Anal. Chem. **2005,** *77,* 3511−3515

Characterization of Nanocrystalline CdSe by Size Exclusion Chromatography

Karl M. Krueger, Ali M. Al-Somali, Joshua C. Falkner, and Vicki L. Colvin*

Department of Chemistry, Rice University, MS-60 6100 Main Street, Houston, Texas 77005

High-performance size exclusion chromatography (HPSEC) is a powerful tool for probing the size and size distribution of complex materials. Here we report its application to the analysis of cadmium selenide nanocrystals produced in organic solvents. If nanocrystal−column interactions are minimized, this method provides an accurate measure of nanocrystal hydrodynamic diameter directly in solution; such information is complementary to TEM in that it can measure the thickness of various capping agents. While the resolution of single-pass HPSEC is limited to 1 nm, we show here that recycling size exclusion chromatography can be applied to assess the fine details of a sample's distribution. Finally, semiconductor nanocrystals can be made a variety of shapes whose optical characteristics are difficult to distinguish. HPSEC can be applied to the general problem of shape separations which we demonstrate with a tetrapod material.

Nanocrystalline CdSe quantum dots are a widely studied nanomaterial whose size-dependent optical and electronic properties have inspired both fundamental research and novel applications.[1−6] Control over the nanocrystal diameter and size distribution is critical in this field, and this requires accurate and rapid determination of particle size and size distribution in solution. This paper demonstrates the application of high-performance size exclusion chromatography (HPSEC) to this problem. Unlike transmission electron microscopy (TEM), HPSEC provides a quantitative assessment of the particle diameter directly in the solution phase.[7] In particular, it measures the hydrodynamic diameter of a nanoparticle that includes information about both the core and its surface coating. HPSEC is also an important complement to optical absorption in that chromatographic elution times can be directly related to hydrodynamic size. This permits particle sizing without any reliance on models of nanocrystal optical properties.

The application of size exclusion chromatography to the problem of nanocrystal analysis has been limited, though it is a widely used technique for measuring the properties of complex macromolecules such as polymers and proteins.[8−14] Fischer et al. first evaluated chromatography for separating aqueous colloidal CdS particles over a decade ago.[15] Even though these nanomaterials were not stable for long times in solution, under the appropriate conditions, elution time and particle size were found to be related qualitatively as expected for a size exclusion process.[16−19] This work highlighted the persistent problem of enthalpic particle−column interactions, which could be minimized but not removed by the use of additives in the running phase.[20] More recently, the application of HPSEC to gold nanocrystals has been reported.[19,21−25] This work extends past efforts on colloidal CdS by illustrating methods for quantitative analysis of HPSEC data; in particular, careful calibration of columns permit nanoparticle elution time to be directly related to hydrodynamic size. Most recently, aqueous SEC was used for the coarse separation of very different sizes of nano-CdSe/protein conjugates.[26]

* To whom correspondence should be addressed. E-mail: colvin@rice.edu. Phone: 713-348-5741. Fax: 713-348-2578.

(1) Huynh, W. U.; Dittmer, J. J.; Alivisatos, A. P. *Science* **2002**, *295*, 2425−2427.
(2) Colvin, V. L.; Schlamp, M. C.; Alivisatos, A. P. *Nature* **1994**, *370*, 354−357.
(3) Gao, X. H.; Chan, W. C. W.; Nie, S. M. *J. Biomed. Opt.* **2002**, *7*, 532−537.
(4) Chan, W. C. W.; Nie, S. M. *Science* **1998**, *281*, 2016−2018.
(5) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.
(6) Klimov, V. I.; Mikhailovsky, A. A.; Xu, S.; Malko, A.; Hollingsworth, J. A.; Leatherdale, C. A.; Eisler, H. J.; Bawendi, M. G. *Science* **2000**, *290*, 314−317.
(7) Yu, W. W.; Qu, L. H.; Guo, W. Z.; Peng, X. G. *Chem. Mater.* **2003**, *15*, 2854−2860.
(8) Bello-Perez, L. A.; Roger, P.; Baud, B.; Colonna, P. *J. Cereal Sci.* **1998**, *27*, 267−278.
(9) Chang, T. Y. *Adv. Polym. Sci.* **2003**, *163*, 1−60.
(10) Hutchens, T. W.; Gibbons, W. E.; Besch, P. K. *J. Chromatogr., A* **1984**, *297*, 283−299.
(11) Skvortsov, A. M.; Gorbunov, A. A.; Berek, D.; Trathnigg, B. *Polymer* **1998**, *39*, 423−429.
(12) Trathnigg, B. *Prog. Polym. Sci.* **1995**, *20*, 615−650.
(13) Trathnigg, B.; Feichtenhofer, S.; Kollroser, M. *J. Chromatogr., A* **1997**, *786*, 75−84.
(14) Krebs, V. K. F.; Wunderlich, W. *Angew. Makromol. Chem.* **1971**, *20*, 203−205.
(15) Fischer, C. H.; Weller, H.; Katsikas, L.; Henglein, A. *Langmuir* **1989**, *5*, 429−432.
(16) Fischer, C. H.; Giersig, M. *Langmuir* **1992**, *8*, 1475−1478.
(17) Fischer, C. H.; Giersig, M.; Siebrands, T. *J. Chromatogr., A* **1994**, *670*, 89−97.
(18) Fischer, C. H.; Kenndler, E. *J. Chromatogr., A* **1997**, *773*, 179−187.
(19) Fischer, C. H.; Giersig, M. *J. Chromatogr., A* **1994**, *688*, 97−105.
(20) Fischer, C. H.; Siebrands, T. *J. Chromatogr., A* **1995**, *707*, 189−197.
(21) Jimenez, V. L.; Leopold, M. C.; Mazzitelli, C.; Jorgenson, J. W.; Murray, R. W. *Anal. Chem.* **2003**, *75*, 199−206.
(22) Siebrands, T.; Giersig, M.; Mulvaney, P.; Fischer, C. H. *Langmuir* **1993**, *9*, 2297−2300.
(23) Wilcoxon, J. P.; Provencio, P. *J. Phys. Chem. B* **2003**, *107*, 12949−12957.
(24) Wilcoxon, J. P.; Martin, J. E.; Provencio, P. *J. Chem. Phys.* **2001**, *115*, 998−1008.
(25) Wilcoxon, J. P.; Martin, J. E.; Provencio, P. *Langmuir* **2000**, *16*, 9912−9920.
(26) Ding, S. Y.; Jones, M.; Tucker, M. P.; Nedeljkovic, J. M.; Wall, J.; Simon, M. N.; Rumbles, G.; Himmel, M. E. *Nano Lett.* **2003**, *3*, 1581−1585.

10.1021/ac0481912 CCC: $30.25
Published on Web 04/16/2005

Here we report HPSEC characterization of CdSe quantum dots in organic solutions and demonstrate methods for the quantitative analysis of these data. Our methods were shaped by previous reports of nanoparticle chromatography, which highlighted the challenges of reducing enthalpic column/particle interactions.[20] We found that nanocrystalline CdSe in organic solution has persistent interactions with most column materials. These enthalpic interactions preclude quantitative analysis of HPSEC data; however, by adding surface capping agents to the mobile phase that react with both the surface Cd and Se atoms, we have successfully realized size exclusion separations. Cadmium passivation is easily achieved using long-chain thiols that present inert carbon chains to the column.[27] Trioctylphosphine acts in a similar, although weaker, fashion and we speculate this interaction occurs through association with surface selenium atoms. With these modifications in standard HPSEC operation, we were able to achieve the first purely size exclusive and quantitative separation of CdSe nanocrystals.

EXPERIMENTAL SECTION

Chemicals. CdO, Se powder, oleic acid, 1-octadecene (ODE), trioctylphosphine (TOP), trioctylphosphine oxide (TOPO), hexanethiol, octanethiol, decanethiol, dodecanethiol, and octadecanethiol were all purchased from Aldrich and used as received. Toluene was used as the primary mobile phase (Fischer Chemicals, Optima). Preprepared standards (Easi-Cal, Polymer Labs, molecular weights 580−377 400) were used for calibrating the columns.

CdSe Nanoparticle Synthesis. CdSe quantum dots were produced in high-temperature liquid-phase reactions using noncoordinating solvents, in this case, ODE.[28] A typical reaction is as follows: CdO, 0.255 g, oleic acid, 1.88 g, and ODE, 80 mL, were loaded into a 250-mL three-neck flask and heated to 300 °C under N_2 flow. When the solution became optically clear, a selenium solution containing 0.08 g of Se powder dissolved in 0.42 g of TOP and 3 mL of ODE was rapidly injected. To obtain varying sizes, the reactions were quenched with 40 mL of ODE at room temperature at variable reaction times. Control of size a priori was not possible; instead reactions were analyzed postsynthesis for size and distribution. The 1-mL fractions of the reaction mixtures were purified by first adding 1 mL of acetone to precipitate impurities. After 15 min, samples were centrifuged at 3200 rpm for 10 min to remove unreacted cadmium oleate; the decantate, which contained the soluble nanocrystals, was then treated with excess acetone, and the mixture was centrifuged at 3200 rpm for 20 min to recover all nanocrystals. These orange-red solids were redissolved in 1 mL of toluene and treated with ∼20 μL of 1-dodecanethiol to add a surface cap. This addition resulted in quenched fluorescence. A final purification in toluene was completed by centrifugation at 14 000 rpm for 15 min to remove any undissolved particles. The optically clear solution resulting from this procedure was used for all chromatographic measurements.

A polydisperse sample of CdSe containing dots, rods, and tetrapods was synthesized also.[29] CdO, 0.051 g, oleic acid, 0.375 g, and TOPO (99%), 16.0 g, were loaded into a 100-mL three-neck flask and heated to 300 °C under N_2. When optically clear, a selenium solution containing 0.064 g of Se powder dissolved in 1 g of TOP was added in four separate 0.25-mL injections spaced 5 min apart. The reaction was run for a total of 1 h 30 min, at which point heat was removed and the nanocrystals were precipitated with 30 mL of acetone/10 mL of 200 proof ethanol. Nanocrystals were collected by centrifugation and rewashed with an additional 30 mL of acetone. After drying under N_2, the sample was redissolved in 20 mL of toluene. The 1-mL fractions were treated with ∼20 μL of 1-dodecanethiol and centrifuged for 10 min at 14 000 rpm. The decantate was taken for HPSEC analysis.

HPSEC Apparatus. A Waters Alliance 2690 chromatographic system with a photodiode array (model 996) and differential refractive index (model 410) detectors was used to gather chromatograms and ultraviolet−visible spectra (UV−visible). Chromatographic and UV−visible resolutions were 1.0 s and 1.2 nm, respectively. Typical injection volumes were 50 μL. There was no influence of injection volume on retention time. A porous, cross-linked, polystyrene column (300 × 7.5 mm) of 1000-Å pore size and 5-μm particle size (Polymer Labs PLgel 5 μm, model 1110-6530) was used for all separations. The mobile phase was a 0.1 M solution of trioctylphosphine in toluene with a flow rate of 1.0 mL/min. Prepared in air, the mobile phase was stable for several weeks and showed no signs of degradation. No column conditioning was necessary other than allowing 1 h for the column to equilibrate after a solvent switch. Column temperature was held constant at 30 °C. An Agilent 1100 series HPLC equipped with Valco switching valves was used to gather data of recycling SEC. This alternate recycling setup utilized one 1000-Å and one 500-Å PLgel column from Polymer Labs.

Transmission Electron Microscopy and Particle Sizing. Each sample was independently sized using a JEOL TEM, model JEM-2010, operating at 100 kV. Samples were evaporated from toluene onto 400-mesh ultrathin carbon/Formvar-coated copper grids (Ted Pella, 01822). A total of 60 digital images were gathered for each sample in three separate sessions at 100000×. Nanocrystal diameters were sized using ImagePro analysis software (Media Cybernetics version 5.0) resulting in over 1000 data points per sample.

RESULTS AND DISCUSSION

Figure 1 shows representative size exclusion chromatograms of two sizes of nanocrystalline CdSe and their respective absorption spectra. Contrary to traditional enthalpic mode chromatography, size exclusion chromatography separates solutes based on their entropic interaction with a porous stationary phase.[9,30−32] Upon entering pores, the conformational degrees of freedom of a solute, in this case CdSe, are reduced resulting in a loss of conformational entropy. This diffusion into the porous stationary phase is driven by the solute concentration gradient between the interstitial and pore volumes. Large solutes suffer a greater loss in conformational entropy than small solutes when entering pores of the stationary phase. As a result, larger solutes do not enter the pores resulting in an overall shorter path length and shorter

(27) Colvin, V. L.; Goldstein, A. N.; Alivisatos, A. P. *J. Am. Chem. Soc.* **1992**, *114*, 5221−5230.
(28) Yu, M. W.; Peng, X. G. *Angew. Chem., Int. Edit.* **2002**, *41*, 2368−2371.
(29) Qu, L. H.; Peng, Z. A.; Peng, X. G. *Nano Lett.* **2001**, *1*, 333−337.
(30) Churms, S. C. *J. Chromatogr., A* **1996**, *720*, 151−166.
(31) Trathnigg, B. In *Encyclopedia of Analytical Chemistry*; Meyers, R. A., Ed.; John Wiley & Sons Ltd.: Chichester, 2000; pp 8008−8034.
(32) Sadao, M.; Barth, H. G. *Size Exclusion Chromatography*, 1st ed.; Springer Laboratory: Berlin, 1999.

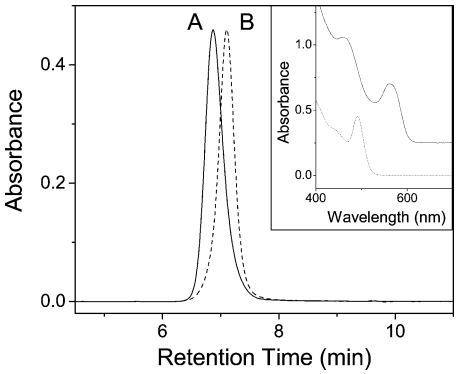

Figure 1. Representative chromatograms of (A) 3.56 (±0.52) and (B) 2.58 (±0.42) nm diameter CdSe nanocrystals monitored at their first absorption peak, 565.0 and 488.0 nm, respectively. The larger particles elute first as expected for a size exclusion process. Inset: absorption spectra of the two samples.

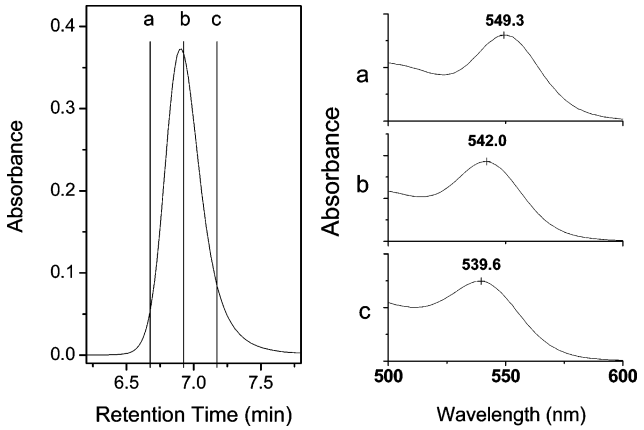

Figure 2. Temporal evolution of the absorption spectrum within one HPSEC chromatogram. Within one chromatogram, the absorption spectrum smoothly shifts from the red to the blue, reflecting the different elution times of the subcomponents within the monodisperse sample. The initial absorbance in this sample was 542.0 nm.

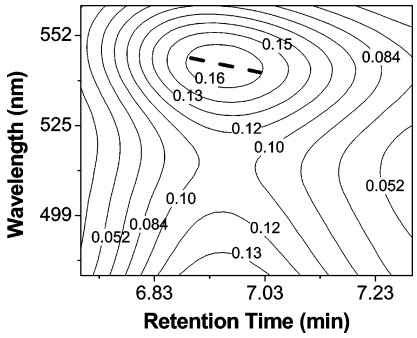

Figure 3. Contour plot of the entire absorbance of the sample versus retention time. The higher energy absorption features of samples early and late in the chromatographic peak of one sample are similar in shape; only small shifts in the exciton absorption peak are observed as indicated by the dashed line.

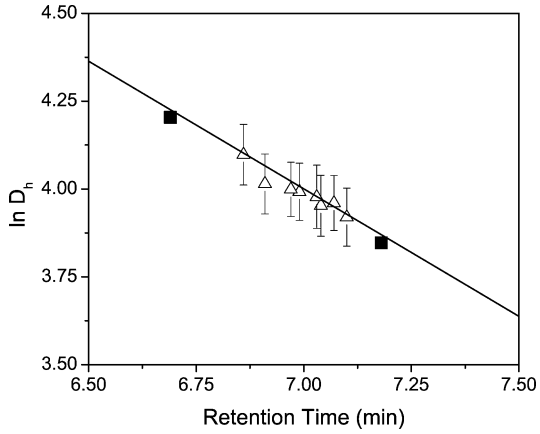

Figure 4. Quantitative calibration of the size exclusion column used in these separations. The relationship between retention time and hydrodynamic diameter is found precisely through the use of polystyrene calibration standards (■). The core diameter of the CdSe samples were found from TEM imaging ($N > 1000$). The hydrodynamic diameter was then calculated by adding a 1-dodecanethiol (△) capping group.

elution time. As Figure 1 illustrates, smaller particles elute at later times than the larger ones; additionally, the data show that HPSEC can easily distinguish between these two samples, which differ in diameter by only 0.98 nm.

We can qualitatively confirm that only a size exclusion, as opposed to enthalpic, separation is operative by analyzing the optical absorption spectrum at different retention times within a chromatographic peak. In Figure 2, optical absorption spectra from various times in the chromatogram are shown. The first absorption peak clearly blue shifts with increasing retention time. This indicates a size exclusion separation occurs even within one sample, with slightly larger CdSe nanocrystals eluting early and slightly smaller nanocrystals eluting later. Instead of viewing spectra for only selected retention times, we may also view them for every retention time (1.0-s spacing) to see the continuous evolution of the optical data (Figure 3). At shorter wavelengths, the overall shape of the spectrum is remarkably constant with retention time; only the exciton absorption peak shifts systematically with time as highlighted by the dashed line. This asymmetry captures the clear and continuous blue shift of the first absorption peak with retention time illustrated in the single absorption spectra shown in Figure 2.

Peak retention time from HPSEC can be quantitatively related to the hydrodynamic diameters (D_h) of analytes if the column is properly calibrated; such data also provide further confirmation that size exclusion effects are dominant in a nanocrystal separation.[23−25] Illustrated in Figure 4, monodisperse polystyrene standards of known hydrodynamic diameter are used to generate a linear calibration curve relating D_h to peak retention time. Peak retention time is taken at the chromatogram apex, and the full calibration plot is available in the Supporting Information. We compared the retention times from nanocrystalline CdSe samples of known core sizes to this calibration line as shown in Figure 4. To do this, core diameters were determined by transmission electron microscopy after counting over 1000 particles. We then converted particle core diameters to hydrodynamic diameter by assuming that the particle surfaces were capped with 1-dodecane thiol (L = 12.3 Å); nanocrystals were treated with it prior to separation.[23−25] We believe the thiol shares the surface with trioctylphosphine, a shorter ligand, but that the longer thiol ligand determines the shell thickness. This model provides quantitative agreement with the calibration of this column as shown in Figure 4.

Once the relationship of retention time to hydrodynamic diameter is established with polymer standards, HPSEC chro-

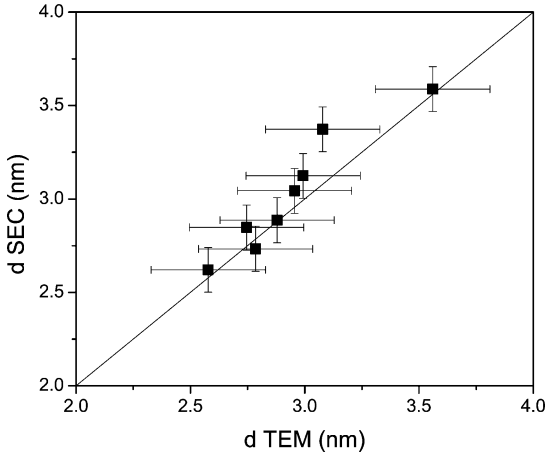

Figure 5. Comparing sample diameter determined from TEM and HPSEC. The line represents the 1 to 1 correlation.

Table 1. Diameter and Dispersity of CdSe Nanocrystal Samples from TEM Imaging and High-Performance Size Exclusion Chromatography (c12sH Thickness Substrated)

sample	D_{tem} (nm)	% dispersity (TEM)	count	D_{sec} (nm)	% dispersity (SEC)
CdSe1	2.58	0.16	2901	2.62	0.17
CdSe2	2.79	0.15	1066	2.73	0.16
CdSe3	2.75	0.16	3487	2.85	0.15
CdSe4	2.88	0.17	3786	2.89	0.16
CdSe5	2.96	0.15	1090	3.04	0.14
CdSe6	2.99	0.14	1053	3.12	0.14
CdSe7	3.08	0.15	5109	3.37	0.15
CdSe8	3.56	0.15	1123	3.59	0.17

matograms can be used to determine the absolute core diameter in a nanocrystal sample. Sample retention time is first converted to hydrodynamic diameter by the calibration curve (Figure 4) and then core diameter may be found by subtracting the shell thickness. This core diameter from HPSEC (D_{sec}) can be compared to independently measured core diameters from TEM (D_{tem}) as shown in Figure 5; there is excellent agreement between core diameters obtained from TEM and HPSEC. Table 1 details the sizing information on these samples and lists the measured D_{tem} and D_{sec} for all sizes. On average, diameters provided from HPSEC and TEM differ by less than 1 Å. Table 1 also compares percent dispersity (σ/diameter) in diameters gathered from TEM and HPSEC and we see good agreement here as well. (The standard deviation in a sample's size can be found from $\sigma = w/2$, where w is the chromatographic peak width at 0.607 × peak height.[32] To obtain an accurate standard deviation we must correct for instrumental broadening. $\sigma^2_{Total} = \sigma^2_S + \sigma^2_I$ (1). Variance in an eluted peak (σ^2_{Total}) is the sum of sample variance (σ^2_S) and instrumental variance (σ^2_I). Instrumental variance was determined by injecting a monodisperse polystyrene standard so that all peak variance was due to instrumental variance. By subtracting instrumental variance from the total we obtain only the sample variance.)

HPSEC used in conjunction with TEM allows the total thickness of the surface cap to be determined. Figure 6a shows one sample capped with thiols of differing chain length. The retention time decreases as expected with an increase in chain length. These retention times are first converted to hydrodynamic

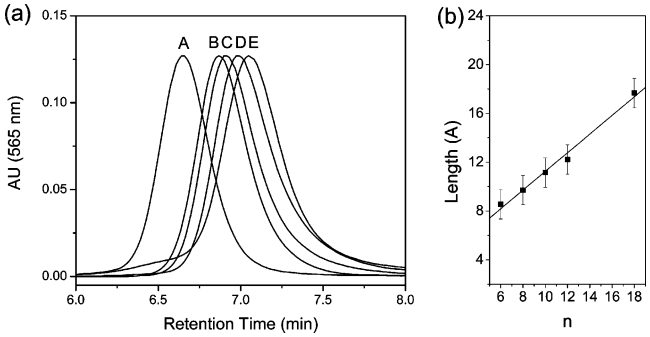

Figure 6. (a) Single core size sample (CdSe8) with long-chain thiol capping agents of different lengths: (A) c18 sH, (B) c12 sH, (C) c10 sH, (D) c8 sH, and (E) c6 sH. Retention time decreases with increase in thiol chain length. (b) Linear relationship of thiol length to number of carbon atoms (n) determined by HPSEC.

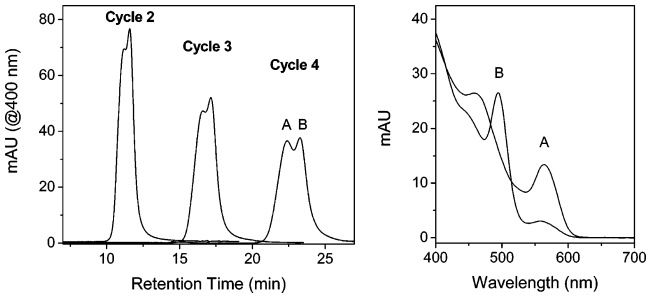

Figure 7. Recycling size exclusion chromatography of a bimodal sample: (A) 3.56 nm; (B) 2.58 nm. Separation increases with cycle number. Separation is confirmed by UV−visible spectra at peak apexes.

diameter using the calibration curve. Then the CdSe core diameter (3.56 nm) is subtracted and the thiol length deduced: 8.5 (C6sH), 9.7 (C8sH), 11.1 (C10sH), 12.3 (C12sH), and 17.6 Å (C18sH). C6sH capped nanocrystals were unstable and precipitated after several hours. All longer chain thiols showed good stability. Figure 6b plots thiol length in terms of n, or number of carbon atoms. The trend is linear as expected, and the slope indicates an increase in chain length of 0.76 Å for each additional carbon atom. For thiols in monolayers, this value ranges from 0.56 to 1.5 Å.[25,33] Clearly, average lengths of thiol chains depend on packing and interchain interactions.

While HPSEC is clearly useful for analysis, its limited resolution may preclude more precise characterization of these highly monodisperse samples. The technique of recycling size exclusion chromatography (RSEC) allows enhanced resolution and separation of nanoparticle samples. Previously applied by our group to gold nanoparticles, RSEC recycles the analyte through the column several times to improve separation without increased back pressure.[34] Figure 7 shows the evolution of a bimodal sample (2.58 and 3.56 nm) with successive passes through the column. Unlike in Figure 1 where two monodisperse samples were analyzed separately, here we now mix to create a bimodal sample. Initially, the single broad peak shows little evidence of being composed of two sizes. However, the non-Gaussian distribution becomes more evident with each pass, and this separation can be confirmed by

(33) Porter, M. D.; Bright, T. B.; Allara, D. L.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **1987**, *109*, 3559−3568.
(34) Al-Somali, A. M.; Krueger, K. M.; Falkner, J. C.; Colvin, V. L. *Anal. Chem.* **2004**, *76*, 5903−5910.

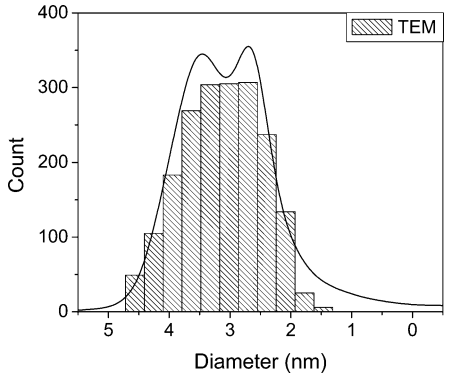

Figure 8. Corresponding TEM histogram data overlaid on the fourth cycle of a bimodal sample. Recycling data show the sample bimodality more clearly than TEM.

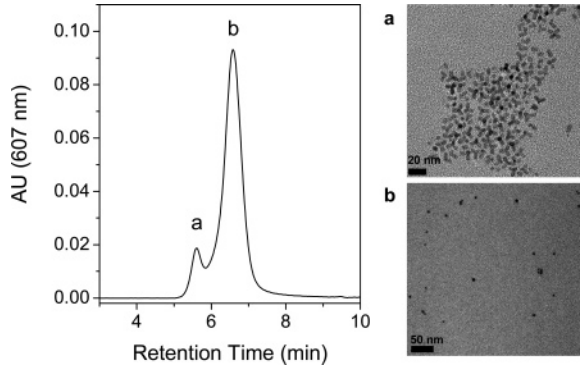

Figure 9. Single-pass separation of polydisperse sample into multipods and quantum dots. TEM was performed on collected fractions of peaks a and b.

the optical absorption data for each peak (A and B). Figure 8 overlays the fourth pass chromatogram of Figure 7 on the TEM histogram data for this sample. Clearly, the TEM cannot capture the bimodality of this sample, a mixture of the monodisperse CdSe1 and CdSe8. In contrast, HPSEC clearly shows in multiple passes the presence of two distinct sample types.

Because separation in HPSEC is based on hydrodynamic diameter, we hypothesized that the technique would be valuable for separating nanocrystals of differing shape. A primitive shape separation of gold nanorods has been shown, although it was plagued by severe enthalpic particle/column interactions.[35] We prepared a distribution of cadmium selenide shape particles using standard methods that have been reported to produce rods, tetrapods, and dots in some statistical distribution.[29] Figure 9 illustrates the separation of one of these samples; the images with the chromatogram are representative images from samples collected from each peak in the chromatogram. Clearly the larger tetrapods elute earlier than the spherical dots. Quantitative analysis of these data is the subject of ongoing efforts, but this result illustrates the generality of HPSEC to the problem of shape purification in nanoscience.

The quantitative size and shape separations shown in this work required complete passivation of nanoparticle surfaces to eliminate particle/column interactions. As compared to gold nanoparticles, this issue was challenging to address because of the heterogeneity of the particle surface. This requires complete capping for *both* surface Cd and Se atoms. For cadmium binding, we used long-chain thiol well known to form bonds to cadmium of nanoparticles.[27] However, particles only treated with thiols showed significant column interactions leading to chromatograms with low intensity, late elution, and asymmetric peak shape. We hypothesized that free selenium at particle surfaces was the cause. Inclusion of TOP in the mobile phase eliminated this issue, presumably because of the strong interactions between Se and P. Chromatography generally is a sensitive measure of surface−column interactions. Further study of this issue may provide more detailed information about the solution-state surface chemistry of nanoparticles.

CONCLUSIONS

We report here the size exclusion chromatography of nanocrystalline CdSe; these data yield quantitative information about nanocrystal size, shape, and size distribution. This is shown through the size-dependent optical properties of nanocrystalline CdSe as well as confirmed by direct comparisons between particle sizes and distributions obtained from TEM and HPSEC. In addition to particle size information, HPSEC provides information about the surface cap thickness when the core diameter is known. Also, recycling SEC techniques can be employed to improve the resolution of analysis and provide more precise information about sample modality than TEM. Shape separation is also possible with this technique. The information provided by HPSEC, coupled with its low cost, fast operation, and reliability, make size exclusion chromatography an attractive method for standardizing nanocrystalline materials directly in the solution state.

ACKNOWLEDGMENT

This work was supported by the Center for Biological and Environmental Nanotechnology under NSF Grant EEC-0118007 and the R.A. Welch foundation (C-1342). Special thanks to William Yu for helpful discussions.

SUPPORTING INFORMATION AVAILABLE

An expanded view of the column calibration plot shown in Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review December 7, 2004. Accepted March 22, 2005.

AC0481912

(35) Wei, G. T.; Liu, F. K.; Wang, C. R. C. *Anal. Chem.* **1999**, *71*, 2085−2091.