Anal. Chem. **2004,** *76,* 5903−5910

Recycling Size Exclusion Chromatography for the Analysis and Separation of Nanocrystalline Gold

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

Department of Chemistry, Rice University, MS-60 6100 Main Street, Houston, Texas 77025

Recycling size exclusion chromatography (RSEC) provides a high-resolution technique for the analysis and separation of materials based on size. We show here the application of this method to gold nanocrystals stabilized by thiols. Alternate recycling is more effective at separating nanomaterials as compared to closed-loop recycling because of its improved efficiency and high resolution. With the use of this technique, we find the resolution ratio of nanocrystal separation increases with the square root of the cycle number, in good agreement with theory. The increased resolution of the size exclusion chromatograms permits the use of RSEC in the baseline separation of nanocrystals which differ by only 6 Å in size. In addition to separations, RSEC is valuable as an analytical tool. For example, after recycling processes an initially broad and chromatographic feature from a gold nanocrystal solution resolves into three distinct peaks. Transmission electron microscopy of collected fractions reveals that these peaks correspond to distinct populations of gold nanoparticles with narrowly defined sizes.

This work aims to use size exclusion chromatography, or SEC, as a tool for evaluating and improving the quality of gold nanocrystals. Like many other nanomaterials, the physical sizes of gold nanocrystals are among their most important attributes. Characteristics ranging from the nanoparticle melting point to its chemical reactivity are a sensitive function of nanocrystal diameter.[1,2] These size-dependent properties are often exploited in applications, as for example in the use of gold clusters below 3 nm for catalytic oxidation of carbon monoxide.[3−7] Generally, many nanocrystal systems, including semiconductor quantum dots,[8−10] magnetic iron oxides,[11−13] and other noble metals, show great tunability in properties with size.[14−17] Advances in nanocrystal synthesis as well as applications will thus require effective tools for measuring and controlling particle size distributions.

Size exclusion chromatography (SEC) offers an ideal solution to the problem of routine nanoparticles characterization and separation in the solution phase.[18−21] In contrast to other forms of chromatography, SEC relies on purely entropic interactions for excluding materials from the pores of a column; peak elution times can thus be quantitatively correlated to the hydrodynamic volume of the analytes. Though this ease of interpretation comes at the cost of chromatographic resolution, SEC is the best method for the analysis of complex mixtures where surface−column interactions are minimal. In the polymer sciences, SEC is used both for the separation as well as the analysis of polymer molecular weight distributions.[18,19] Biotechnologists also use SEC for high-resolution purification and preparation of viruses[22] and larger proteins.[23−25]

Given these features, size exclusion chromatography (SEC) would seem to be an ideal tool for both characterizing and purifying nanocrystal solutions. Its use for this application, however, has not been extensively reported. Wei et al first explored the tool for separating differently shaped gold nanocrystals; their works highlight how strong nanoparticle−column interactions make the realization of purely size exclusion processes quite challenging.[26,27] Enthalpic interactions are the basis of the

* To whom correspondence should be addressed. E-mail: colvin@rice.edu.

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10.1021/ac049355h CCC: $27.50
Published on Web 09/08/2004

chromatographic analysis in another report which uses this information to examine the surface chemistry of monolayer-protected clusters (MPCs) of gold coated with different passivating agents.[28] Purely size exclusive separations have been shown in the work of Wilcoxon et al; these data demonstrate the use of conventional SEC as an analytical tool and illustrate the advantages of SEC over transmission electron microscopy for determining nanoparticles size.[16,29−31]

These existing reports illustrate the general value of chromatography in nanoparticle analysis, but the low resolution of SEC limits its application to nanomaterial separations. Great effort has been expended in developing synthetic methods which produce narrowly dispersed nanocrystalline materials; in order to improve the distributions of such samples, a separation process must be capable of distinguishing between particles of less than 10% difference in size. Conventional SEC cannot meet this condition. Commercially available columns offer theoretical plates ranging from 12 000 to 18 000; the translation of this performance into a size resolution depends in detail on the system conditions (e.g., pore volume, detector choice, flow rate, column packing particles size). In the few cases where the size discrimination of SEC has been carefully measured, a resolution in size of 10% in hydrodynamic diameter is commonly reported (where resolution is defined by the size difference needed to discriminate between the peaks of two samples chromatograms).[32] These observations are consistent with data already reported on gold nanocrystals, which show that the SEC features of samples different in size by 10 Å are clearly overlapping.[30] Overlapping peaks are not problematic if SEC is to be used for size analysis; however, it does complicate evaluation of particle size distributions.[16,30] More definitively it precludes the use of conventional SEC in preparative processes. For this application, better resolution between particle populations is required. Conventional SEC is unlikely, thus, to improve upon the 10−20% size distributions achieved directly by many chemical syntheses.[15,17,33,34]

Here we use recycling strategies to improve the resolution of conventional size exclusion chromatography and thus extend its application in nanoscience.[19,35] Recycling SEC is a practical method for increasing the column length in a SEC system. It overcomes the increase in back pressure associated with long columns by recycling the column output back into the column. The resolution increases with each pass through the column and exhibits a square root dependence on the number of cycles.[19,35] Other methods can also be employed to increase SEC resolution, such as the use of preparative columns with larger internal diameter and, therefore, higher pore volume. However, larger particle sizes are used in the packing of these columns to increase sample loading, since the effect of sample volume on the broadening is more pronounced for the smaller size packing material, and hence, the number of theoretical plates is usually smaller than that of analytical columns.[18,36] For example, in a typical preparative SEC column of 25-mm internal diameter (i.d.) and 300-mm long, packed with a 10-μm beads, the injection volume can be increased 10−20 times with 10 times concentrated sample relative to an analytical column of 7.5-mm i.d.[36] Therefore, rapid and high yield of separated fractions compensates for the relatively low efficiency, which can be improved by recycling. Because preparative columns are relatively expensive, method development is usually carried out using analytical columns and then scaled up to higher sample loading and mobile phase flow rate in a preparative column packed with the same beads.[36]

The existing uses of recycling chromatography suggest it may provide a general method for the purification of nanocrystal samples.[37,38] Because recycling SEC trades speed and peak capacity (e.g., number of peaks separated at a given time) for resolution it is well suited for the separation of closely eluting peaks. In one case, recycling chromatography permitted the separation of benzene and deuterated benzene.[39] Recycling chromatography is also widely applied in the pharmaceutical industry where such techniques (e.g., "moving bed" chromatography) are used for the purification of drugs.[27,40] In principle, its application to sharpening nanostructure distributions should be possible if nanocrystals undergo size exclusive interactions with columns, making the addition of an additive to the mobile phase necessary in some cases. For example, alkanethiol is added to mobile phase whenever the size is greater than 5 nm in the Brust method to minimize interactions with the column. Modification of surface chemistry may be necessary, in some cases, to eliminate or reduce enthalpic interactions with the column. Though there are remarkable synthetic reactions which can produce some nanocrystals of very narrow size and shape distributions, universal and scalable methods for generating "monodisperse" nanoparticles of arbitrary material types are not available.[33,34,41]

In this paper, we demonstrate the application of both closed-loop and alternate-recycling size exclusion chromatography to the analysis and separation of thiol-passivated gold nanocrystals. The alternate-recycling method provided far better performance than the closed-loop configuration. The resolution and efficiency of alternate-recycled chromatograms increased as the square root of the cycle number in agreement with existing models. Confirmation of nanoparticles separation was obtained by transmission electron microscopy (TEM) analysis of the size distributions of sample fractions collected at different times in the chromatographic separation. With the use of alternate recycling, we achieved baseline separation ($R = 1.3$) of populations of particles in a polydisperse gold nanoparticle sample. The chromatographic resolution of a separation is defined here as

$$R = \frac{(2.35/2)(t_a - t_b)}{(w_{0.5(a)} + w_{0.5(b)})} \quad (1)$$

where t_a and t_b are the retention times of two adjacent peaks

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having peak widths of $w_{0.5(a)}$ and $w_{0.5(b)}$ at full width at half-maximum (fwhm). We use the term "baseline separation" to define a separation where $R > 1.25$. In particular, the smallest nanoparticle population, consisting of less than 40 atoms,[42] was completely isolated from a sample originally characterized by a 2.6-nm average diameter and distribution width of over 2.0 nm.

EXPERIMENTAL SECTION

Chemicals. The gold precursor, gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), tetraoctylammonium bromide (TOAB, ≥99%), decanethiol, tetradecanethiol, and octadecanethiol were all purchased from Aldrich and used as received. An alkaline solution of sodium borohydride (approximately 12 wt % $NaBH_4$ in aqueous 14 M NaOH) was used for reducing the gold salt after diluting to a final concentration of 0.4 M. Solvents for all reactions and separations were distilled water ($R = 18.2$ MΩ) and toluene (Fisher Chemicals, HPLC grade). Pre-prepared standards (Easi-Cal, Polymer Labs, Molecular weights 580−377 400) were used for calibrating the columns.

Gold Nanoparticles Synthesis. A procedure based on the work of Brust et al was used to prepare gold nanoparticles.[43] A large excess of thiols was added (2−3 mL) to a two-phase solution containing both organically modified gold in toluene and water. Prior to reduction the mixture was a deep-brown color, and after thiol addition the solution became colorless in less than 40 s. This results in the formation of very small but monodisperse nanoparticles. To prepare the sample used in the recycling process, we intentionally made a broad size distribution by adding the reducing agent (25 mL) to the solution over approximately 3 min. The size distribution was further increased by adding the thiol before and during the reduction process in small amounts. To achieve even greater polydispersity, gold-1-decanethiol nanoparticles produced with varying TOAB/Au molar ratios (0.075, 0.22) were mixed together. All the samples were purified with solid-phase extraction cartridges (Waters Corp., Sep-Pak silica or alumina) prior to the SEC experiments.

SEC Apparatus. An Agilent high-performance liquid chromatograph (HPLC), 1100 series, equipped with a fixed wavelength UV−visible detector was used to record the recycled SEC chromatograms. In all experiments, the UV−visible wavelength was set at 520 nm for detection. The HPLC was outfitted with two six-port, two-position, high-speed (<100 ms) Valco valves with microelectric actuators (Model EHCA-CE). The two-position microelectric actuator has stall-sensing circuitry which prevents valve−actuator misalignment. A major factor in the equipment design is the minimization of dead volume; dead volume in recycling SEC leads to peak broadening and low detector response. We used valves with small port sizes, only 0.75 mm, for this reason; a fill port connected to one of the valves, fitted with a 20-μL loop, was used to introduce the samples into the column. Tubing with an internal diameter (i.d.) of 0.25 mm (0.010 in.), with the shortest possible length, was used for all connections. To avail the maximum separation efficiency of SEC analytical columns, it is recommended that the injected volume should not exceed 0.5% of the total volume bed of analytical SEC columns, which is about 50 μL in our case.[44]

Porous hydrophobic microgel (Polymer Labs PLgel 1110 series) columns of a cross-linked polystyrene (300 mm × 7.5 mm, 5-μm particles size) of either 500 or 1000 Å pore size were used in all experiments. Toluene was always the mobile phase, and the flow rate was kept at 1.0 mL/min except in the closed-loop recycling experiment, where it was 0.5 mL/min. Calibration of the columns was completed on a Waters HPLC (Alliance, separation module 2690) equipped with differential refractive and PDA detectors. In those cases where fractions were collected during SEC operation, manual operation of the valves permitted collection using the minimum tubing length possible of 0.010 in. from the outlet of the detector, where the total volume was kept at approximately 10 μL. Larger ID tubing could result in the mixing of chromatographic peaks after multiple cycles. The SEC system employed for these experiments was analytical in its design; this limited the volume of material collected to 100 or 200 μL. The retention volume for every peak is generally greater than the injected volume, because of the diffusional process that tend to spread the sample in the stationary phase each time the sample is reintroduced to the recycling system.

Transmission Electron Microscopy and Particle Size Analysis. A JEOL TEM, model JEM-2010, operating at 100 kV and equipped with a single tilt, multisample holder, was used to record images of nanoparticle samples. Samples were prepared by evaporating one drop (~50 μL) of a gold nanocrystal sample in toluene onto ultrathin carbon-coated copper grids (Ted Pella). Typical magnification levels used for size analysis were 80 000, 100 000, and 150 000×; at least 15 digital images were collected for each sample type. Particle size distributions were generated from these data using ImagePro analysis software (Media Cybernetics version 5.0), which permits the automated identification, sizing, and analysis of particle images. Large sample populations ($N > 750$) were used in all cases to ensure a high confidence ($P > 0.95$) in the reported size distributions.[45]

RESULTS AND DISCUSSION

SEC as an Analytical Tool. SEC is well suited for routine online characterization of dispersed nanocrystals, provided that the separation of the materials in a column is based purely on a size exclusion mechanism. Figure 1 shows typical size exclusion chromatograms for three gold nanocrystal samples ($d = 1.5$ nm, $\sigma < 5\%$) in a toluene mobile phase capped with 1-dodecanethiol (C10), 1-tetradecanethiol (C14), and 1-octadecanethiol (C18); the surface-capping agents contribute substantially to the overall hydrodynamic diameter of these samples which are 2.19 nm (C10), 2.87 nm (C14), and 3.67 nm (C18).[30] The smaller particles elute later than the larger particles as expected for a size exclusion mechanism. Moreover, this conventional SEC illustrates that the method can distinguish between particle populations different in

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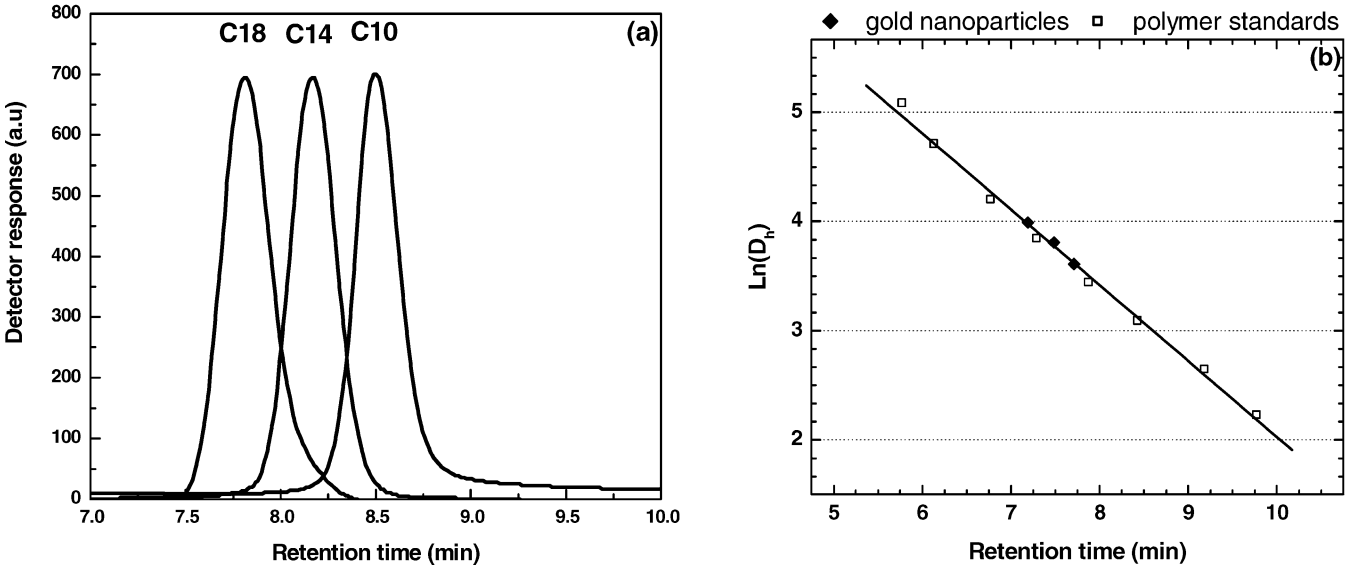

Figure 1. Conventional size exclusion chromatograms of gold nanoparticles. (a) Gold nanoparticles of fixed core size (1.5 ± 0.11 nm) elute at different times because of the thickness of their surface coatings (C18 1-octadecanethiol, C14 1-tetraoctanethiol, and C10 1-decanethiol). (b) The elution times of materials through the 1000 Å column vs the natural log of the hydrodynamic diameter. Known polymer standards are shown as open squares (□) while the elution times of the materials in (a) are shown as closed diamonds (◆). The hydrodynamic diameters of gold nanoparticles were found from their core diameter, as determined by transmission electron microscopy and the lengths of their alkanethiols surface measurements (ref 30).

size by 8 Å. Although there is more than a 15% difference in the hydrodynamic diameter between successive peaks, the features are not completely resolved in this experiment.

With calibrated columns such chromatograms can confirm that a size exclusion separation is operative and provide the hydrodynamic diameters of the nanoparticles. We used commercially available polymer standards to calibrate the microgel columns; a plot of the elution time versus the natural log of the D_h (hydrodynamic diameter) is linear for purely size exclusion separations (Figure 1b). With the use of the universal calibration model, this SEC data permits the determination of the hydrodynamic diameter of a particle from its elution time.[18] After correcting for the thickness of the capping group (1-decanethiol, $T = 21.9$ Å) the core diameter can be calculated.[30] For these samples, the agreement between the core size as determined from TEM and the core size as found from SEC analysis is within 10% in all cases.

Even though gold nanocrystals are often monodisperse materials, the peak widths of the chromatograms are large relative to the separation between the differently sized nanocrystals (Figure 1b). Several experimental factors lead to the low resolution of conventional SEC.[32] First, the time scale of particle transport through columns is naturally determined not only by a particle's ability to sample pore volumes (size exclusion) but also by natural diffusive processes. The latter factor leads to an intrinsic broadening of peak elution times and thus limits chromatographic resolution.[40] Moreover, SEC resolution is further limited by the size of the column packing material, column dimensions, and eluent linear velocity. While these effects are minimized in commercial columns and SEC instruments, they still constrain the efficiency of the separation. Different approaches for correcting chromatograms for the instrumental response have been published, but it is a challenge to extract quantitative information about particle quality for samples with relatively narrow size distributions.[32,46−48]

Recycling SEC. One way to improve the resolution of SEC is to extend the column length; this is an impractical solution for a single column as back pressure in the system increases linearly with column length. However, if solutes are recycled at the end of a run back into the inlet, the column can be reused. Figure 2a depicts the design of a typical closed-loop recycling chromatography system. This is the simplest recycling concept and it places the pump, detector, and column in a closed loop. The solid line in the switching valve indicates the direction of the flow when the system is in the recycling mode, while the dotted line indicates the direction of the flow when the valve is set to the collection mode. This configuration requires only one two-position, four-port valve. Because the output of the detector is directly connected to the control valve, a 0.5 mL/min (34-bar back pressure) flow rate is used to protect the cell from the high back pressure, which has a pressure limit of 40 bar.

Figure 2b shows the resulting chromatogram from a closed-loop recycling process completed on a gold nanocrystal sample passivated with 1-decanethiol. Although the resolution increases with each cycle (chromatographic resolution improves from 0.5 to 1.2 in the data shown (Figure 2b)), this method suffers from severe limitations. The chromatographic peaks are skewed, and extensive broadening of the chromatogram results in a low detector response. Moreover, the peak capacity in this case is substantially reduced due to the dead volume of the pump (100 μL in our case). The success of closed-loop recycling depends,

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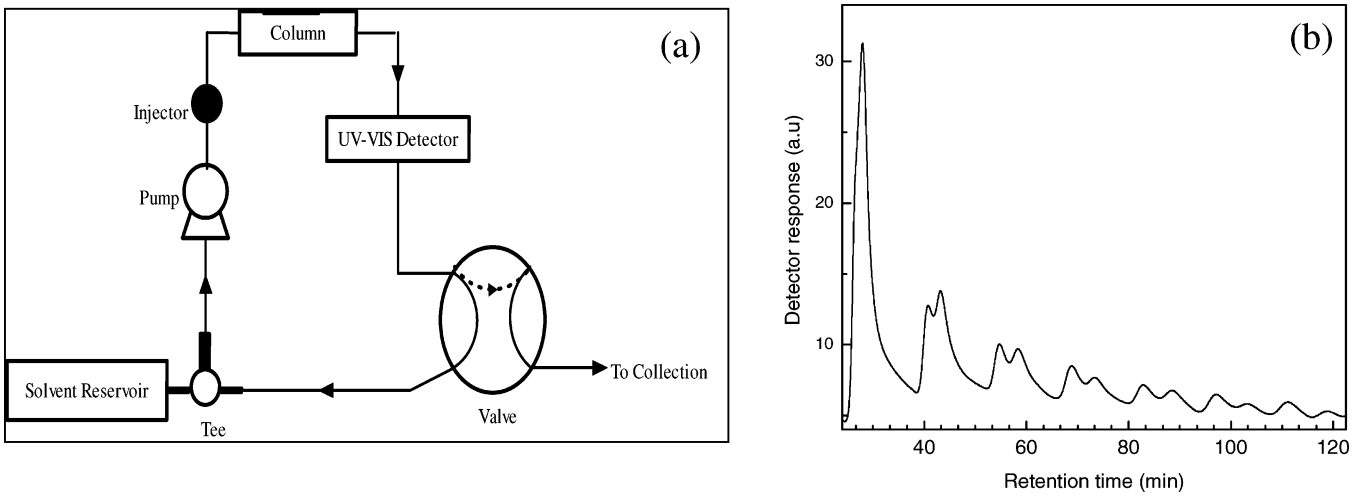

Figure 2. Closed-loop recycling chromatography of broadly distributed 1-decanethiol-Au nanoparticles. (a) The configuration of the closed-loop recycling system. The detector, pump, and the column are all in a single closed loop. The dotted line in the valve indicates the direction of the flow when the valve is switched for collection. (b) Closed-loop recycling chromatogram of 1-decanethiol-Au nanoparticles taken on a 1000 Å column with flow rate of 0.5 mL/min. The initially broad peak resolves into two features which quickly dissipate due to extensive broadening introduced by recycling.

apparently, on the mode of separation (e.g., enthalpic vs size exclusion) and the specific design of the pump.[35,37]

Alternate-Recycling SEC. In the alternate-recycling mode, two columns are used in tandem, and solute flow through each is regulated by a low-volume, high-speed valve.[18,19,35] The process increases the effective column length of the system and thus improves the resolution of closely spaced peaks substantially. We adopted the alternate pumping recycling protocol used by Kucera and Manius;[39] a schematic of the apparatus is shown in Figure 3. In contrast to closed-loop recycling where the column eluent is continually diverted into the mobile phase, alternate recycling uses two columns and no solvent recycling. Initially, a chromatogram is begun conventionally by introduction of a sample into a mobile phase at zero time. As the peaks of interest elute off the column they are immediately directed into a second column via the six-port valve. The eluent from this second pass is then directed back into the first column. One disadvantage of alternate recycling is that it requires a mobile phase flowing continually throughout the separation and does not reuse solvent; this disadvantage over closed-loop recycling is greatly outweighed in this case by the improvement in the nanocrystal separations.

Practically, alternate recycling can lead to improvements of up to 3 in chromatographic resolution.[49−51] In the work of Kucera and Manius, for example, two peaks of deuterated and normal benzene are unresolved in a conventional chromatogram but after only five passes are baseline separated.[39] Measuring the efficiency and resolution of RSEC is straightforward using the number of theoretical plates, N, typically defined for the last recycling peak

$$N = 5.54\left(\frac{t_R}{w_{0.5}}\right)^2 \qquad (2)$$

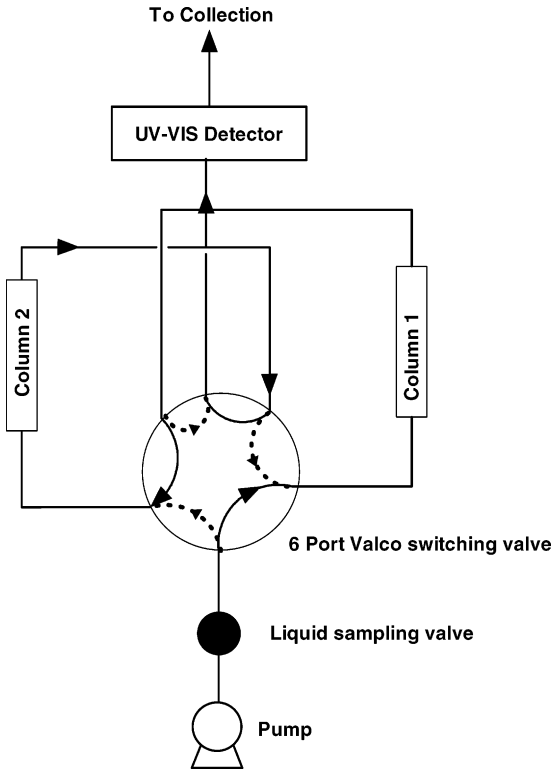

Figure 3. Experimental diagram for alternate-recycling chromatography. Here, solvent is continually run through two columns in alternating turns. Each column must be identical in dimensions (7.4 mm × 300 mm) and pore size to avoid high-pressure fluctuations when the flow direction is changed from one column to another. Solid arrows in the valve indicate the direction of the flow in position one, where column 1 is on the high-pressure side. The dotted arrows indicate the direction of the flow when the valve is switched to position two, and column 2 is on the high-pressure side in this case.

where t_R is the retention time of the peak at the apex and $w_{0.5}$ is the width of the peak at full width at half-maximum. If resolution is defined as in eq 1, then the resolution ratio of peaks 2 and 3,

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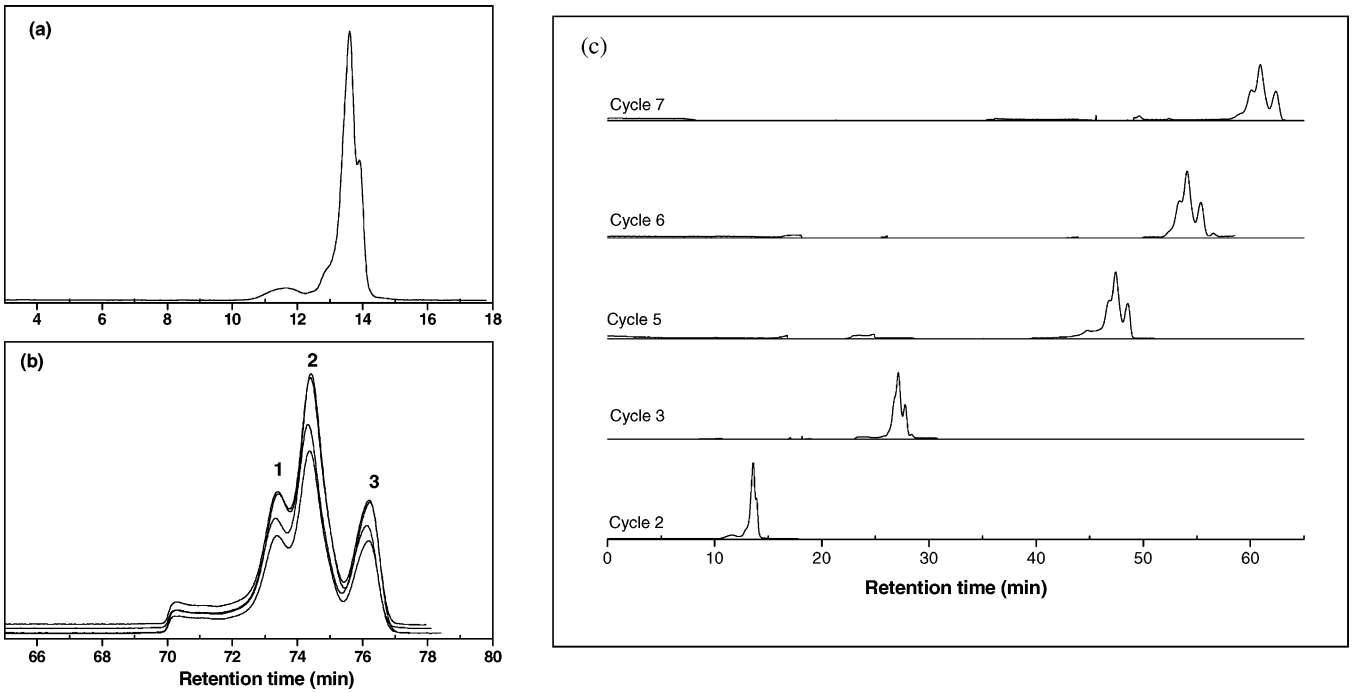

Figure 4. Alternate-recycling chromatograms for a broadly distributed 1-decanethiol-Au nanoparticle sample at a flow rate of 1.0 mL/min. (a) SEC chromatogram of cycle 2. Notice the small peak at 11.6 min which corresponds to the separation of larger gold particles. (b) Four overlaid chromatograms after cycle 8, showing the stability and reproducibility of the system. (c) The ongoing evolution of the RSEC data is shown here as a function of cycle number.

$R(n)/R(2)$, depends on the square root of the recycling passes and is given by[39]

$$\frac{R(n)}{R(2)} = \frac{\sqrt{n}}{\sqrt{(1+p^2)}} \tag{3}$$

where $R(2)$, $R(n)$ is the resolution at cycle 2 and n ($n \geq 2$), respectively, n is the number of cycles, and p is the fractional increase in peak width after each pass through the chromatographic system. Here, p is a measure of the degree of inefficiency in the recycling process and is equal to σ_v/σ, where σ_v is the bandwidth due to the switching valve and tubing while σ is the bandwidth due to the stationary phase.

In this work, the p value of the separation approached its ideal value of 0 because of several experimental factors. First, both columns were matched in dimension and pore size to keep a constant pressure after valve switching. Additionally, in alternate recycling the pump is removed from the recycling loop to minimize dead volume and thus peak broadening.[49,51] Finally, besides using a valve with a very small port volume, the detector cell was kept out of the loop to further minimize dead volume. Thus, the progress of recycling could only be assessed once the final fraction of a separation was collected.

Alternate recycling of a model gold nanocrystal sample, gold-decanethiol, is shown in Figure 4. In conventional SEC, the chromatogram of this sample presented only one asymmetric peak; its single pass elution time defined the automatic valve switching times for the subsequent recycling processes. Figure 4a shows the chromatogram after passing the sample through two columns, e.g., without switching the valve. In the next trace, Figure 4c, the sample was redirected through the columns again, and after this process several peaks become apparent in the chromatogram. Their separation improves smoothly with increased recycling. Moreover, the peak heights in this recycling scheme remain constant throughout the process indicating that there is little loss of material during the separation. After eight cycles all three features present in the initial peak are clearly resolved; the reproducibility and stability of the system is illustrated in Figure 4b, which shows four overlaid chromatograms after cycle 8.

In any recycling process, elution times are effectively magnified after each pass; this limits the range of elution times that can be processed, resulting in a lower peak capacity as compared to a single pass experiment. In this case, a less intense peak at 11.6 min in cycle 2 (Figure 4a) is lost during the recycling process due to the reduction in the peak capacity. However, this is of little consequence as the peak was well resolved even after only one pass, and thus recycling offers little additional information. We note that as compared to closed-loop recycling, alternate recycling permits a higher peak capacity and thus a larger range of separation length scales.

The improved resolution of alternate-recycling SEC can be quantified from the data shown in Figure 4. In Figure 5, both the number of theoretical plates and resolution ratio of the chromatograms are displayed versus the square root of the cycle number. As expected from eq 2, the data have a linear relationship; the slope of the resolution ratio curve is equal to the efficiency of the separation which for these data is 0.97, corresponding to a p value of approximately zero. The inclusion of a detector cell, or even a dual detector cell, to monitor the progress of recycling process would be easily accommodated given the high separation efficiency of this system.

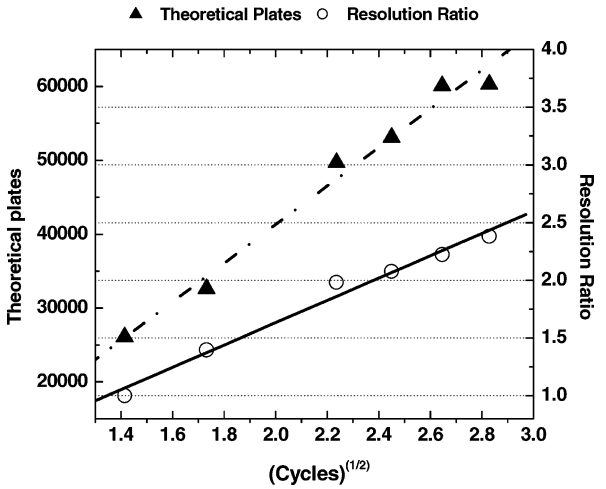

Figure 5. The efficiency and resolution ratio of alternate-recycling chromatography as a function of cycle number. In this graph efficiency and resolution, namely, the number of theoretical plates and the resolution ratio (eq 3), are shown vs the square root of the cycle number. For these data, peaks 2 and 3 in Figure 4 were used for comparison. The resolution ratio line has a slope of 0.97 ($R = 0.99$) or a p value of 0.03 indicating the near 100% efficiency of the recycling process.

In principle the resolution of alternate recycling can be increased without limit as the cycle number increases; however, there are practical reasons to limit the process to fewer than 10 operations. First, additional cycles limit the peak capacity or the range of sizes examined in a single chromatogram. It is possible to increase the peak capacity, however, by collecting only the last peak while keeping other peaks in the loop. Moreover, remixing of the peaks may occur as widely spaced peaks catch up with the area of interest.

Given the high resolution of the alternate-recycling SEC process, samples were collected from the eluting peaks so as to demonstrate the baseline separation of differently sized nanocrystal populations. For these purposes, the initial nanocrystal sample was evaluated using transmission electron microscopy to determine the sample size and distribution shape. This information is displayed in the bottom histogram in Figure 6. Between 5 and 10 images per sample were recorded at five different magnifications to cover the broadly distributed materials, and several hundred particles were counted. The quantitative analysis of particle dimensions below 5 Å (0.5 nm) was less reliable given the limit of our electron microscope. For this reason, histogram data is only displayed for particle sizes greater than 5 Å. The particle size is peaked at 1.4 and 4.2 nm in the original material, and the size distribution is not symmetric. There is a substantial tail in the distribution to smaller sizes.

Recycling SEC reveals that the shoulders in the original nanoparticle size distribution as determined by electron microscopy resolve into distinctive subpopulations of gold nanocrystals after RSEC. Three distinct and narrow features in the recycling chromatogram are observed after recycling (Figure 4b). Transmission electron microscopy of material collected from these peaks reveal that these three features correspond to differently sized nanoparticle populations, of mean diameter of 2.7, 1.5, and 0.9 nm (Figure 7, Table 1). In RSEC, the smaller-sized nanocrystals elute at longer times than the larger particles as expected for a

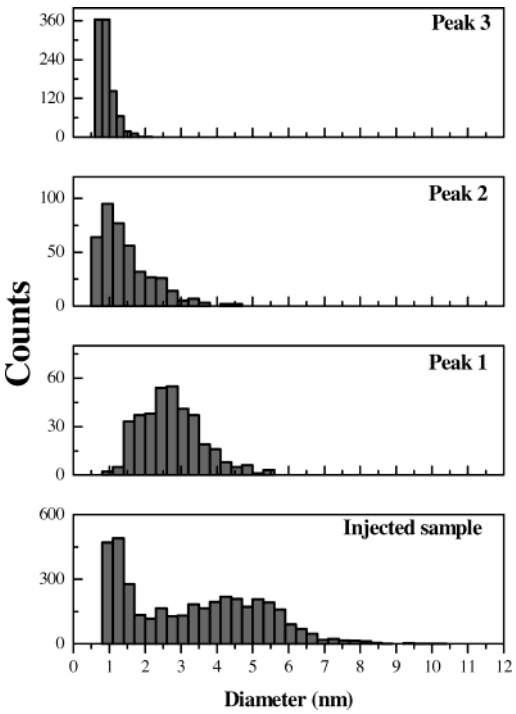

Figure 6. Transmission electron microscopy analysis of the core sizes of peaks collected from the recycling process. The original sample has a broad size distribution characterized by several populations of materials ($d = 2.6 \pm 2.0$ nm). Solution collected after completion of eight cycles show three distinctive sample size distributions, with average sizes of 2.7 ± 0.8 nm (peak 1), 1.5 ± 0.7 nm (peak 2), and 0.9 ± 0.2 nm (peak 3).

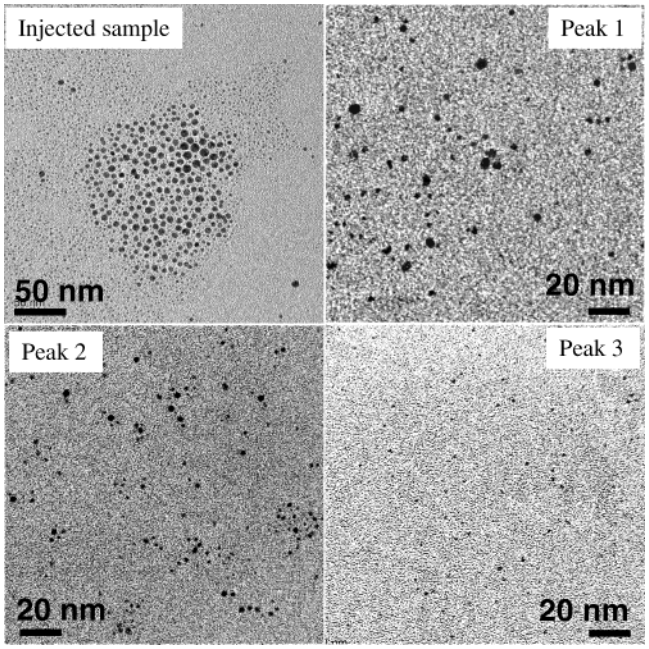

Figure 7. Representative electron microscopy images of the injected sample and collected fractions. The changes in the particle size distributions are visually apparent in the TEM data as shown here. The fractions were collected at the rise of each peak close to the apex, except peak 3 where collection was approximately the full baseline width.

size exclusion separation process. A more quantitative analysis of the RSEC elution times to determine size (see for example Figure 1b) would require that a calibration curve be developed

Table 1. TEM Size Distribution Analysis of the Collected Fractions

sample analyzed[a]	average diameter (nm)	no. of particles analyzed
peak 1	2.7 ± 0.8	360
peak 2	1.5 ± 0.7	410
peak 3	0.9 ± 0.2	967
injected sample	2.6 ± 2.0	5341

[a] Peak number refers to peaks appearing in cycle 8 (in Figure 4b).

from known standards. Commercially available polymer standards are different enough in size that the low capacity of the recycled SEC precludes their use in calibration. Still, the presence of three fractions in what was apparently a broadly distributed gold nanocrystal sample is informative; additionally, quantitative collection of these peaks is rather simple with the high-speed and precise Valco valves, and the ability to collect material for TEM analysis demonstrates that sample recovery from columns is practical.

An important issue for the use of RSEC as a preparative tool is its ability to affect a complete separation between nanoparticle populations; generally, a baseline separation is defined for chromatographic peaks with a resolution greater than 1.25.[18] Peaks 1 and 2 have a resolution of only 0.83; while not baseline separated, their collection is quite efficient and there is minimal overlap in their particle size distributions (Figure 6). Peaks 2 and 3, after eight cycles, have a measured chromatographic resolution of 1.3 and are nonoverlapping and baseline separated. While it is difficult to size the particles in peak 3 accurately because of their small diameter, an inspection of the TEM data reveals that these populations have average nanoparticle core diameter of 1.5 and 0.9 nm

The appearance of three distinct sizes of gold nanoparticles in the RSEC data is surprising but can be understood in the context of recent studies of gold nanocrystal formation. Typically, one would expect particles to be continuously distributed in size if their growth obeys the classic LaMer mechanism.[52] In RSEC, a continuous distribution would result in a wide peak, which would broaden and dissipate with recycling. Our work shows instead three distinct and narrow peaks emerging from the initial chromatogram. This result is consistent with other work on very small gold nanocrystals. Below 3 nm, an evolution occurs in gold nanoparticle growth in which very specific structures of clusters, ranging in size from less than 20 to 800 gold atoms, dominate the particle distributions.[42,53−56] The reported sizes and molecular weights for the most prevalent gold clusters are similar to our own results for this polydisperse sample.

While RSEC data cannot make an exact assignment of the cluster type, the TEM data on the collected fractions permits a direct sizing of the cores. The last peak, with a size of less than 1 nm, may correspond to one of the smallest cluster fractions, namely the 8 kDa molecular weight gold nanoparticle (~1 nm); these gold clusters are known to be very monodisperse.[42,57] Since TEM is a poor analytical tool for these very small systems, we estimated the size distribution of this fraction using a monodisperse gold sample of known size distribution (σ < 5%). For this model sample, our Agilent SEC instrument produced a single elution peak with a fwhm of 0.69 min at cycle 8. The fwhm of peak 3 in our data was 0.80 min; using the law of variance addition we estimate the size distribution for the last peak to be less than 7%.[30]

Our TEM size measurements of the larger particles, corresponding to peaks 1 and 2, find an average diameter of 1.5 and 2.7 nm, which correspond roughly to the 146 and 800 gold atoms clusters, respectively.[53,58] The broad peak at short times, or larger sizes, occurs where particle size distributions are known to adopt a more continuous form. Confirmation of these assignments will require the structural analysis of recovered fractions by mass spectrometry or other methods.[53,54,59] However, their general existence is expected. Additionally, the presence of various cluster species (different number of gold atoms) suggests that recycling SEC with high-resolution columns of smaller pore size may be used to effectively separate these very small gold nanoclusters before their use in applications.

CONCLUSIONS

For the first time we report a baseline physical separation of nanocrystals different by 6 Å in diameter using alternate-recycling SEC. The chromatographic peaks observed after recycling reflect the noncontinuous distributions of gold nanocrystals in the sample. Alternate-recycling SEC is a very powerful and cost-effective tool for separating nanocrystals according to size. The method could be scaled up for preparative applications providing that the enthalpic interactions of nanocrystals with columns can be minimized.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Center for Biological and Environmental Nanotechnology Grant EEC-0118007 for funding this research. A.M.S. thanks Saudi Aramco for a graduate fellowship.

SUPPORTING INFORMATION AVAILABLE

Alkanethiol-protected nanocrystals core dimensions and approximate number of atoms. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review April 30, 2004. Accepted July 8, 2004.

AC049355H

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