 exhibited non-fluorescent properties. Small, fluorescent CdSe nanoparticles are initially formed. As the selenium concentration increases, the fluorescence becomes weaker due to the higher Se : Cd ratio on the surface of the nanoparticles. Therefore, one can conclude that the surface composition of the CdSe QDs and the particle stabilisation by the glycodendrimers are two important factors that determine the fluorescent properties.

The transmission electron microscopy (TEM) micrographs in Fig. 2 and the ESI (Fig. 1-ESI†) reveal only a weak correlation between the generation of the dendrimer and the average particle size. Histograms of the particle size, as determined from TEM measurements using each dendrimer **1–4**, show only slightly increasing particle sizes at higher generations of dendrimers. The average particle diameter is

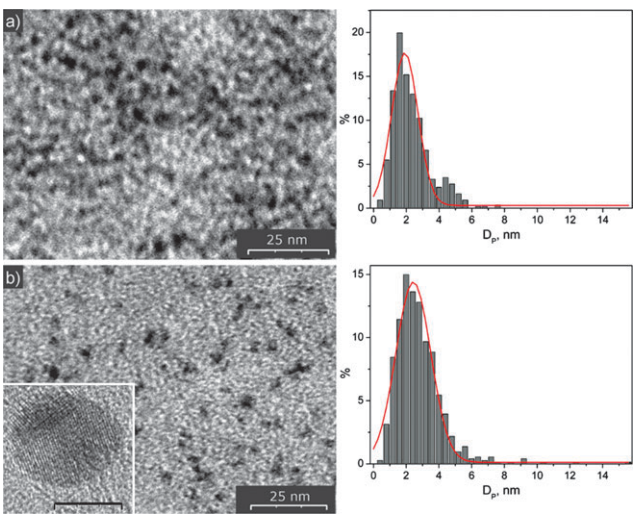

Fig. 2 TEM micrographs and corresponding size distribution histograms of (a) **1**- and (b) **4**-stabilized CdSe nanoparticles. The inset in (b) shows a HR-TEM micrograph of an individual CdSe nanoparticle (scale bar 5 nm).

Table 1 The hydrodynamic radii (R_h) of dendrimers **1–4** and the diameters of CdSe particles (d_{CdSe}), as determined by TEM

Dendrimer	Generation	R_h/nm	d_{CdSe}/nm
1	2nd	1.7[37]	1.8 ± 0.8
2	3rd	2.5[27]	1.9 ± 0.7
3	4th	3.0[27]	2.1 ± 0.5
4	5th	3.4[27]	2.3 ± 0.9

around 2 nm, with standard deviations of 0.7 nm. The particle diameters are summarized in Table 1; the given error represents the standard deviation of the respective distribution.

The similar particle sizes and comparable optical properties of the colloidal, dendrimer-stabilized CdSe nanoparticles allow some consideration of their formation and stabilization mechanisms. The nature of dense-shell glycodendrimers (Scheme 1) has a decisive effect on the particle formation, their dimensions and their stabilization that is only weakly dependent on the generation of the dendrimer. Considering that the glycodendrimers are not able to undergo structural rearrangement, such as amphiphilic PPI dendrimers[29] with aliphatic surface groups do, the hydroxyl groups of the maltose units actively participate in the stabilisation of the CdSe particles by an interfacial uptake process of the glycodendrimers. Particle nucleation takes place at the terminal maltose units of the dendrimer molecules. Firstly, the Cd(II) precursor can interact with maltose units in aqueous solution, forming Cd(II)–maltose complexes in the outer shell of the glycodendrimers.[36] With these pre-loaded glycodendrimers, upon adding NaSeH, the particle growth commences in the proximity of the maltose shell. The glycodendrimers act as a ligand to stabilize the growing CdSe nanoparticles. Additionally, hydrogen bonds can be formed among dendrimers adsorbed onto the nanoparticle surface, leading to a confined environment, in which the particles grow, surrounded by densely packed glycodendrimers. The size of the available volume for particle growth is defined by R_h and the packing of the dendrimers, and limits the final particle size and size distribution. As a result, slightly larger particles are obtained in the presence of higher generation dendrimers (Table 1). The size distribution of the nanoparticles is relatively narrow, regardless of the dendrimer's generation, because the monodispersity of the glycodendrimers in terms of molecular weight and R_h leads to the formation of uniform spaces available for particle growth.

In conclusion, we have developed a simple concept to synthesize *in situ* water-soluble CdSe particles at room temperature with average diameters of 2 nm, stabilised by maltose-modified PPI dendrimers. The optical properties of these CdSe QDs were investigated, yielding strong fluorescence bands at around 530 nm. Remarkably, there was no significant difference in the emission wavelength of particles stabilised by different generations of dendrimers. This observation correlates with the fact that the particle size was almost independent of the dendrimer generation. The growth and stabilisation of uniform CdSe particles could be related to the formation of similarly-sized spaces based on densely packed glycodendrimers, which were adsorbed onto the nanoparticle surface. Future work will focus on the investigation of specific biointeractions of these glycodendrimer-stabilised CdSe particles in solution and on surfaces with various species. Furthermore their biological properties, *e.g.* in terms of toxicity, stabilisation under physiological conditions and transfection properties, are under investigation.

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Experimental

Materials

All substances (2nd generation poly(propylene imine) dendrimer (DAB-Am8), 3rd generation poly(propylene imine) dendrimer (DAB-Am16), 4th generation poly(propylene imine) dendrimer (DAB-Am32), 5th generation poly(propylene imine) dendrimer (DAB-Am64), sodium borate, selenium powder, cadmium acetate dihydrate, sodium borohydride and ethanol) were used as purchased from SyMO-Chem (Eindhoven, The Netherlands), Aldrich, Acros or Fluka. EtOH (ethanol), $Cd(OAc)_2{\cdot}2H_2O$ (cadmium acetate dihydrate), $NaBH_4$ (sodium borohydride), NaSeH (sodium hydroselenide) and PPI poly(propylene imine) are used as abbreviations.

Synthesis

Maltose-modified PPI dendrimers **1–4** were synthesized as described, at which reductive amination of amination-terminated PPI dendrimers in the presence of excess maltose was carried out (Scheme 1).[27,37]

CdSe nanoparticles

A wet chemical method was used under an N_2 atmosphere to prepare colloidal solutions of dendrimer-stabilised CdSe nanoparticles in aqueous solution. This procedure consisted of three steps at room temperature: (A) $Cd(OAc)_2{\cdot}2H_2O$ (2.40 mg, 9×10^{-6} mol) was dissolved in a 10 mL aqueous solution (0.3 mM) of maltose-modified PPI dendrimer, leading to dendrimer-complexed Cd(II) ions with a Cd(II)/dendrimer ratio of 3 : 1. (B) Selenium powder (4 mg) was suspended in 10 mL ethanol. A colourless solution of NaSeH was prepared by slowly adding $NaBH_4$ ($\leq$20 mg) under an N_2 atmosphere. (C) 0.9 mL of the NaSeH solution was added to the solution of dendrimer-complexed Cd(II) ions to obtain yellow solutions of colloidal CdSe nanoparticles.

Characterization

UV-vis and PL spectra. These spectra were recorded on Varian Cary 50 and Varian Eclipse photospectrometers (Varian Inc.). The monochromator slit width was 5 nm.

TEM. Thin films were spin-coated onto carbon-coated copper grids (400 mesh/AGAR Scientific) using a solid substrate support. The copper grid was then peeled off the substrate and analysed in a TECNAI Biotwin (FEI Ltd.) transmission electron microscope at 100 keV. The instrument was operated at low beam intensities to prevent electron damage of the polymer samples. HR-TEM measurements were performed using a JEOL JEM-2110F instrument

operated at 200 kV; the instrument was equipped with an Orius SC1000 digital camera.

The hydrodynamic radii, R_h, of the glycodendrimers were determined *via* dynamic light scattering, details of which can be found in the literature indicated in Table 1.

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