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Size-Dependent Dissociation pH of Thiolate Ligands from Cadmium Chalcogenide Nanocrystals

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Abstract: A method, pseudo steady-state titration, is introduced for determining the precipitation pH of nanocrystals coated by electron-donating ligands. CdSe nanocrystals coated with hydrophilic deprotonated thiol (thiolate) ligands were studied systematically. For comparison, CdTe and CdS nanocrystals coated with the same types of ligands were also examined. The results show that the precipitation of the nanocrystals is caused by the dissociation of the nanocrystal—ligand coordinating bonds from the nanocrystal surface. The ligands are removed from the surface due to protonation in a relatively low pH range, between 2 and 7 depending on the size, approximately within the quantum confinement size regime, and chemical composition (band gap) of the nanocrystals. In contrast, the redispersion of the nanocrystals was found to be solely determined by the deprotonation of the ligands. The size-dependent dissociation pH of the ligands was tentatively used as a means for determining the size-dependent free energy associated with the formation of a nanocrystal—ligand coordinating bond.

Introduction

Colloidal nanocrystals are nanometer-sized fragments of the corresponding bulk crystals, a class of metastable species in solution. The metastable feature of nanocrystals implies that they need to be kinetically stabilized, typically by a monolayer of organic ligands. Thus, colloidal nanocrystals refer to the nanocrystal—ligand complex, including both the inorganic core and the organic ligand shell. The weakest point of a colloidal nanocrystal is generally the interaction between the ligands and the surface atoms of the inorganic core. This means that the nature and strength of the interaction between the ligands and the surface atoms of nanocrystals determine the stability of the nanocrystal—ligand complexes. The stability of nanocrystal—ligand complexes has become a focus of study in recent years because several promising applications of nanocrystals, such as biomedical labeling[1,2] and light-emitting diodes[3-5] using semiconductor nanocrystals, all seem to be limited by their stability. At present, most of the related efforts on ligand chemistry of semiconductor nanocrystals are focused on the development of new types of ligands[6-10] and different passi-

vation strategies[1,11-15] to satisfy stability requirements for certain types of applications. To our knowledge, there is very limited information on quantitative understanding of the stability of nanocrystal—ligand complexes.

Typical ligands for colloidal nanocrystals, such as thiolates (deprotonated products of thiols), amines, phosphonates (deprotonated products of phosphonic acids), and carboxylates (deprotonated products of carboxylic acids), etc., are all Lewis bases. In principle, if the pH at the nanocrystal—ligand interface decreases to a certain value, the ligands should be protonated and detach from the nanocrystals. This will destroy the nanocrystal—ligand complexes. Potentially, this is a general issue for nanocrystals with different compositions in solutions. For example, the average pH of human stomach juices is approximately 2. The detachment of the ligands will not only destabilize the colloidal system but also cause possible cytotoxic problems. Recent studies revealed that cadmium based semiconductor nanocrystals did not affect the biological functions if they were completely coated with organic ligands.[15,16] After

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the ligands were detached, the nanocrystals became extremely toxic.[16]

The interaction between a ligand, a Lewis base, and the surface cations, Lewis acids, can be regarded as a special type of coordinating bond. When hydrogen ions are added into the system, the hydrogen ions, another set of Lewis acids, will compete for the Lewis base, the surface ligand, with the nanocrystal. Therefore, the dissociation of the ligands from the surface of nanocrystals by lowering the pH of the solution can be considered as a displacement reaction, which is a general way to determine the formation constant of a complex.

The above considerations invited us to explore the possibility of using traditional titrations by adding a strong acid into solutions for the quantitative determination of the formation constant and the related free energy change of nanocrystal-ligand complexes. However, slow diffusion of hydrogen ions through the ligand monolayer on a nanocrystal technically prevented us from using common titration techniques. However, different from the photochemical stability of CdSe nanocrystals studied previously,[17] precipitation of semiconductor nanocrystals must be examined for systems close to thermodynamic equilibrium conditions, instead of kinetics of the diffusion. For this reason, a pseudo steady-state titration was developed.

The precipitation pH of nanocrystals with different chemical compositions and/or with different ligands was examined. This report will concentrate on one type of the most studied colloidal nanocrystals in the literature: cadmium chalcogenides semiconductor nanocrystals. We verified that the precipitation pH of all model systems, CdS, CdSe, and CdTe nanocrystals coated with deprotonated hydrophilic thiol ligands, did not depend on the concentration of the nanocrystals, the concentration of the free thiol ligands, or the nature of the anion of the strong acids used for the titrations [HCl, H_2SO_4, or trifluoroacetic acid (TFA)]. Within the pH range tested, the nanocrystal precipitates were found to be recoverable, without any noticeable change in their absorption spectra, by adjusting the pH of the solution to the pK_a of the thiol ligands. The experimental results suggest that the precipitation of nanocrystals was solely dependent on the dissociation of the ligands from their surfaces.

The most surprising observation of this study is that the dissociation pH of hydrophilic thiolate-coated semiconductor nanocrystals was found to be significantly size- and band-gap-dependent. For a given type of nanocrystal composition, the smaller the size of the nanocrystals was, the lower the dissociation pH. Additionally, if the ligands and the size of the nanocrystals were the same, the dissociation pH of CdS, CdSe, and CdTe nanocrystals increases as the bulk band gap of the semiconductor decreases.

According to the seminal work of Brus,[18] a chemical explanation of quantum confinement of semiconductor nanocrystals is the enhanced bonding strength between the internal atoms of a semiconductor nanocrystal as particle size decreases. At present, it is not clear whether the strength of the coordinating bond between the ligands and the surface atoms is also size-dependent. This issue is difficult to be addressed theoretically because it is challenging to quantitatively define the surface energetics of nanocrystals.[19] Thermal analysis of powder samples[20] has yielded some interesting information, but the configuration change and decomposition of the surface ligands has made it complex to extract quantitative information for surface bonds. With all these in mind, we tried to extract the free energy change ($\Delta_r G°$) determined by the formation constant of the coordinating bond between a nanocrystal and its ligands from their size-dependent ligand dissociation pH. The calculations based on the titration results from our model suggest that this $\Delta_r G°$ value is significantly size-dependent in the quantum confinement size regime. The information provided in this report should be of importance for both synthesis and manipulation of colloidal nanocrystals.

Experimental Section

Chemicals. Technical grade (90%) trioctylphosphine oxide (TOPO), technical grade (90%) trioctylphosphine (TOP), technical grade (90%) 1-octadecene (ODE), technical grade (90%) oleic acid (OA), cadmium acetate hydrate (99.99+%), cadmium oxide (99.99+%), sulfur powder (99.98%), selenium powder (−100 mesh, 95%), anhydrous toluene (99.8%), 3-mercaptopropionic acid (MPA, 99+%), 3-mercapto-1-propanol (MPOH, 95%), 2-(dimethylamino)ethanethiol hydrochloride (95%), deuterium chloride (99.5% atom D), and deuterium oxide (99.9% atom D) were purchased from Aldrich. 1,4-dithio-*d*,l-threitol (DTT), *n*-tetradecylphosphonic acid (98%), and tellurium powder (−325 mesh, 99.998%) were purchased from Alfa. 1-Octadecylamine (ODA, 98%+) was purchased from Lancaster. *n*-Octadecylphosphonic acid was purchased from PolyCarbon Industries, Inc. Acetone, anhydrous ethyl ether, chloroform, toluene, ethyl acetate, and methanol were purchased from EM Science. Both the ethyl acetate and methanol were of HPLC grade.

Semiconductor Nanocrystals. CdS and CdSe were obtained from NN-Labs. CdTe was synthesized according to previously published methods.[21] Prior to surface ligand exchange with hydrophilic thiols, the side products and unreacted chemicals were carefully purified away using the standard methods.

Typical Synthesis of Thiol-Coated Semiconductor Nanocrystals. A procedure slightly modified from that previously published[17] was applied. 3-Mercapto-1-propanol (MPOH, 100 mg) was placed into 15 mL (11.85 g) of methanol in a 25 mL three-neck flask. The pH of the solution was adjusted to about 10 with the addition of tetramethyl-ammonium hydroxide pentahydrate. CdSe nanocrystals (40 mg) were added into the reaction vessel under argon. The vessel was heated under reflux at 65 °C for 6 h. After reflux, the reaction was allowed to cool to room temperature, removed from argon, and stored in the dark. Nanocrystal aliquots (2 mL) were taken from the reaction and precipitated with ethyl acetate. The aliquots were centrifuged, and the supernatant was removed. The precipitate was redissolved in methanol and precipitated with ethyl acetate 2 more times. It was verified that the base, thiol, and original ligands on the surface of nanocrystals were all soluble in methanol/ethyl acetate mixture. After the final precipitation, the nanocrystals were dissolved in water at a high concentration to create a stock solution for use in the titration experiment.

The above procedure was followed to prepare thiol-coated CdS and CdTe, as well as CdSe coated with the other ligands mentioned in the Results section. However, the stock solution of CdTe nanocrystals coated with thiols was made by dissolving the purified nanocrystals in aqueous NaOH solution with pH ≈ 10 because of the instability of thiol-coated CdTe nanocrystals in distilled water (pH = 5−6).

Precipitation of Nanocrystals in Acidic Aqueous Solution Monitored with a UV−vis Spectrophotometer. A typical acidic solution was prepared by adding 0.1 M HCl dropwise to ∼20 mL of Millipore

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H$_2$O and then adjusting the pH to the desired range with addition of more Millipore H$_2$O. For a given set of titration experiments, at least six acid solutions with different pH values were used. A similar procedure was used for the preparation of sulfuric and trifluoroacetic acid solutions. The volume of nanocrystal stock solution required for each titration experiment was calculated from the intensity of the first exciton absorption peak of a concentrated aqueous stock solution. A 100 μL aliquot from the stock solution was diluted 10- to 20-fold, and its absorption intensity was used to calculate the optical density of the initial stock solution. The simple formula below was then used to determine the amount of nanocrystal solution that needed to be added to the aqueous acidic solution for a typical titration experiment:

$$(OD_{stock})(V_{stock}) = (0.3)(5 + V_{stock})$$

where OD$_{stock}$ is the absorbance (optical density) of the stock nanocrystal solution, and V_{stock} is the volume of stock solution required to achieve 0.3 optical density in an ~5 mL HCl solution.

All titration experiments were performed using a VWR Model 9100 pH/Temp/mV meter. The pH electrode was placed in the aqueous acid solution after nanocrystals had been added and stirred to achieve equilibrium. Each titration solution was placed in 20 mL vials with a sample volume of ~5 mL. The UV−vis absorption spectra were recorded for each titration solution after pH reached equilibrium. Samples that showed scattering were centrifuged, and the spectra of their supernatants were measured. Centrifugations were performed in an IEC clinical centrifuge 50/60 Hz 115 VAC model. The samples were typically left to centrifuge for 5 min, although samples centrifuged for longer times (~10 min) did not show significant change in the pH value before and after centrifugation. Certain samples that were soluble and optically clear were centrifuged for extended periods of time (>20 min), and no significant variation in their absorbance intensity was noted. Samples that showed scattering were also filtered using a Whatman 0.45 μm GMF syringe filter. Similar reproducible results were obtained using the filtering and centrifugation techniques. The OD values of the first exciton absorption peak of the nanocrystals from a set of titration experiments were used to generate a titration curve that allowed the determination of the equilibrium precipitation pH for all the nanocrystal systems studied.

Reverse Titration. A stock solution of CdSe−MPOH nanocrystals was precipitated by the addition of a strong acid, HCl, H$_2$SO$_4$, or CF$_3$COOH. The precipitated nanocrystals were then stirred to achieve a uniform suspension, and aliquots of this stock were put into NaOH solutions in a certain pH range. The NaOH solutions for a given set of reverse titration experiments were made in a similar manner as that described for HCl solutions. The intensity of the first exciton peak was again used to generate titration curves that indicated the equilibrium pH for dissolution of the nanocrystals back into water.

NMR Experiments. Purified CdSe−MPOH nanocrystals (20 mg) were dissolved in 0.5 mL of D$_2$O. The nanocrystals had previously been dried in a vacuum oven in the dark for 2 h. The nanocrystals were then titrated with deuterium chloride inside a test tube (8.5 cm length, 1.5 cm diameter), and their pH was monitored. Nuclear magnetic resonance (NMR) spectra were taken at points before and after nanocrystal precipitation with a JEOL 270 MHz NMR spectrometer. After the nanocrystals were precipitated, the sample was centrifuged and the NMR spectrum of the supernatant was measured.

Gel Electrophoresis. A nanocrystal solution (20 μL, 10 mg/mL) coated with MPA and MPOH, respectively, was placed in the wells of a 2% agarose gel. The nanocrystals were dissolved in the buffer solution right after they were precipitated out from the methanol reaction solution. The gel was run in 1× TAE buffer for 10 min at 150 V, and special care was taken to avoid direct light onto the nanocrystals. A solubility experiment was performed by mixing 0.5 mL of 10 mg/mL solutions of the nanocrystals coated with their respective ligands with 0.5 mL of a 2% agarose suspension with 1× TAE buffer. The
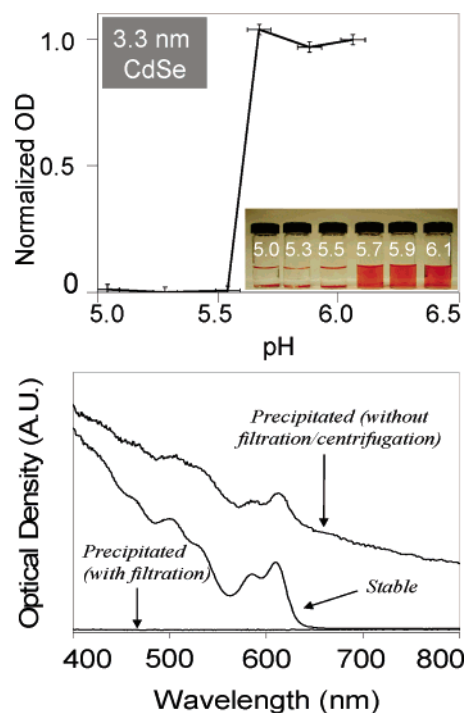
Figure 1. (Top) A typical titration curve obtained by plotting the normalized optical density (OD) of each sample in the titration series (inset) vs their final pH. (Bottom) Typical UV−vis spectra of the samples. Note: a significant scattering background is evidenced in the precipitated sample before filtration/centrifugation.

nanocrystals were mixed with the agarose suspension and then centrifuged for 5 min at 14 000 rpm. The supernatant was then recovered and filtered with a 0.45 micrometer syringe disk filter. Both of the solutions with MPA- and MPOH-coated nanocrystals were still soluble in the agarose suspension after centrifugation and optically clear nanocrystal solution were recovered after filtration. A small amount of precipitation corresponding to nanocrystals that became trapped in some of the undissolved agarose was observed for both cases.

Results

Pseudo steady-state titration, placing ligand-coated nanocrystals into a series of aqueous solutions with different pH values (Figure 1, top (inset)), was found to be essential for studying the stability of semiconductor nanocrystals against pH change. The ligand monolayer on each nanocrystal makes the diffusion of solvated hydrogen ions a relatively slow process. As a result, it was very difficult to determine when to add the next drop of acid solution for a regular titration experiment. In addition, it was not easy to judge the precipitation of the nanocrystals. Consequently, the results obtained through regular titration experiments were not reproducible.

On the contrary, the pseudo steady-state titration technique does not have a time constraint. In fact, it can even be performed under air-free and/or under dark conditions to avoid possible photo-oxidation. The pH equilibrium between the bulk solution and the nanocrystal−ligand interface was thus achieved by leaving the titration samples in the dark for a necessary amount of time, which took a few seconds to a few hours depending upon the chain length of the ligands and the composition of the solutions. Centrifugation or filtration further helped the clarification of the precipitation of nanocrystals (Figure 1, bottom). After centrifugation or filtration, accurate measurement of the optical density of the unprecipitated nanocrystals in each sample of a
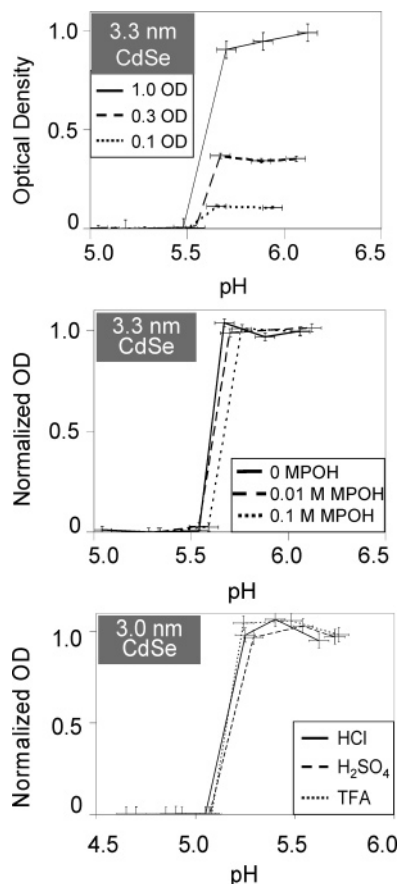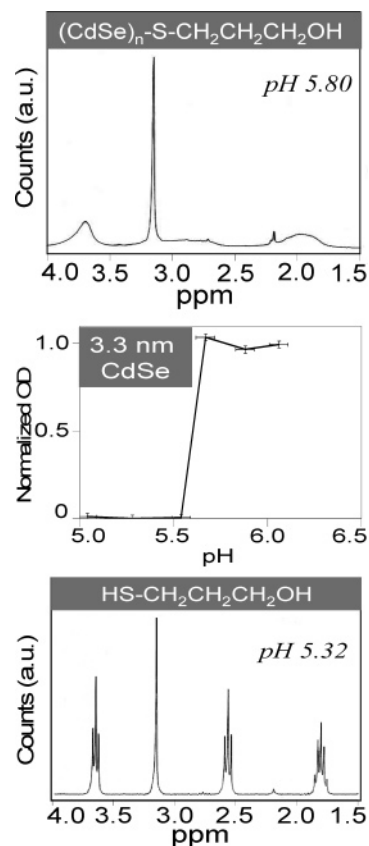
Figure 2. (Top) Precipitation of 3.3 nm CdSe nanocrystals coated with MPOH with different nanocrystal concentrations, (middle) different free ligand concentrations, and (bottom) different acids.

Figure 3. Status of the ligands before (top) and after (bottom) precipitation occurred.

given titration series became possible. Fully reproducible titration curves within experimental error were obtained using the pseudo steady-state titration technique.

Varying the concentration of the nanocrystals with a given sized nanocrystal coated by a given type of ligands in the titration solutions did not affect the pH at which the nanocrystals precipitated. As shown in Figure 2 (top), the precipitation pH of 3.3 nm CdSe nanocrystals coated with 3-mercapto-1-propanol (CdSe-MPOH nanocrystals) with different concentrations was essentially the same within the experimental error. The concentration range of the nanocrystals in this specific experiment was approximately between 0.0008 mmol/L and 0.008 mmol/L calculated from the optical density[22] (Figure 2, top), which was a 10-fold difference.

Free ligands added into the titration solutions did not change the equilibrium precipitation pH of the nanocrystals against pH change, although high concentrations of free ligands did make the precipitation take longer. In other words, the concentration of free ligands could only change the precipitation kinetics but not the thermodynamics. Figure 2 (middle) shows that an aqueous solution of MPOH-coated CdSe nanocrystals with OD = 0.3 and 0.1 M free MPOH concentration had the same stability and equilibrium pH as a similar solution with no free MPOH or 0.01 M MPOH added.

Counterions of the strong acids used for the precipitation should bind onto the surface of the nanocrystals after the original

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ligands were protonated. For most counterions of strong acids, this interaction should be very weak in comparison to the thiolate–cadmium bonds. Therefore, the nature of the counterions of the strong acids used for titration should not affect the precipitation pH of the nanocrystals. To verify this hypothesis, three different strong acids, HCl, H_2SO_4, and CF_3COOH, were tested for the titration experiments. The differences between the corresponding anions are that Cl^- is monovalent, SO_4^{2-} is divalent, and CF_3COO^- is organic and very bulky. The results shown in Figure 2 (bottom) revealed that the titration curves obtained with these three different acids were not distinguishable within experimental error. This confirmed that the interaction between the surface of nanocrystals and the counterions of the strong acids used for titration is negligible in determining the precipitation pH of the nanocrystal–ligand complexes.

Dissociation of the ligands from the surface of the nanocrystals at the precipitation pH was confirmed by ^{1}H NMR. Figure 3 (top) shows the NMR spectrum of the bound MPOH ligands on the surface of 3.3 nm CdSe nanocrystals, in a solution with the pH above the precipitation pH of this specific sample. As expected, when the pH of the solution was higher than the precipitation pH of the nanocrystals, the NMR features of the thiolate ligands on the CdSe surface are broad and shifted from their positions in the standard spectrum of the free protonated thiol ligands (Figure 3, top). In comparison, the NMR peak of the countercations (tetramethylamonium ions) at about 3.15 ppm is relatively sharp. After the solution pH went below the precipitation pH of the nanocrystals, the NMR peaks of the ligands in the supernatant were found to be always identical to those of the free thiol ligands (standard NMR spectrum of
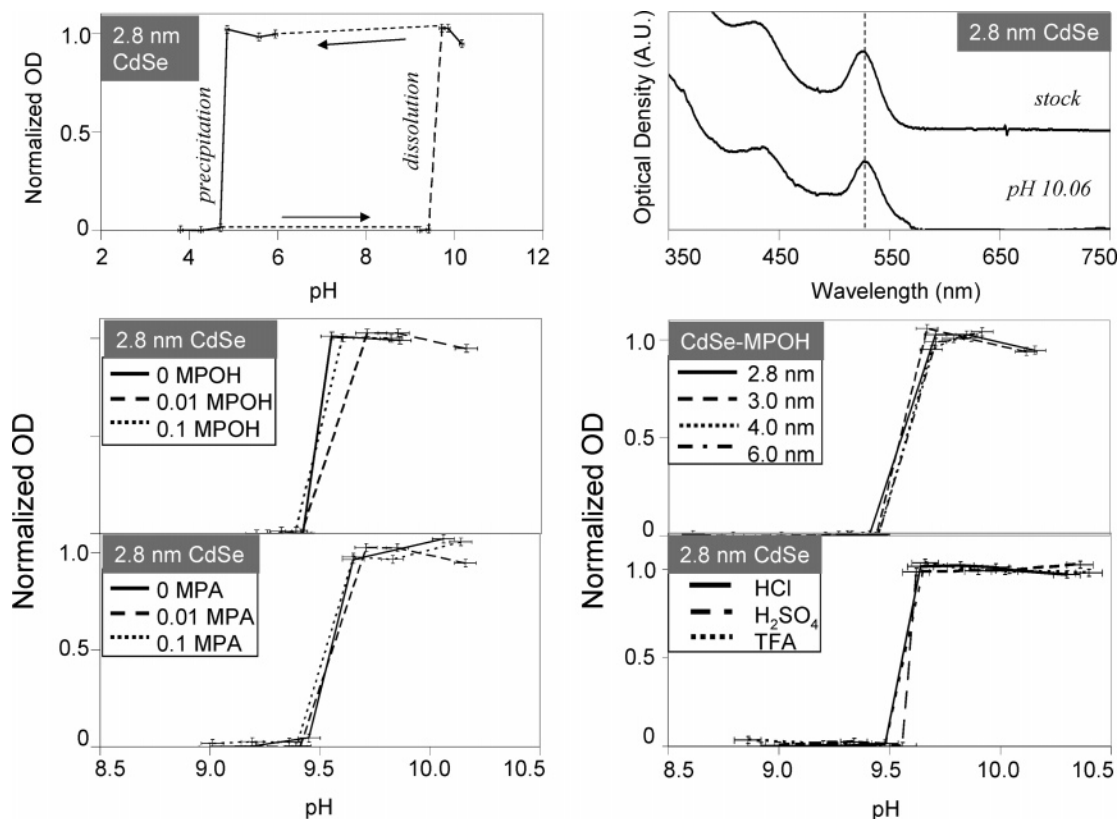
Figure 4. Redispersion of the nanocrystal precipitates under different conditions. Except for the one labeled differently (top left), all titration curves represent the redispersion of CdSe nanocrystals.

MPOH available in Aldrich NMR spectra book), with sharp peaks at exact positions (Figure 3, bottom). This indicates the dissociation of the ligands from the nanocrystal surface in the form of free thiols, instead of thiolates, which is expected by protonating the ligands at relatively acidic pH. This experiment also confirmed that no photo-oxidation of the ligands had occurred,[17] because no disulfide formation was evidenced by the NMR spectrum after nanocrystal precipitation.

Furthermore, the NMR data was applied to quantify the percentage of ligands removed by titration. The resonance at 3.15 ppm corresponding to tetramethylammonium group added in as the base during the surface ligand exchange (see Experimental Section) was used as a internal standard to compare the integration areas of the MPOH signals. No additional purification or separation of the solution/nanocrystal was performed for the NMR study. Therefore, the amount of the tetramethylammonium ions should be a constant. This peak should be a good internal reference because solubility of these ions in water should not change significantly with pH. Because the peaks of the thiolates on the surface of nanocrystals before precipitation were very broad, integration of the peaks could not be as accurate as the ones for the free ligands after the precipitation of the nanocrystals. However, the results revealed that at least 80% of the surface ligands had been removed when the precipitation of the nanocrystals occurred.

The integrity of the semiconductor nanocrystals upon precipitation was confirmed by UV–vis absorption spectrum of the recovered nanocrystals. As shown in Figure 4 (right top), the size of the inorganic core of the redispersed nanocrystals (see details for redispersion below) was the same as the original nanocrystals, indicated by the identical peak position of the absorption spectra. We consider this result to be consistent with the extremely small k_{sp} of the inorganic semiconductors. For example, the k_{sp} of CdSe bulk crystals in water is about 10^{-37}, which means that the equilibrium cadmium and selenium ion concentrations in the solution would be about 10^{-21} mol/mL, only several hundreds of ions per milliliter. This is completely negligible in comparison to the total cadmium and selenium units contained in all nanocrystals in each titration solution.

The reversibility of the precipitation process was tested under different conditions (Figure 4). As shown in Figure 4 (top left), redispersion of the inorganic nanocrystals occurred if the pH of the solution was sufficiently high. This pH value is approximately the same as the pK_a values of the thiol groups of the ligands as determined by this group. There is a significant hysteresis between the precipitation curve and the redispersion curve (Figure 4, top left). The results shown in Figure 4 revealed that the redispersion of the nanocrystal precipitates is solely determined by the pK_a of the thiol ligands and independent of the nanocrystal size, free ligand concentration, the terminal groups of the thiol ligands, and the nature of the counterions of the strong acids. These results are consistent with the observation that it was necessary to adjust the pH of the solution above the pK_a of the thiol group when the original ligands, either phosphine oxide or other ligands, on the surface of nanocrystals were replaced by the hydrophilic thiol ligands.[17]

Size and band gap effects on precipitation pH of semiconductor nanocrystals were observed with CdS, CdSe, and CdTe nanocrystals approximately in their strong quantum confinement size range (Figure 5). For a given semiconductor with a given ligand, the precipitation pH of the nanocrystals within its strong quantum confinement size regime increased
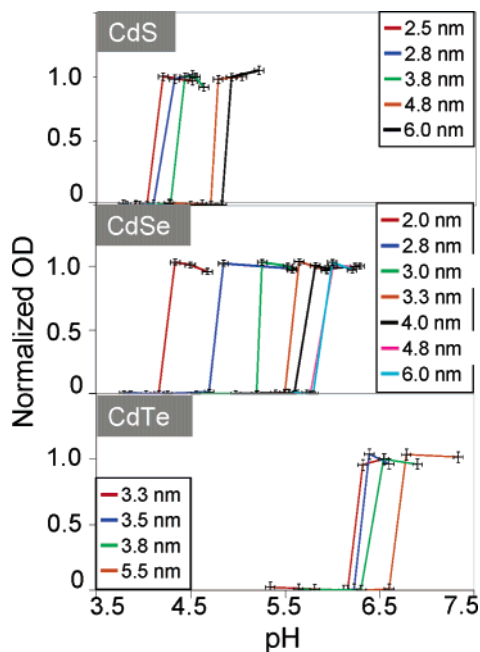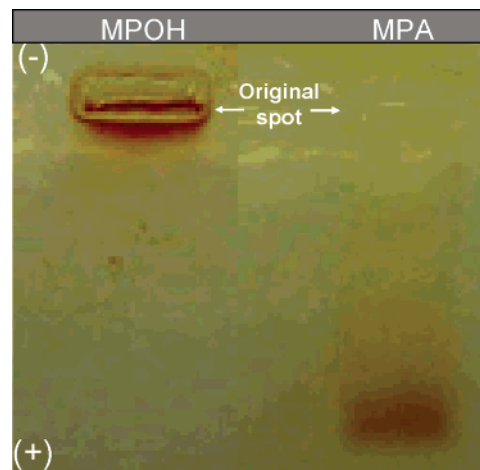
Figure 5. Size-dependent titration curves (precipitation curves) for different semiconductor nanocrystals.

Figure 6. Gel image of MPOH-coated and MPA-coated CdSe nanocrystals after 10 min (details in Experimental Section).

as the size of the nanocrystals increased (Figure 5). When the size of the nanocrystals reached approximately the top limit of strong quantum confinement size of the semiconductor, the precipitation pH reached a plateau. For example, 4.8 and 6.0 nm CdSe−MPOH nanocrystals had the same equilibrium precipitation pH. Experiments performed with 5.2, 5.3, and 8.5 nm CdSe−MPA nanocrystals all showed the same equilibrium precipitation pH as well (Supporting Information).

Furthermore, the precipitation pH of the nanocrystals was also dependent on the bulk band gap of the semiconductors if the size of the nanocrystals was the same. For example, the CdSe and CdS nanocrystals, both 2.8 nm in size, precipitated at pH 4.6 and 4.1, respectively (Figure 5). Similarly, the precipitation of CdTe and CdSe nanocrystals with 3.3 nm in size was observed at pH 6.2 and 5.5, respectively (Figure 5).

The charge of nanocrystals is an important factor for understanding the experimental data described above. The existence of the tetramethylammonium ions, indicated by the sharp peak at 3.15 ppm, implies that the nanocrystal/ligand complexes might be charged. As reported previously, the original CdSe nanocrystals used in this study with amines and TOPO as the surface ligands should not be charged.[23] When these neutral ligands were replaced by the charged thiolates, the nanocrystals might become charged. However, the integration of the NMR peaks indicates that, for MPOH-coated nanocrystals (Figure 3), the ratio between the ammonium ions and the thiol ligands is only about 1:5, which is much below the expected 1:1 ratio. This means that the ammonium ions might come from unpurified base which is poorly soluble in ethyl acetate and the thiolate-coated CdSe nanocrystals might thus be neutral. To confirm this, gel electrophoresis experiments were performed for CdSe nanocrystals coated with MPOH and MPA (Figure 6). Evidently, the MPA-coated CdSe nanocrystals were negatively charged and moved to the positive direction (Figure 6) because of the −COO⁻ terminate group of MPA at

pH = 7.2. On the contrary, the MPOH-coated CdSe nanocrystals did not show a significant move under the same conditions. Additional experiments were performed to confirm that both MPA-coated and MPOH-coated CdSe nanocrystals were stable in the gel medium and could be recovered back into the buffer solution from the gel (see detail in Experimental Section), which means that if nanocrystals were charged, they should move under the experimental conditions.

Discussion

The size-dependent equilibrium precipitation pH (Figure 5) is not the result of the size-dependent attractive interaction between the nanocrystals. This concern comes from the fact that the molecular interaction increases with the volume of the nanocrystals with the same structure and chemical environment.[24−27] This could result in a size-dependent precipitation pH for the nanocrystals because the volume of the nanocrystals increases approximately 8 times as the diameter of the nanocrystals doubles. However, the result shown in Figure 5 clearly indicates that the size dependence of the precipitation pH is not a cubic function. For example, the volume of the 6.0 nm CdSe core is about 2 times bigger than that of the 4.8 nm CdSe core. However, the equilibrium precipitation pH of the nanocrystals with these two sizes was about the same (Figure 5). The results for MPA-coated CdSe nanocrystals also support the same conclusion. In that case, CdSe nanocrystals 5.2, 5.3, and 8.5 nm in size all precipitated with the same pH within experimental errors (Supporting Information).

The concentration independent precipitation pH shown in Figure 2 further supports this conclusion. In principle, if the interparticle interaction was playing a role for the precipitation of the nanocrystals, the distance between particles (or concentration of the particles) should strongly affect the precipitation pH of the nanocrystals. This is so because interparticle interactions strongly rely on the interparticle distance.

All experimental results indicate that the precipitation of the nanocrystals is caused by the dissociation of the surface thiolate

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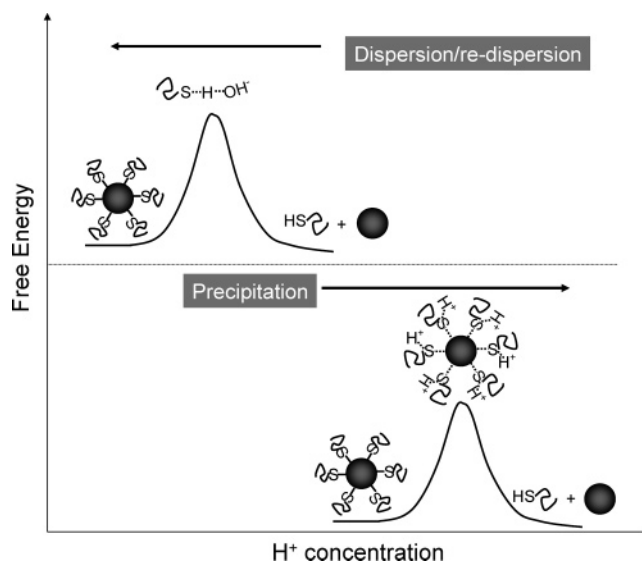
ligands. NMR measurements (Figure 3) and the redispersion experiments (Figure 4) revealed that the detachment of the ligands was due to the protonation of the thiolate ligand with the hydrogen ions provided in the aqueous solution with low pH. The same experiments further provided no evidence of formation of disulfides caused by the photocatalytic oxidation studied previously.[17] Within the pH range tested, dissolution of the inorganic nanocrystal cores, or shrinkage of the inorganic nanocrystals, was not detected (Figure 4, top right). The interaction between the nanocrystal precipitates and the anions from the strong acids used for titrations made a negligible contribution to the precipitation pH of the nanocrystals (Figure 2, bottom). Therefore, it is safe to conclude that the equilibrium precipitation pH of the nanocrystal−ligand complexes determined by the pseudo steady-state titration method introduced here is the dissociation pH of the surface ligands from a nanocrystal. This pH should thus reflect the bonding nature and strength of the coordinating bond between the ligands and the nanocrystals.

The redispersion experiments (Figure 4) support that the precipitation of nanocrystals caused by the dissociation of the ligands is reversible. However, the significant hysteresis between the precipitation titration and redispersion titration shown in Figure 4 (top left) indicates that the reversibility of the dissociation of the ligands by lowering the pH of the solution is not exactly the same as that of the traditional acid−base titration.

We noticed that a hysteresis also exists for the well-studied solid−solid phase transitions of nanocrystals.[28] In the case of phase transition, the hysteresis is believed to be caused by the thermodynamic metastability of the nanocrystals.[29] The surface free energy of the nanocrystals changes the chemical potential of the nanocrystals, which in turn varies the stability of a given phase.[30] However, the hysteresis observed in Figure 4 seems to be different from that observed in the phase transitions of nanocrystals. For the phase transition experiments, the transition pressures in both directions vary with the size of the nanocrystals.[31] In contrast, the redispersion curve is independent of the size of the nanocrystals, although the precipitation curve is strongly size-dependent (Figures 4 and 5).

Experimental results (Figure 4) revealed that the redispersion of the nanocrystal precipitates occurred when the pH reaches approximately the pK_a of the free thiols, which means a significant amount of deprotonated thiols exist in the solution. The same situation occurred when the original hydrophobic nanocrystals were made to be water-soluble by replacing the hydrophobic ligands by hydrophilic thiols.[17] These facts indicate that the deprotonation of thiol ligands is a prerequisite for the coordination of the deprotonated thiol ligands and the nanocrystals. One possible reason for this prerequisite may be that the free ligands and the nanocrystal precipitates were not in the same phase. Another cause is likely the steric factor at the inorganic nanocrystal−ligand interface.

Based on the above analysis, we consider that the hysteresis between the precipitation and redispersion of the nanocrystals (Figure 4) is solely caused by the different paths between the two events. For the precipitation experiments, the relative stability of the nanocrystal−ligand complexes decreases as the hydrogen ion concentration increases. At the equilibrium pH, the chemical interaction between thiolate groups and the surface cadmium atoms should be approximately the same as that between the thiolate groups and the hydrogen ions. Further increase of the hydrogen concentration shifts this metastable equilibrium to the formation of thiols and uncoated nanocrystal precipitates (Figure 7, bottom). In the case of dispersion/redispersion, surface coordination, a heterophase process, only occurs after the deprotonation of the thiol ligands at a relatively high pH takes place (Figure 7, top). In a certain sense, the deprotonated thiols, thiolates, can be viewed as an activated species for the desired surface coordination. Therefore, the redispersion titration only measures the dissociation of the thiol groups.

Figure 7. Schematic illustration of the precipitation and dispersion/redispersion processes.

Above discussions qualitatively support that the stability of the nanocrystal−ligand complexes increases as the size of the nanocrystals decreases as indicated by the size-dependent dissociation pH shown in Figure 5. The remainder of the discussion is intended to establish a quantitative understanding based on two assumptions that are supported by experimental results.

The precipitation equilibrium studied in this work can be written as follows using CdSe nanocrystals as the example.

$$(CdSe)_n-L_m + mH^+ \rightleftharpoons ((CdSe)_n)^{m+} + mHL, \qquad K_{eq} \quad (1)$$

where $((CdSe)_n)^{m+}$ and $(CdSe)_n-L_m$ correspond to bare nanocrystals (precipitates) and nanocrystals coated with ligands, respectively. The positive charges of the bare nanocrystals in the precipitates should be balanced by the negative charges of the counterions of the strong acid used for titration. K_{eq} is the equilibrium constant of equilibrium 1. Equilibrium 1 can be expressed as the two equilibria listed below.

$$HL \rightleftharpoons H^+ + L^-, \qquad K_a \qquad (2)$$

$$((CdSe)_n)^{m+} + mL^- \rightleftharpoons (CdSe)_n-L_m, \qquad (K_s)^m \quad (3)$$

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Equilibrium 3 is a general equation for the formation of a complex and $(K_s)^m$ is the formation constant for $(CdSe)_n-L_m$. We assume that the equilibrium constant K_s, the formation constant of a single nanocrystal–ligand coordinating bond, in equilibrium 3 is the same for all cadmium sulfur bonds between a surface cadmium atom on the surface of a given sized nanocrystal and its deprotonated thiol ligands. Another way to state this assumption is to view K_s as an average value of the formation constant of all nanocrystal–ligand coordinating bonds on a given nanocrystal. There are two pieces of evidence to back up this assumption. One, the precipitation of nanocrystals occurred quite abruptly as evidenced by the titration curves (Figures 1, 2, and 5). Two, NMR data (Figure 3) revealed that approximately all ligands detached from the surface of the nanocrystals at the precipitation point. With this assumption and the relationship among the above three equilibriums, equilibrium 3 plus m times of equilibrium 2 being equal to negative equilibrium 1, K_s can be expressed as follows.

$$(K_s)^m = 1/[(K_a)^m K_{eq}] \qquad (4)$$

and

$$K_s = 1/[(K_{eq})^{1/m} K_a] \qquad (5)$$

Equilibrium 1 tells us that

$$K_{eq} = \{[HL]^m[((CdSe)_n)^{m+}]\}/\{[(CdSe)_n-L_m]([H^+]_{eq})^m\} \qquad (6)$$

Combining eqs 5 and 6, we obtain eq 7.

$$K_s = \{[(CdSe)_n-L_m]^{1/m}[H^+]_{eq}\}/\{[HL][((CdSe)_n)^{m+}]^{1/m}K_a\} \qquad (7)$$

Interestingly, all concentration terms except the $[H^+]_{eq}$ can be eliminated from eq 7. $[((CdSe)_n)^{m+}]$ can be assumed as a constant because nanocrystals with no thiol ligands precipitated out of the solution as shown in the experiments. The experimental results (Figure 2, top and middle) revealed that the precipitation of the nanocrystals is not affected by the concentration of the additional free ligands ([HL]) in solution or by the concentration of the nanocrystals themselves ([$(CdSe)_n-L_m$]).

A reasonable explanation for this concentration independence revealed by the results in Figure 2 (top and middle) is that the dissociation of the coordinating bond occurred at the interface between the inorganic core and the ligands. Because of the significant size of the nanocrystals and the dense coating of the ligand monolayer on the surface of nanocrystals, other nanocrystals and the free ligands in the bulk solution cannot participate in the event occurring on the surface of a given nanocrystal. Even for small ions, such as hydrogen ions, it took a significant amount of time, from a few seconds to a few hours as mentioned above, to reach an equilibrium value between the nanocrystal–ligand interface and the bulk solution. The fact that the equilibrium precipitation pH of the nanocrystals did not depend on the concentrations of the nanocrystals and the free ligands implies that eq 7 can be simplified as eq 8.

$$K_s = [H^+]_{eq}/K_a \qquad (8)$$

Equation 8 enables the calculation of the average reaction Gibbs
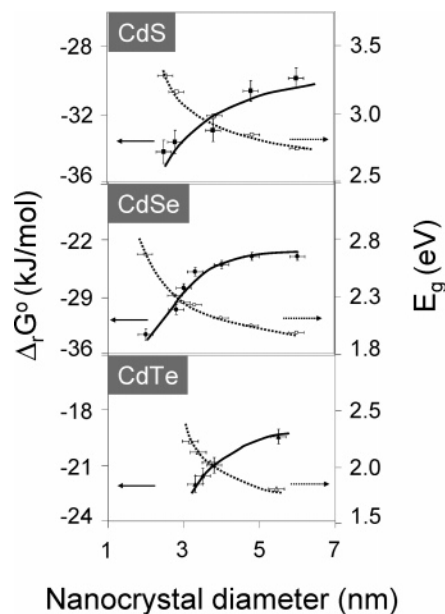
Figure 8. Size-dependent reaction Gibbs energy ($\Delta_r G°$) for the formation of the nanocrystal–ligand coordinating bond (solid lines) for MPOH-coated nanocrystals. In comparison, the size-dependent optical band gap of the nanocrystals is shown as dashed lines.

free energy of the formation of a nanocrystal–ligand coordinating bond ($\Delta_r G°$) using the following equation.

$$\Delta_r G° = -RT \ln K_s = -RT \ln([H^+]_{eq}/K_a) \qquad (9)$$

Equation 9 reveals that the Gibbs energy change solely depends on the equilibrium dissociation pH determined by titration experiments. The pH at the interface and the bulk solution after reaching diffusion equilibrium should be the same. However, we did observe that when some net charges exist on the outer surface of the ligand monolayer, the equilibrium pH was different from that in the bulk solution because of the screening and trapping effects (Supporting Information).

As seen in Figure 5, the equilibrium precipitation pH of the nanocrystals was found to be size-dependent approximately in their strong quantum confinement size regime. Consequently, a similar size dependence of the $\Delta_r G°$ defined using eq 9 is obtained (Figure 8). For comparison, the size-dependent band gap of semiconductor nanocrystals reported in the literature (experimental data) [22] is also illustrated in Figure 8.

Figure 8 demonstrates that the Gibbs free energy change for the formation of nanocrystal–ligand coordinating bonding ($\Delta_r G°$) decreases as the size of the nanocrystals increases for a given type of semiconductor. $\Delta_r G°$ of the nanocrystals with the same size increases as the bulk band gap of the semiconductor increases. To our knowledge, the values in Figure 8 are reasonably consistent with the free energy of the Cd–S bond for the complexes of cadmium ions with thiol ligands reported in the literature.[32–34] More specifically, our results are slightly smaller than the bonding free energies (−44.1 and −47.1 kJ/mol) reported for a complex of Cd^{2+} with 2-dimethylamino-ethanethiol.[35]

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To the best of our knowledge, there is no established literature on the bonding strength of the coordinating bonds between ligands with the surface atoms of semiconductor nanocrystals. A very recent report studied the bonding free energy between Au nanocrystals and thiol ligands in aqueous solutions. The value was found to be about 49 kJ/mol, which is close to the values reported for thiol and Au bulk crystals, 20−40 kJ/mol.[36] Evidently, the results shown in Figure 8 are reasonably similar to these values.

Theoretical simulation for the ligand−nanocrystal interaction has not been reported yet. However, we considered that the results shown in Figure 8 are reasonable based on the simple picture of quantum confinement. Brus[18] pointed out a very interesting fact regarding quantum confinement of semiconductor nanocrystals. The chemical indication of quantum confinement is that, within the quantum confinement size regime, the bonding strength between interior atoms in a nanocrystal increases as the size of the nanocrystals decreases. As nanocrystal size decreases, the potential energy curve of the ground state becomes deep and steep, increasing the "hardness" of the material.

The results shown in Figure 8 imply that the bond enhancement inside nanocrystals in the Brus picture may have some connection to the nanocrystal−ligand coordinating bonds on the surface of inorganic nanocrystals. However, it should be pointed out that the $\Delta_r G°$ determined in this work is associated with the formation of the coordinating bonds between negatively charged thiolate ligands and positively charged nanocrystal core. The fundamental reason for the size-dependent $\Delta_r G°$ may be much more complex than this simple model implied. Substantially more experimental and theoretical work will be helpful for further clarification and understanding of the results shown in Figure 8.

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Conclusions

Pseudo steady-state titration was found as a valuable method for the determination of stability of colloidal nanocrystals upon change of the pH in the solutions. The dissociation of the ligands from the surface of the nanocrystals was confirmed as the main cause for the precipitation of the nanocrystals in relatively acidic solutions. The equilibrium pH value for this dissociation process was found to be dependent on the size (band gap) of the semiconductor nanocrystals. This dissociation process was confirmed as a reversible process, although there is a significant hysteresis between the dissociation and bonding pH. This hysteresis is caused by the different path for the dispersion/redispersion process. The size-dependent dissociation pH of the nanocrystal−ligands complex was applied for calculating the average formation constant and associated $\Delta_r G°$ for the nanocrystal−ligand coordinating bonds on the surface of nanocrystals, which was found to be dependent on the size of the nanocrystals in their quantum confinement size regime.

Supporting Information Available: Including titration curves of CdSe coated with mercaptopropionic acid, comparison between bidentate and monodentate ligands, and effects of the terminal groups. This material is available free of charge via the Internet at http://pubs.acs.org.

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