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Size-exclusion chromatography of metal nanoparticles and quantum dots

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ABSTRACT

This review presents an overview of size-exclusion chromatographic separation and characterization of noble metal nanoparticles (NPs) and quantum dots (QDs) over the past 25 years. The properties of NPs and QDs that originate from quantum and surface effects are size dependent; to investigate these properties, a separation technique such as size-exclusion chromatography (SEC) is often needed to obtain narrow-distribution NP populations that are also separated from the unreacted starting materials. Information on the size distributions and optical properties of NPs has been obtained by coupling SEC to detection methods such as ultraviolet–visible and/or fluorescence spectroscopy. Problems associated with the sorption of NPs and QDs onto various SEC stationary phases, using both aqueous and organic eluents, are also discussed.

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Contents

1. Introduction

Nanoparticles (NPs) are particles of any shape with size, in at least one dimension, ranging between 1 and 100 nm. Small NPs (≤10 nm), which are also referred to as nanoclusters or nanocrystals, are defined as clusters of atoms with atom numbers ranging anywhere from 3 to 10^7. In theory, NPs can be produced in two ways: cleaving the bulk material into nanoscopic material (although this is rarely done experimentally) or condensing atoms into clusters and NPs. Due to the nanometer-range size of NPs, they display properties representative both of atoms and of bulk solid- or liquid-state materials. The unique properties of NPs originate from quantum effects and surface effects, both of which are size dependent. The investigation of quantum effects focuses on how electronic and structural NP

Abbreviations: CTAB, cetyltrimethylammonium bromide; DLS, dynamic light scattering; DRI, differential refractive index; FFF, field-flow fractionation; FL, fluorescence; HDC, hydrodynamic chromatography; ICP-MS, inductively coupled plasma mass spectrometry; LEDs, light-emitting diodes; MALS, multi-angle static light scattering; *N*, plate number; NPs, nanoparticles; PDA, photodiode array detector; PEG, polyethylene glycol; QDs, quantum dots; QELS, quasi-elastic light scattering; R_G, radius of gyration; R_H, hydrodynamic radius; SDS, sodium dodecyl sulfate; SEC, size-exclusion chromatography; SLS, static light scattering; TEM, transmission electron microscopy; THF, tetrahydrofuran; UV–Vis, ultraviolet–visible.

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properties such as ionization potentials, binding energies, chemical reactivity, crystallographic structure, melting temperatures, or optical properties vary as a function of particle size. Surface effects are related to the fraction of atoms at the surface of NPs. A broader introduction to NP synthesis and properties can be found in various excellent books and review articles on the topic, such as the review by Roduner [1] and the book by Kreibig and Vollmer [2].

Among metal NPs, gold (Au) and silver (Ag) NPs are most widely studied due to their numerous applications as sensors and nanocarriers, and in cosmetics [3–5], among others. The applicability of these NPs stems from their visible absorption bands, straightforward method of synthesis (with high degree of size and shape control), stability, biological compatibility, and easy functionalization with various (bio)molecules. Nanocrystals composed of semiconductor materials, and which exhibit quantum mechanical properties, are also known as quantum dots (QDs); they include materials consisting of cadmium sulfide (CdS), cadmium selenide (CdSe), cadmium telluride (CdTe), and zinc sulfide (ZnS). QDs are applied in, for example, biological imaging and labeling, lasers, light-emitting diodes (LEDs), and solar cells [6–8]. Both metal NPs and QDs are usually stabilized or coated to prevent aggregation and to modify their surface properties for targeted applications. The size of Ag and Au NPs ranges from a couple of nanometers (nanoclusters) to several tens of nanometers. In general, QDs are in the range of nanoclusters smaller than 10 nm.

As indicated by the first paragraph of this Introduction, many properties of both metal NPs and QDs are size dependent. Thus, study of the size-dependent properties of NPs requires high-purity samples with narrow size dispersion. Generally, these types of samples are obtained by fractionation of the bulk sample via, most commonly, size-exclusion chromatography (SEC). This review focuses on the SEC analysis of different metal NPs and QDs by summarizing the work conducted over the past 25 years in this field. During this period, research on all aspects of NPs increased significantly. In addition to summarizing the existent literature on SEC separation and characterization of NPs and QDs, our aim is to discuss the challenges related to their SEC separation and detection. Because we intend this review for a broad audience, before discussing the NP applications of SEC, we briefly introduce this separation technique and the associated detection methods used in NP analyses.

2. SEC instrumentation for characterization of metal NPs and QDs

2.1. General principles of SEC, column types, and mobile phases

SEC is a column liquid chromatographic technique commonly used for the separation of macromolecules in solution. Typically, SEC columns are packed with small, rigid porous particles of size ranging from 3 to 20 μm and pore size from 50 to 10^7 Å. SEC separates molecules according to their size in solution or, more specifically, their hydrodynamic volume. The larger molecules in a sample elute before the smaller molecules because larger molecules either enter fewer pores or sample a smaller pore volume of the column packing material (depending on whether the column is of a mixed-bed or individual pore size), than their smaller counterparts. Unlike other chromatographic techniques, which rely mainly on enthalpic interactions between the stationary phase and analytes, SEC is primarily an entropy-controlled process; the separation is based on exclusion of the molecules from the pores of the SEC stationary phase, and ideally no interaction between the analytes and stationary phase occurs [9,10]. In practice, this entropic dominance is sometimes difficult to achieve and, as we shall see, the separation of two Au NPs with different shapes could be achieved only when two mechanisms, size-exclusion and adsorption, were combined within a single separation.

SEC column stationary phases are commonly either polymer based (e.g., styrene/divinylbenzene) or silica based. A wide selection of mobile phases (both aqueous and organic, depending on the procedure used for NP or QD synthesis) can be used with either type of stationary phase. Indeed, many different combinations of SEC columns and mobile phases have been employed for NP and QD analysis, as summarized in Table 1 (in this review, for the sake of simplicity, SEC in aqueous solution will be referred to as "aqueous SEC", whereas SEC employing organic eluents will be referred to as "organic SEC"). As can be seen in Table 1, both polymer- and silica-based columns (note that Nucleosil is a silica column, whereas Nucleogel and PL/PLgel columns are polymer based) are used in aqueous solution, whereas polymer-based columns are commonly used only with organic solvents.

The most significant challenge in the SEC analysis of metal NPs and QDs is their adsorption to the column packing material. Adsorption can cause several problems in the SEC analysis of NPs and QDs. First, if adsorption occurs, due to incomplete analyte recovery (i.e., the amount of material that elutes from the columns is less than the amount injected), the results will not be quantitative. Second, the hydrodynamic diameters obtained by the calibration of column(s) using size standards will be biased, because of the shift in retention volumes caused by analytes interacting with the column stationary phase. Researchers have attempted to overcome these adsorption limitations using columns with large pore size (small surface area) and by modifying the mobile phase with additives that reduce the enthalpic interactions between the NPs and the column stationary phase. As can be seen in Table 1, mobile-phase additives include surfactants, molecules that have been used as coating/stabilizing agents for the NPs, and modifiers containing the cations that constitute NP (*e.g.*, cadmium perchlorate for the analysis of CdS).

2.2. Detection

As can be seen in Table 1, all SEC studies on metal NPs and QDs employ ultraviolet–visible (UV–Vis) spectroscopy (either single or multiple wavelength) as a detection method. For example, Au NPs have a surface plasmon band at 520 nm, hence the commonality of this wavelength for the detection of these NPs. In addition to UV–Vis, other detectors used to detect NPs include differential refractive index (DRI), fluorescence (FL), and conductivity. Because the sensitivity of DRI is rather low compared to that of UV, the latter has mainly been used for the detection of NPs, whereas the former has been used in the detection of residual nonabsorbing chemicals from the NP synthesis. FL detection has been used in the investigation of NP and QD photoluminescence, as we shall see in the following sections. Transmission electron microscopy (TEM), which is the most common size determination technique for NPs and QDs, has been used off-line from SEC, to determine the sizes of each of the fractions eluted from the chromatographic column. In some cases, the size results from TEM were compared to the results from SEC obtained by calibrating the columns using well-characterized size standards (either NP standards or polymer-based standards). The results from these comparisons are discussed in Sections 3 and 4.

3. Aqueous SEC of Au NPs and QDs

3.1. Gold nanoparticles

The majority of research on aqueous SEC of metal NPs has been conducted using Au NPs. The first reports on the SEC characterization of colloidal Au NPs were published in 1993 and 1994 by Siebrands et al. [11] and by Fischer and Giersig [12]. For the first time, those studies demonstrated the use of SEC for the separation and size characterization of Au NPs with a size range of 2.9–20 nm. A plot of the logarithm of particle size (as determined by

Table 1
SEC methodology used for separation and characterization of metal NPs and QDs

Metal nanoparticle (stabilizer/capping agent)	SEC column(s)	Eluent	Detection	Column calibration standard for size determination	Remarks	Reference(s)
SEC in aqueous solution						
Au (citrate)	Nucleosil 500 + Nucleosil 1000 C4 (Machery-Nagel	10^{-3} mol L^{-1} sodium citrate	PDA	Synthesized Au NPs (diameter determined by TEM)		[11]
Au (citrate)	Nucleosil 500 + Nucleosil 1000 C4 (Machery-Nagel)	10^{-3} mol L^{-1} sodium citrate	PDA			[12]
Au (citrate)	TSK3000 (Toyo)	H_2O	UV–Vis (λ_0 = 525 nm) and FL (em = 440 nm, ex = 230 nm)	Commercial PEG, polysaccharides	SEC used for separation of AuNPs from impurities prior investigation of AuNP photoluminescence	[13]
Au (citrate)	Nucleogel GFC 1000-8 (Machery-Nagel)	H_2O, 0.1 mmol L^{-1} SDS, 1 mmol L^{-1} SDS, 5 mmol L^{-1} SDS, 80 mmol L^{-1} SDS	PDA	Commercial Au NPs (diameter determined by the manufacturer)	5 mmol L^{-1} SDS authors' choice from the tested mobile phases (used also when calibrating the column)	[14]
Spherical and rod-shaped Au (CTAB)	Nucleogel GFC 1000-8 (Machery-Nagel)	H_2O, 40 mmol L^{-1} SDS, 40 mmol L^{-1} SDS + 30 mmol L^{-1} Brij-35	PDA		40 mmol L^{-1} SDS + 30 mmol L^{-1} Brij-35 gave best resolution when separating spherical and rod-shaped AuNPs	[15]
Au (citrate)	Nucleosil, 100-nm pore size and 7-μm particle size (Machery-Nagel)	5 mmol L^{-1} sodium citrate, 20 mmol L^{-1} sodium citrate, NaCl (concentrations used not reported), 15 mmol L^{-1} SDS, 50 mmol L^{-1} SDS	UV–Vis (λ_0 = 520 nm)		The adsorption of AuNPs on C_{18}-capped silica gel was found in separate TEM studies	[16]
Au (citrate)	Nucleogel GFC 60-8 (Machery-Nagel)	10 mmol L^{-1} SDS	UV–Vis (λ_0 = 520 nm)	Commercial AuNPs (diameter determined by the manufacturer)		[17–21]
Phenylethynyl-bridged AuNP dimers and trimers (citrate)	Silica microsphere GPC columns, 500 and 350 Å (Alltech, Inc.)	40 mmol L^{-1} SDS	UV–Vis (λ_0 = 525 nm)			[22]
Au/Pd core/shell (SDS)	Nucleogel GFC 60-8 (Machery-Nagel)	10 mmol L^{-1} SDS	UV–Vis (λ_0 = 520 nm)	Commercial AuNPs (diameter determined by the manufacturer)		[19,23]
Au/Pt core/shell (SDS)	Nucleogel GFC 60-8 (Machery-Nagel)	10 mmol L^{-1} SDS	UV–Vis (λ_0 = 520 nm)	Commercial AuNPs (diameter determined by the manufacturer)		[24]
CdS (polyphosphate)	Nucleosil 500 C4 + Nucleosil 1000 C4 (Machery-Nagel)	10^{-3} mol L^{-1} $Cd(ClO_4)_2$ + 10^{-3} mol L^{-1} hexametaphosphate	PDA	Narrow-dispersity CdS (diameter determined by TEM)		[12,25–29]
ZnS (polyphosphate)	Nucleosil 500 C4 + Nucleosil 1000 C4 (Machery-Nagel)	10^{-3} mol L^{-1} $Zn(ClO_4)_2$ + 10^{-3} mol L^{-1} hexametaphosphate	PDA	Narrow-dispersity ZnS (diameter determined by TEM)		[27]
CdSe (amphiphilic polymer)	Superdex 200 (GE Healthcare Life Sciences)	0.1 mol L^{-1} NH_4HCO_3, pH 7.4	UV–Vis, FL, ICP–MS		Column recoveries reported	[30]
CdSe/ZnS core/shell (amphiphilic polymer)	Superdex 200 (GE Healthcare Life Sciences)	0.1 mol L^{-1} NH_4HCO_3, pH 7.4	UV–Vis, FL, ICP–MS		Column recoveries reported	[30]
SEC in organic solvent						
Au (octane/ tridodecylmethylammonium chloride/hexanol)	PL-500 (Agilent/Polymer Laboratories)	THF	PDA (λ_0 = 520 nm) and FL (em = 520 nm, ex = 300 nm)			[13]

(*continued on next page*)

Table 1 (*continued*)

Metal nanoparticle (stabilizer/capping agent)	SEC column(s)	Eluent	Detection	Column calibration standard for size determination	Remarks	Reference(s)
Au (various alkylthiols)	PL-1000 or PL-500 (Agilent/ Polymer Laboratories)	0.01 mol L^{-1} dodecanethiol in toluene	PDA (λ_0 = 520 nm), DRI, conductivity	Linear alkanes and polystyrene standards	DRI and conductivity detection used to demonstrate that AuNPs had no charge and that AuNPs were separated from the nonabsorbing chemicals	[31–34]
Au (octadecanethiol/ tetraoctanethiol/ decanethiol)	PLgel 1110 (Agilent/Polymer Laboratories)	Toluene	UV–Vis (λ_0 = 520 nm), DRI	Polymer standards (not specified)	Recycling SEC used in addition to conventional SEC	[35]
Au (polystyrene coated)	PLgel 1110 (Agilent/Polymer Laboratories)	Toluene	PDA (λ_0 = 505 nm), DRI	Polystyrene standards		[36]
Ag (tetraoctylammonium bromide/ tetraoctylammonium chloride/trioctylphosphine	PL-1000 (Agilent/Polymer Laboratories)	0.01 mol L^{-1} dodecanethiol in toluene	PDA (λ_0 = 400 nm), DRI, conductivity	Linear alkanes and polystyrene standards	DRI and conductivity detection used to demonstrate that AgNPs had no charge and that AgNPs were separated from the nonabsorbing chemicals	[32]
CdS (dodecanethiol)	Nucleosil 500 + Nucleosil 1000 (Machery-Nagel)	1 mmol L^{-1} $Cd(ClO_4)_2$ + 1 mmol L^{-1} dodecanethiol in THF	PDA			[27]
CdSe (alkylphosphines)	PL-50 or PL-1000 (Agilent/ Polymer Laboratories)	THF	PDA, DRI, FL		DRI was used to detect nonabsorbing chemicals	[37]
CdSe (alkylthiols)	PLgel 1110 (Agilent/Polymer Laboratories)	0.1 mol L^{-1} trioctylphosphine in toluene	UV–Vis	Polystyrene standards	Recycling SEC used in addition to conventional SEC	[38]
CdSe with a ligand of poly(dimethylaminoethyl methacrylate) labeled with pyrene	AM Gel Linear/5 (American Polymer Standards Corporation)	*N*-methyl-2-pyrrolidinone	UV–Vis (λ_0 = 343 nm), DRI			[39]
Polystyrene coated CdSe	PLgel 1110 (Agilent/Polymer Laboratories)	0.1 mol L^{-1} trioctylphosphine in toluene	PDA (λ_0 = 517 nm), DRI	Polystyrene standards		[36]
CdSe/ZnS core/shell (alkylphosphines)	PL-50 or PL-1000 (Agilent/ Polymer Laboratories)	THF	PDA, DRI, FL		DRI was used to detect nonabsorbing chemicals	[37]
CeO_2 (unmodified and surface modified)	poly(methyl methacrylate brush immobilized silica monolith columns/Shodex KF-803L and Shodex KF-805L	THF	DRI		poly(methyl methacrylate) brush stationary phase was synthesized by authors	[40]
Pd (decanethiol/dodecanethiol/ hexadecanethiol)	JAIGEL-W253 (Japan Analytical Industry Co., Ltd)	Toluene	UV–Vis (λ_0 = 290 nm)	Polystyrene standards	Recycling preparative SEC	[41]
Qdot® 545 and 705	poly(methyl methacrylate brush immobilized silica monolith columns/Shodex KF-803L and Shodex KF-805L	THF	DRI		poly(methyl methacrylate_ brush stationary phase was synthesized by authors Qdot 545 is CdSe/ZnS (core/shell) and Qdot 705 is CdSeTe/ZnS (core/ shell)	[40]

TEM) as a function of SEC elution time was linear, supporting the notion that the NP separation was based on a size-exclusion mechanism. The SEC columns used in those studies had a large particle size, ranging from 15 to 25 µm (Nucleosil 500 and Nucleosil 1000 C4, respectively), because Au NPs were found to be adsorbed irreversibly onto silica columns of particle size lower than 15 µm. The first studies on aqueous SEC of Au NPs had already indicated the adsorption problem that continues to limit the widespread use of SEC for accurate characterization of NPs in aqueous solution.

Aqueous SEC was also used by Wilcoxon et al. [13], who used SEC coupled with UV–Vis and FL detection to purify small gold nanoclusters and investigate their photoluminescence. Novak et al. [22] used SEC/UV ($\lambda_0 = 525$ nm) for separating phenylethynyl-bridged Au NP dimers and trimers from excess monomer particles. The sizes (or more precisely, the aggregate type and proportion in each separated fraction) of the SEC-separated Au NPs were determined off-line, using TEM. Before attempting the separation of these NPs, the SEC system was tested by injecting a mixture of pure 10-nm, 20-nm, and 30-nm Au NPs and a mixture of 10- and 30-nm Au NPs onto the columns. The 30-nm particles were meant to mimic the trimers of 10-nm-sized monomers. The resolution between the 10- and 30-nm particles was 0.8. This resolution was higher than that obtained when injecting the phenylethynyl-bridged Au NPs, where a resolution of 0.5 was measured between monomers and trimers. This difference in resolution is not surprising, given that the monomeric components in the trimers are arranged in a triangular fashion, rather than a straight line, where the former geometry occupies a smaller hydrodynamic volume than does the latter. In the separations, two silica-based SEC columns (500 and 350 Å) were used in series with 40 mmol L^{-1} of sodium dodecyl sulfate (SDS) in water as the mobile phase.

The majority of papers on aqueous SEC of Au NPs were written by Wei and Liu, and Liu [14–21,23,24,42]. These authors' early work focused on finding the analytical conditions for the separation of Au NPs and gold nanorods [14–16]. In said studies, different mobile-phase additives (in water) were evaluated to find an eluent that would prevent the irreversible adsorption of Au NPs (citrate stabilized and hexadecyltrimethylammonium bromide (CTAB) stabilized) to the SEC column packing material. The mobile-phase additives tested included SDS, an anionic surfactant, at varying concentrations; a mixture of SDS and Brij-35, a neutral surfactant; sodium chloride; and sodium citrate. For the separation, both a polymer-based (styrene/divinylbenzene) and a silica-based column were evaluated. The authors found that, when either NaCl or sodium citrate were used as mobile-phase additives, the Au NPs both aggregated with themselves and adsorbed onto either column packing material. The addition of surfactants appeared to enable the separation of Au NPs on both types of column. According to the authors, SDS acted to prevent the adsorption of the analytes onto the columns by both increasing the negative charge of the packing material and changing the charge of the NPs from positive to negative. Adsorption is thus prevented by charge–charge repulsion between the analyte and stationary phase, that is, through ion-exclusion effects. Conversely, Brij-35 was added to increase separation resolution between the spherical and rod-shaped analytes. It was postulated that the neutral Brij-35 replaced some of the charged SDS on the NP surface, decreasing the negative charge on the NPs and, thus, leading to a controlled increased adsorption of NPs onto the stationary phase in a size- and/or shape-dependent manner. The separation process was thus considered to be governed both by size-exclusion and by adsorption mechanisms (Fig. 1). In all of these studies, elution of Au NPs was monitored with UV–Vis detection; it should be noted that observations regarding Au NP elution using various SEC analysis conditions were based on the differences in UV peak areas. Thus, the results obtained from all studies were only qualitative; the absolute Au NP concentrations eluted from the columns were not determined. The sizes of Au NPs used in these studies were determined using TEM.

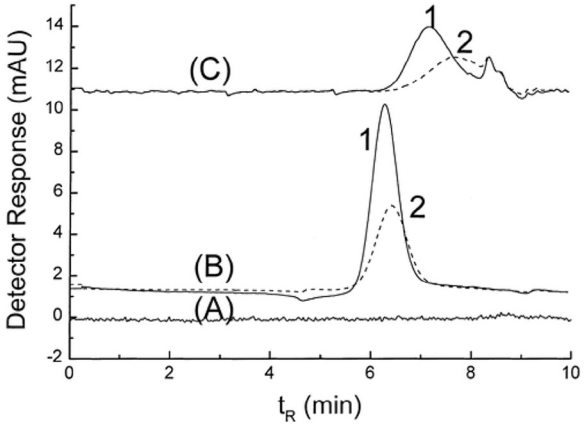

Fig. 1. Effect of mobile-phase additives on the SEC separation of AuNPs with different shapes (nanorods, detected at $\lambda_0 = 920$ nm, solid line (1) in the chromatogram, and spherical, detected at $\lambda_0 = 520$ nm, dashed line (2) in the chromatogram). (A) H_2O, (B) 40 mmol L^{-1} SDS, and (C) 40 mmol L^{-1} SDS and 30 mmol L^{-1} Brij-35. Reprinted with permission from Ref. [15].

Later studies from the same group report the use of aqueous SEC for the separation and size characterization of Au NPs synthesized using various procedures [17,18,24]. SEC was also used in stability studies of Au NP solutions [19–21]. A polymer-based (styrene/divinylbenzene) column and 10 mmol L^{-1} SDS mobile phase were used in all of these studies. Size information on the studied Au NPs was obtained by calibrating the column using commercial Au NP standards with known diameters ranging from 12 to 79 nm. The elution of Au NPs was monitored using UV–Vis detection at $\lambda_0 = 520$ nm, which, as mentioned earlier, is the surface plasmon band of spherical Au NPs.

3.2. Quantum dots

Although aqueous SEC, in the context of metal NP characterization, is mainly used for Au NPs, it is also used for the separation of polyphosphate-stabilized semiconductor particles of CdS [12,25–29] and ZnS [27]. The relatively short SEC analysis time enables the characterization of rapidly growing colloidal species in solution and allows studies on the stability and growth mechanism of semiconductor particles. For SEC analysis of these particles, the eluent contained both polyphosphate (also used as stabilizer of semiconductors in solution) and either cadmium perchlorate (for CdS) or zinc perchlorate (for ZnS). Narrow-dispersity CdS and ZnS particles, the diameters of which were determined using TEM, were used to calibrate the SEC columns with respect to the particle diameter.

Although SEC was successfully employed for the separation and size characterization of semiconductor particles in aqueous solution, concentration-dependent adsorption of NPs onto the SEC columns was observed, as indicated by a comparison of the UV peak areas obtained using different CdS concentrations [28]. It was found that the adsorption problem was more severe when the NP concentration exceeded a certain molarity (>5 mmol L^{-1} for CdS). In addition to adsorption, at CdS concentrations >5 mmol L^{-1}, the retention times increased due to a decrease in the thickness of the electrical double layer surrounding the NPs. It should be noted that the adsorption phenomenon was found to be both irreversible and reversible, with the latter being recognized by an increase in baseline drift as a function of elution time, attributed to the release of semiconductor particles adsorbed onto the stationary phase. This

adsorption activity was shown to be lower when the stationary phase was already coated by CdS (i.e., after several injections of a QD sample onto a new column) than for columns with an uncoated stationary phase.

In a later study, Trapiella-Alfonso et al. characterized CdSe and CdSe/ZnS QDs using a Superdex 200 SEC column [30]. The detection techniques included UV–Vis, FL, and inductively coupled plasma mass spectrometry (ICP-MS). Element-specific ICP-MS detection allowed the determination of column recoveries (see Section 5 for further discussion of this last point).

4. Organic SEC characterization of NPs

4.1. Gold and silver nanoparticles

In the first report on the use of SEC in organic solution for Au NP analysis, the photoluminescence of small Au nanoclusters synthesized in inverse micelles was studied by SEC coupled to photodiode array (PDA) and FL detectors using tetrahydrofuran (THF) as the eluent [13]. Later studies of Wilcoxon et al. [31–34] include the purification and investigation of the size distribution, optical properties, and stability of Au and Ag nanoclusters with SEC in toluene containing 0.01 mol L^{-1} dodecanethiol. In these studies, the columns were calibrated for hydrodynamic radius (R_H) using polystyrene standards and linear alkanes. As stated by the authors [31], SEC is more advantageous than TEM as a size determination method for metal nanoclusters in that the total cluster size is obtained (core + shell) with the former, and only the inorganic core is detected by the latter. By using both techniques, SEC and TEM, the size of the shell can be estimated by subtracting the core size obtained from TEM from the total cluster size obtained from SEC. An overlay of the SEC chromatograms of Au nanoclusters with different alkanethiol capping agents (C_6SH, C_{10}SH, and C_{14}SH) is shown in Fig. 2. The size of the Au core was 2.0 nm, as determined by high-resolution TEM. The sizes obtained for alkanethiol shells varied slightly depending on the SEC column used (PL 1000 or PL 500). In general, the sizes determined with SEC were consistent with the assumption that every four carbon atoms added contribute an ≈8 Å increase in size to an alkanethiol.

Because the low resolution of conventional SEC can limit its applicability in nanomaterial separations, where the size difference between closely eluting species is very small, Al-Somali et al. [35] used alternate-recycling SEC (also known as recycle SEC; see Section 15.3 of Ref. [9]), which improved the resolution in the separation

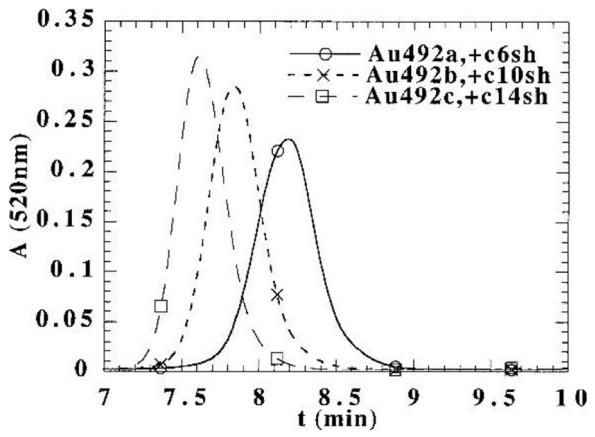

Fig. 2. Effect of alkylthiol capping agents (C_6SH, C_{10}SH, and C_{14}SH) on the SEC retention time of Au nanoclusters. The absorbance at 520 nm from the PDA versus elution time is shown. The size of the clusters was obtained by calibrating the column with polystyrene standards. Reprinted with permission from Ref. [31].

of Au NPs capped with alkanethiols as compared to conventional SEC. In alternate-recycling SEC, the eluate from the first SEC column is directed to a second column (identical to the first one) via a low-volume, high-speed valve. The eluate from the second column is then transported back to the first column. This recycling process increases the effective column length and, thus, increases the resolution of species with only a small size difference between them. Fig. 3 demonstrates the increase in resolution as a function of increasing cycle number obtained in alternate-recycling SEC. The resolution of individual species in a broad-dispersity Au NP sample clearly increases as a function of cycle number. This is observed on comparing Fig. 3a, in which the SEC chromatogram of a Au NP sample is shown after the second cycle, to Fig. 3b, in which an SEC chromatogram of the same sample is shown after cycle number 8. In addition, in Fig. 3b, the chromatograms from four injections overlie neatly upon one another, indicating that the recycle SEC system is both stable and reproducible for this Au NP sample at the analysis conditions used. Fig. 3c demonstrates the improvement in resolution with increasing cycle number.

To date, the only report on the SEC characterization of Ag nanoclusters appears to be that by Wilcoxon et al. [32] Ag nanoclusters were prepared by an inverse micelle technique in organic solvents and separated with SEC using 0.01 mol L^{-1} dodecanethiol in toluene as the eluent. The aim of the study was to compare differences in the optics of Au and Ag nanoclusters arising from the different energies of the inter-band transition onsets of these two metals. The spectra of separated nanoclusters were recorded with a PDA detector, and the sizes of the Ag nanoclusters were obtained using the same calibration curve, constructed with linear alkanes and polystyrene standards, as described in the previous section for the size determination of Au nanoclusters. The results showed that both broadening and red shift of the absorbance spectrum were observed for Ag nanoclusters with decreasing cluster size, whereas broadening was accompanied by blue shifting as a function of decreasing cluster size for Au nanoclusters.

4.2. Quantum dots

The first studies on the SEC of semiconductor particles in organic solvents date back to the early 1990s, when the first report on the separation of CdS nanocrystals was published by Fischer et al. [27] They demonstrated the feasibility of SEC of CdS QDs stabilized with alkanethiols, in THF containing 1 mmol L^{-1} $Cd(ClO_4)_2$ + 1 mmol L^{-1} dodecanethiol. Later studies by Wilcoxon and Provencio [37], Wang et al. [39], and Kruger et al. [36,38] showed that SEC reveals various characteristics and size-dependent properties of CdSe and CdSe/ZnS core/shell nanocrystals, as described later.

Wilcoxon and Provencio [37] used SEC in THF with three on-line detectors, FL, diode array detection, and differential refractometry, to separate both CdSe and CdSe/ZnS QDs and to investigate their size-dependent optical and chemical properties. SEC column calibration was not used for the determination of hydrodynamic size, unlike in earlier studies on Au NPs by this group, due to specific chemical interactions between the QDs and the column material (styrene/divinylbenzene), interactions that violated the ideal SEC separation mechanism. Rather, off-line dynamic light scattering (DLS) was used for the size determination of CdSe and CdSe/ZnS QDs. Although size could not be determined directly by SEC, chromatographic analysis allowed isolation of the discrete size populations present, with the various on-line detectors providing insight into the optical properties of the individual fractions.

Krueger et al. [36,38] were able to apply SEC with toluene containing 0.1 mol L^{-1} trioctylphosphine for the hydrodynamic size determination of polystyrene- and alkylthiol-coated CdSe nanocrystals. Polystyrene standards were used for size calibration of the SEC column. Sizes were also determined with TEM, the results from

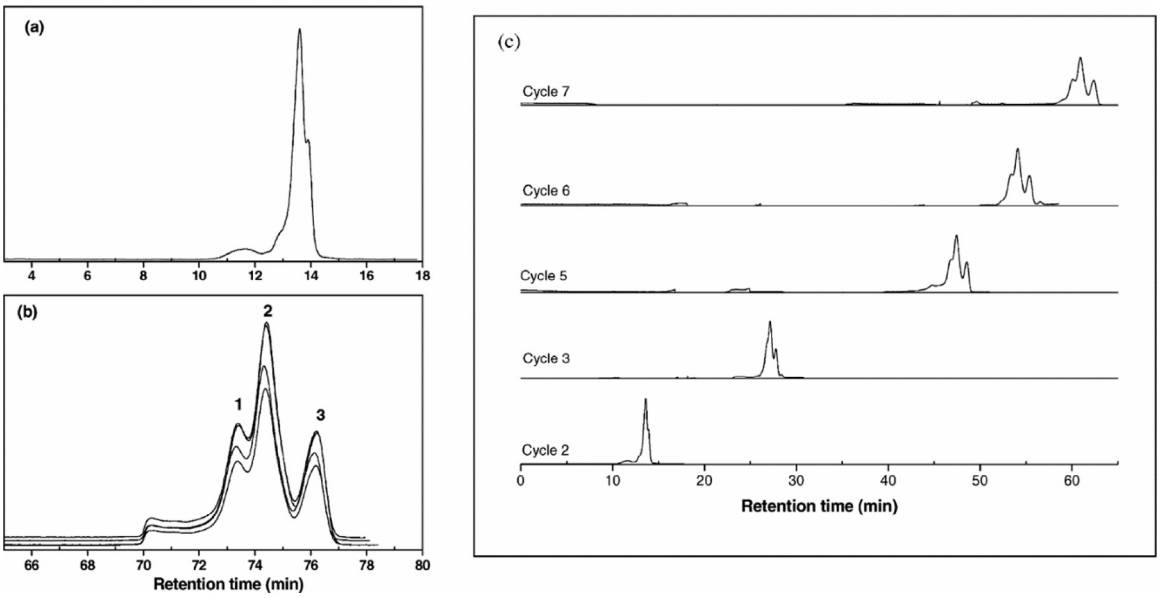

Fig. 3. Alternate-recycling SEC chromatograms of a broadly dispersed Au NP sample. (a) A chromatogram of cycle 2, (b) four overlain chromatograms of cycle 8, and (c) the evolution of the data as a function of cycle number. Reprinted with permission from Ref. [35].

both methods being consistent for nanocrystals with sizes ranging from ≈2.5 to ≈4.0 nm. The successful SEC separation of alkylthiol-coated CdSe nanocrystals, however, required complete capping for both Cd and Se atoms to eliminate particle–column interactions. An additional aim of this study was to investigate the change in the length of polystyrene chains when attached to CdSe versus unbound [36]. When linked to a metal core at full coverage (full coverage was defined by the maximum NP size measured by SEC), polystyrene was found to assume a brush conformation and is 44% longer than that in the unbound polymer in solution. Fig. 4a illustrates the total hydrodynamic diameter (HD), which is defined as the sum of the core diameter (D_c) and two shell thicknesses (T_{shell}). As mentioned earlier, the core diameter can be determined by TEM, whereas SEC can separate QDs based on the length of the capping agent (Fig. 4b). As seen in Fig. 4c, the hydrodynamic sizes obtained by SEC are consistent with the values calculated by adding the core diameter to the shell thickness values obtained from the literature.

Wang et al. [39] used SEC for the separation of CdSe nanocrystals from the excess polymer (unbound poly(dimethylaminoethyl methacrylate) labeled with pyrene) used as coating material for nanocrystals. They were also able to quantify the amount of polymer bound to the nanocrystals. The polymer was analyzed by SEC at different concentrations, and the peak areas of each run were recorded using a UV detector. To construct a calibration curve for the determination of polymer amounts, UV peak areas were plotted against concentration. This curve was used to determine the amount of free polymer (which was completely separated from the nanocrystal-bound polymer) in a nanocrystal solution. The amount of bound polymer (both concentration and average number of polymer chains bound to each CdSe particle) could also be determined.

In addition to cadmium-based semiconductors, organic SEC has also been used to characterize Pd nanocrystals [41], exploiting the structure and stability of nanocrystals coated with alkanethiols of varying chain lengths. The hydrodynamic sizes of the nanocrystals were determined by calibrating the SEC column using polystyrene standards. The core diameters were determined by TEM.

As reported by many authors who used SEC to separate NPs, analyte adsorption onto the SEC column packing material (induced by the high surface energy density of NPs) has contributed the most to limiting the use of SEC in NP characterization. To overcome this

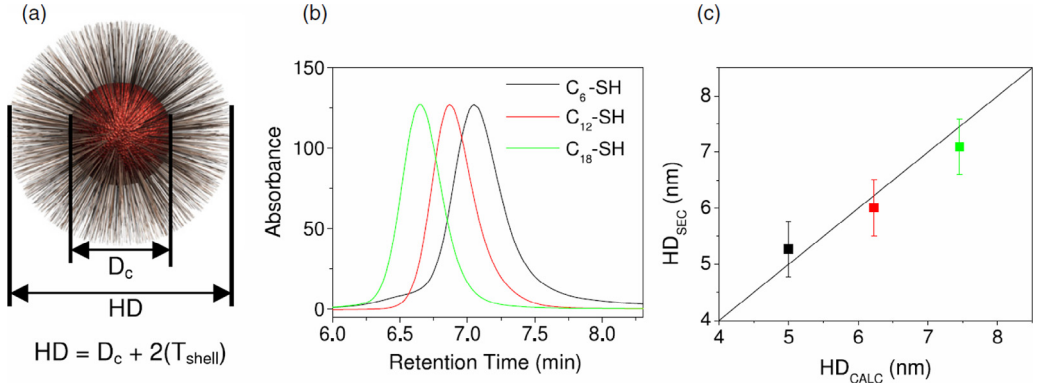

Fig. 4. Geometric model of total hydrodynamic diameter (HD) of NP. (a) The HD is calculated from core diameter (D_c) and surface-coating thickness (T_{shell}). (b) SEC detects the retention time difference resultant from differences in capping agent length between CdSe nanoparticles coated with 1-hexane (C_6), 1-dodecane (C_{12}), and 1-octadecane (C_{18}) thiol (SH). (c) The hydrodynamic diameter of the coated NPs determined from SEC (HD_{SEC}) is compared to expected values for 3.6-nm CdSe core plus literature values for the shell thickness (HD_{CALC}). The line slope is set to 1. Reprinted with permission from Ref. [36].

problem, Arita et al. [40] synthesized a poly(methyl methacrylate) brush stationary phase immobilized onto silica, which showed good column recovery (measured by comparing the DRI peak areas with and without the column) for CeO_2 NP and Qdot samples analyzed in THF. The separation performance was monitored by analyzing the eluted SEC fractions with TEM. The size-based separation was successful for both the CeO_2 NPs and the QDs. The experiments were successful in demonstrating the absence of strong interactions between the column packing material and the NPs and QDs.

5. Challenges and future prospects for SEC characterization of NPs and QDs. Comparison with other separation methods

As stated in the previous sections referring to the literature, the most significant challenge in the SEC separation and characterization of NPs and QDs is their adsorption onto SEC stationary phases. A good laboratory practice in SEC is to measure (and report) column recovery, to ensure that results are quantitative. However, column recoveries were reported in only one of the papers discussed in this review [30]. This practice was omitted probably because quantitatively measuring NP and QD recovery from the column is not a straightforward task, due to difficulties in accurately determining NP concentration in the eluate. It is well known that UV–Vis spectroscopy can be used to determine concentration. If concentration is determined by the measured absorbance, the molar absorptivity at the wavelength used must be determined. However, the molar absorptivity for metal NPs and QDs increases with increasing NP size [43] and, thus, a single value for molar absorptivity cannot be used to calculate the population concentration in the UV–Vis peak of a disperse sample. Comparison of UV peaks can still be useful, however, by providing qualitative information on the recovery (e.g., by comparing peak areas obtained using different concentrations of mobile-phase modifier). Although most spectroscopically based detection methods (such as UV–Vis) fail to determine the absolute concentration eluting from the column and, thus, to quantify column recovery, ICP-MS can be used for concentration determination by element-specific detection. Currently, the coupling of SEC to ICP-MS is rarely used to characterize metal NPs and QDs [30,44]. However, given the relative success with which other separation methods such as field-flow fractionation (FFF) and hydrodynamic chromatography (HDC) [45–50] have been coupled to ICP-MS for the characterization of metal NPs, hyphenated SEC/ICP-MS experiments appear to have great potential in this respect [51–58].

SEC is generally, and most often, used to characterize macromolecules, both synthetic and naturally occurring. Determination of molar mass (the molar mass distribution of a sample as well as its molar mass averages) is perhaps the most common application of SEC in polymer analysis. Molar mass can be determined by calibrating the columns using molar mass standards (ideally, with identical chemical composition and architecture to those of the polymer studied) or by using a static light scattering (SLS) detector together with concentration-sensitive detection (usually UV or DRI). Multi-angle static light scattering (MALS) detection can also be used for the determination of size in the form of radius of gyration (R_G), a parameter that is usually more significant in NP characterization than molar mass is. The radius of gyration can be obtained from the angular dissymmetry of the MALS data. There are, however, limitations to the use of SLS (including MALS) in NP characterization. In the case of MALS, size cannot be determined for small particles ($\lesssim$10 nm) such as QDs, because light scatters from small particles approximately equally at all angles; thus, the angular dissymmetry needed for R_G determination is lacking (i.e., QDs are near-isotropic scatterers). In addition, metal NPs and QDs are strongly light absorbing and, consequently, weakly light scattering. This can affect the signal-to-noise ratio in light scattering when the total mass of injected NPs is low. Quasi-elastic light scattering (QELS), which is also known as DLS, can be used as an alternative to MALS in the determination of molecular size, in the form of R_H. The advantage of QELS over MALS is that QELS is capable of measuring sizes that are below 10 nm. QELS (as well as SLS) can be used in both off-line batch mode and online, coupled to separation techniques. To date, neither SLS nor QELS have been coupled to SEC to characterize metal NPs and QDs. As was the case for ICP-MS, however, both these detection techniques have been used together with FFF for the size determination of separated NP species [52,56]; thus, coupling to SEC appears promising.

As regards other separation methods, the analyte resolution in SEC is generally superior to that in flow FFF, which, in turn, has generally better resolution than HDC. The main reason for this relative ranking is the plate number N achievable by each separation method. Although plate numbers in SEC experiments are much smaller than those in techniques such as reversed-phase liquid chromatography, they are substantially larger than those in flow FFF methods. (Within the family of flow FFF techniques, hollow-fiber flow FFF [47] achieves higher plate numbers than does asymmetric flow FFF [45,51–57], although, in both cases, N are much lower than in SEC.) The ability to use cross-flow gradients in flow FFF does imply that N in these methods is generally larger than that in packed-column HDC, where the main parameter governing plate number is the size of the column packing particles. An in-depth discussion of resolution in SEC can be found in Refs. [9] and [10], whereas the resolution in asymmetric flow FFF is discussed in Ref. [45] and resolution in HDC in Ref. [48]. SEC has also been found to be superior to both HDC and flow FFF in terms of analyte recovery, due to column sorption issues in the case of HDC, and due to an open channel (or open fiber) design that generally precludes 100% analyte recovery in FFF. An additional advantage of SEC and HDC over FFF is the need for specialized instrumentation and a large capital outlay to perform the latter, whereas the former use instrumentation common to most liquid chromatography laboratories.

In an almost unique comparison among techniques, in 1981, Yau and Kirkland contrasted SEC and HDC to time-dependent exponential force-field sedimentation FFF for particle and ultrahigh molar mass macromolecular analysis [59]. The authors demonstrated that this particular FFF method had a 5–10-fold better resolution than did SEC and a 10–50-fold better resolution than did packed-column HDC. For a comparison of other separation techniques such as density gradient centrifugation and analytical centrifugation, at least in theory, to chromatographic and FFF methods, the reader is referred to chapters 6 and 8 of Ref. [60]. More recent discussions of analytical ultracentrifugation of NPs are also presented in Refs. [61] and [62]. A novel NP characterization method was recently introduced, namely electrical asymmetrical flow FFF. The underlying principles and instrumentation, along with application examples, can be found in Ref. [63] and in the supporting information accompanying that paper.

6. Conclusions

Over the past 25 years, SEC has been used to fractionate a variety of Au NPs, Ag NPs, and QDs. Because many NP and QD properties are size dependent, a separation technique such as SEC is often needed to obtain NP populations with a narrow size distribution. SEC experiments provide valuable information on the properties of NPs and QDs, including size, optical properties, and stability. Further, it can also be used to purify NP solutions. The most significant challenge in the SEC analyses of NPs and QDs, in both aqueous and organic solvents, is analyte adsorption onto the column stationary phase. Although some stationary-phase/mobile-phase combinations have been found to work better (less adsorption) than others, the adsorption problem remains for most metal NPs and QDs. Size

information from SEC analyses is commonly obtained by calibrating the columns with well-characterized standards. However, these results are not quantitative if adsorption occurs. Results can also be biased if reversible adsorption occurs, and/or other non-size-exclusion effects are present during the separation. At present, different detectors, such as SLS, QELS, and ICP-MS, in conjunction with SEC can be considered to generate size information without column calibration, information on elemental profiles, and quantitation of column recoveries. Because these techniques have been successfully coupled, for the purposes of NP and QD characterization, to FFF and HDC, they also show great promise as SEC detectors for the types of analyses discussed in this review.

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