Influence of pH on Dendrimer-Protected Nanoparticles

Jie Zheng,[†] Melinda S. Stevenson,[†] Robert S. Hikida,[‡] and P. Gregory Van Patten*,[†]

Department of Chemistry and Biochemistry, Ohio University, Clippinger Laboratories, Athens, Ohio 45701-2979 and Ohio University College of Osteopathic Medicine, Irvine Hall, Athens, Ohio 45701-2979

Highly stable, isolated, and monodisperse Ag nanoparticles, protected by poly(amidoamine) (PAMAM) dendrimers, were prepared via direct chemical reduction. UV−visible absorption, dynamic light scattering, and transmission electron microscopy have been used to study the structure and behavior of the nanocomposites as a function of pH. The data show that pH is a critical determinant of aggregation in PAMAM dendrimers, and that dendrimer aggregation, in turn, strongly influences nanoparticle stability against flocculation. The results allow for a complete understanding of previously reported difficulties in preparing Ag nanoparticles by this method.

Introduction

Dendrimers are molecules with a well-defined primary structure based on a hyperbranched, fractal-like motif emanating from a central core. These molecules have been the subjects of intense study recently due to interesting and potentially useful properties that derive from their unique structural characteristics. Dendrimers have been identified as promising candidate materials for a wide variety of applications that include light-harvesting,[1−3] drug delivery,[4,5] and use as nanoreactors for the preparation of inorganic nanoparticles.[6−13] These molecules are closely related to conventional polymers and can be used as well-defined models or replacements for these molecules in many cases, as well. In this respect, polyelectrolyte dendrimers are particularly interesting because of their similarities to and interactions with biological macromolecules such as proteins and nucleic acids.[14]

A recent focal point in dendrimer research has involved the control of dendrimer structure and function via local environment.[15−17] Recently, some questions regarding the nature of these changes has arisen. Specifically, Nisato et al.[16] reported their surprising find that the size of generation 8 PAMAM (poly-(amidoamine)) dendrimers is relatively insensitive to pH. This find contradicts predictions by Welch and Muthukumar[15] and seems counter to the findings of Chen et al.[17] and Sideratou et al.[5] Although Sideratou et al. did not find direct evidence for conformational changes, they did observe functional changes in polyelectrolyte dendrimers as a function of pH. The ability to control physicochemical properties of dendrimers via enviromental variables, such as pH, is viewed as critical to drug delivery applications, and may also have implications for other applications that rely on these macromolecules.

PAMAM dendrimers have been used extensively as templates for the preparation of inorganic nanoparticles. Nanoparticles of several different metals (Cu, Pd, Pt, Au, Ag) and ceramics (ZnS, CdS, Fe_2O_3, and others) have been successfully encapsulated inside these dendrimers.[6−13] The preparation of PAMAM-protected[18] Cu, Pd, and Pt nanoparticles is extremely simple and was first reported by Zhao et al.[6] Their method depends on complexation of the metal cations with amine groups in the dendrimer followed by reduction with a chemical reductant such as borohydride. Despite the apparent simplicity and generality of this method, the preparation of stable, dendrimer-protected Ag nanoparticles has been reported to be more difficult. The product obtained with Ag generally exhibits anomalous UV−visible absorption spectra and/or precipitates from solution after a short time. Crooks and co-workers were initially unable to prepare stable Ag nanoparticles directly by this method, but developed an elegant solution to this problem involving metal exchange with Cu.[19] Other researchers have reported routes to stable PAMAM-protected Ag nanoparticles, but absorption spectra presented in these reports[10,20] show anomalous, red-shifted peaks uncharacteristic of a well-dispersed, nanometer-size, silver colloid.

In light of the research described above and some of our own observations, we have undertaken an investigation of the effects of pH on the stability of PAMAM-encapsulated nanoparticles. Our findings indicate that the irregular behavior of Ag nanoparticles that has been observed in previous preparations is due in large part to pH-dependent aggregation of the PAMAM dendrimers. In some cases, aggregation of the nanocomposites leads to *reversible* precipitation of the intact nanocomposite. Moreover, pH-induced nanocomposite aggregation is a critical factor in the flocculation and *irreversible* precipitation of bulk Ag metal from these solutions. Aggregation and reversible precipitation of the intact nanocomposites is also observed in the PAMAM/Cu system under high pH conditions, but precipitation of bulk Cu^0 is never observed. The results imply that pH can significantly influence the efficacy of PAMAM dendrimers as nanoscale reactors and as protecting agents for inorganic nanoparticles. In addition, the reversible, environmentally controlled precipitation of the intact nanocomposites may find application, for example, in the facile separation and recovery of the nanocomposites following use as homogeneous catalysts.

Experimental Section

The preparation of dendrimer-encapsulated nanoparticles was similar to the method first reported by Crooks and co-workers.[6]

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* To whom correspondence should be addressed. Electronic mail: *vanpatte@ohio.edu*. Fax: 740-597-1849.
† Department of Chemistry and Biochemistry, Ohio University.
‡ Ohio University College of Osteopathic Medicine.

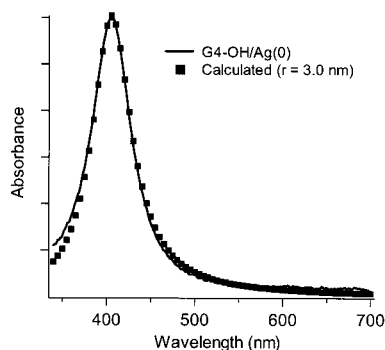Influence of pH on Dendrimer-Protected Nanoparticles

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Figure 1. Solid line: UV−Visible absorption spectrum of G4-OH/Ag(0) after addition of 1.25 eq. of NaBH$_4$. Squares: Calculated UV−Vis spectrum of isolated silver nanoparticles with radius of 3.0 nm (see Supplementary Information for details of calculation).

Hydroxy-terminated, generation 4, PAMAM dendrimers (G4-OH) (10% w/w in methanol) were obtained from Aldrich and used as received. Silver nitrate, sodium borohydride, and nitric acid were obtained from Fisher Scientific and used as received. G4-OH (1 μmol) and silver nitrate (20 μmol) were dissolved in 10 mL organicfree, deionized water (Barnstead filtration system). The pH was adjusted to 5.0 with dilute nitric acid. The resulting solution was stirred for 15 min, followed by the slow addition of up to 100 μmol sodium borohydride dissolved in 2 mL deionized water. The reduction process was followed with a Cary-16 UV−Visible spectrophotometer. G4-OH/Cu(0) nanocomposites were prepared under similar conditions, with a 20:1 metal atom:dendrimer molar ratio. G6-OH/Ag(0) nanocomposites were also synthesized using G6-OH dendrimers. The metal-to-dendrimer molar ratio was adjusted to 60:1 in this preparation.

Dynamic Light Scattering. Sizes of particles in aqueous solution were determined by the dynamic light scattering method (DynaPro MS/X, Protein Solutions Inc., Charlottesville, VA). Solution viscosities were measured with a viscometer and entered into the DynaPro software to correct for the influence of viscosity on particle mobility.

Transmission Electron Microscopy. A drop of the particle suspension was placed onto a Formvar-coated 150-mesh copper grid, allowed to sit for 30 s, then blotted off. The grid was then examined, unstained, in a Zeiss EM 109 electron microscope at 80kV. The microscope had been calibrated with a diffraction grating (2,160 lines per mm). Electron micrographs of selected regions were taken at a direct magnification of 49 200× on Ilford Pan F film (120 mm size). After the film was developed, the particles (200 per photograph) were measured directly from the negatives. The negative was placed upon a light box, covered with a transparency film, and measured with a magnified micrometer divided into 0.1 mm divisions.

Results and Discussion

Preparation of Stable Silver Nanoparticles. Reduction of G4-OH/Ag$^+$ at pH 5 with 1.25 equivalents of borohydride resulted in the formation of a clear, yellow-brown solution of G4-OH/Ag(0). The absorption spectrum of the product solution is shown in Figure 1 (solid line). The intense peak at 405 nm is due to absorption by surface plasmons in the silver nanoparticles.[21] The pH of the solution at this point was approximately 5.5. If the reduction is halted at this point, then the solution remains stable and can be stored on the shelf for months without clouding or precipitation. We confirmed that silver reduction was complete by adding excess borohydride: no further increases in area were observed under the plasmon absorption
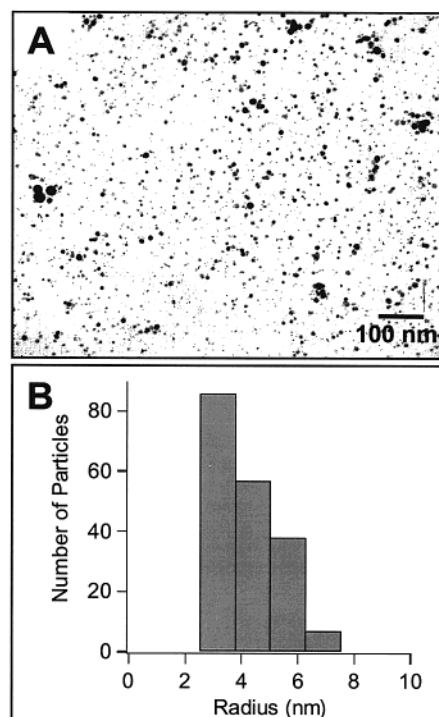

Figure 2. (a) Transmission electron micrograph of G4-OH/Ag(0) at pH 5.5. (b) Particle size histogram ($N = 194$).

peak. The primary effect of excess borohydride was to raise the pH of the solution and reduce its stability against flocculation (see next section).

The position of the plasmon absorption peak in Figure 1 suggests that the nanoparticles are widely dispersed; aggregated metal nanoparticle spectra are characterized by red-shifted peaks.[22−24] The sizes of metal nanoparticles can be roughly estimated from absorption spectra through application of simple Mie theory (see Supporting Information for details of calculations).[21,25,26] The complex dielectric function of the nanoparticles can be approximated using a modified Drude theory. As shown in Figure 1, selection of a 3.0 nm nanoparticle radius results in a good approximation (squares) to the experimental absorption spectrum (solid line).

Figure 2 shows a TEM image of silver nanoparticles from the solution described above. The particles are isolated and monodisperse. Electron density appears to vary from one particle to the next. Also shown is a histogram showing the results of manual measurements of nanoparticle sizes. The mean particle radius was determined to be 3.6 nm. The radius of an individual G4 PAMAM dendrimer has been estimated on the basis of molecular modeling calculations to be approximately 2.3 nm[27] and has been measured in methanol solution by small-angle X-ray scattering to be approximately 1.7−1.9 nm.[28] On the basis of these estimates, most of the particles in the image field are too large to be contained inside a single dendrimer. These particles are most likely capped by multiple dendrimers as has been documented in the literature for similar systems.[11,20]

The average hydrodynamic radius of these particles as measured by DLS at pH 5.5 is 4.6 nm. In comparing DLS and TEM size measurements, it is important to consider several factors that are likely to give rise to discrepancies. First, both measurements are naturally subject to inherent sources of random and systematic error. Second, the size determined from TEM is a number-weighted average, whereas the size determined from DLS is a mass-weighted average. As is the case with polymers, the mass-weighted average should be larger
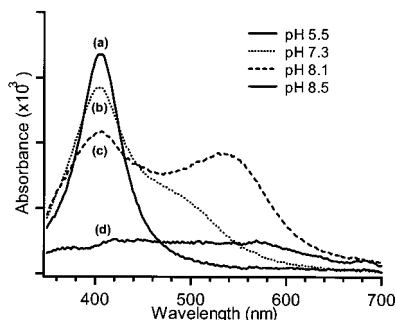
Figure 3. UV−Visible absorption spectra of G4-OH/Ag(0) after addition of (a) 1.25 eq., (b) 2.5 eq., (c) 2.75 eq., (d) 3.0 eq. of NaBH₄. Red-shifted peak is due to pH-induced aggregation.

except in a perfectly monodisperse system. Third, the TEM measurements should be sensitive only to the electron-dense metal particle, whereas the DLS measurements are sensitive to the size of the whole nanocomposite. If the metal particles are encased by any organic material, as seems likely, then the DLS measurements will include this organic matter, whereas the TEM measurements will exclude it. Given these differences in the measurements, the TEM and DLS data are in remarkably good agreement, and both agree quite well with the radius estimated by spectral simulation. Taken together, the data provide conclusive evidence that the G4-OH/Ag(0) nanocomposites at pH 5.5 are well-isolated, spherical, monodisperse, and 7−9 nm in diameter.

pH-Dependent Aggregation and Precipitation. As mentioned above, the primary effect of adding excess borohydride to the G4-OH/Ag(0) solutions is to increase the solution pH. Figure 3 shows the solution absorption spectrum after addition of various amounts of excess borohydride. Figure 3B shows the spectrum after the addition of 2.5 eq of borohydride (pH 7.3). The 405 nm peak has diminished (compare with 3A) and given rise to a shoulder shifted to lower energy that imparts a perceptible reddish tint to the solution. This spectral trend continues with the further addition of borohydride (Figure 3C) until, finally, the reddish solid precipitates from solution (Figure 3D). Addition of small amounts of hydroxide ion in place of borohydride produced the same result. However, the red shift is not simply the result of changes in ionic strength. The addition of NaNO₃ at pH 5.5 (up to concentrations of 0.01 M) does not lead to changes in the solution's absorption spectrum.

The red-shifted absorption peak observed in the solutions at elevated pH is due to the formation of nanocomposite aggregates. Red-shifts of the plasmon peak upon aggregation of metal nanoparticles are predicted by Mie theory and have been the subject of recent calculations by Schatz and co-workers.[21,23−25] The assignment of the red-shifted peak to aggregate species is supported by several observations. First, when G4-OH/Ag(0) is prepared at low concentration (10^{-6} M), this red-shifted peak is not observed. Second, DLS measurements on G4-OH/Ag(0) solutions between pH 7 and 9 show the presence of very large particles with radii ranging from tens to hundreds of nanometers. Murphy and co-workers[11,12] have reported DLS-based evidence for aggregation in PAMAM dendrimers and PAMAM-protected CdS nanocomposites. These authors did not discuss the influence of pH on aggregation. Third, TEM images such as that shown in Figure 4 clearly show the presence of large, multi-particle aggregates.

The pH-induced aggregation and precipitation of the red aggregate are reversible if the pH is lowered within a period of hours after aggregation/precipitation. The re-solvation of the red precipitate at pH 4 is slow compared with the effectively
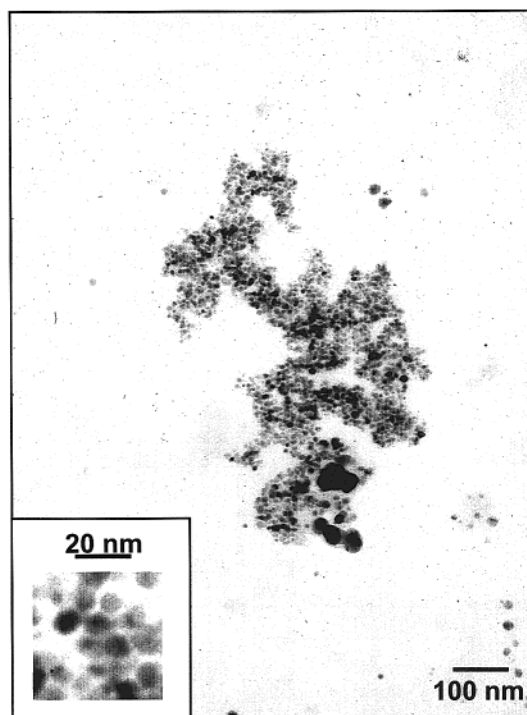

Figure 4. Transmission electron micrograph of G4-OH/Ag(0) at pH 8.

instantaneous formation of the precipitate at high pH, but does occur. The reversibility suggests that the red aggregates result from dendrimer-dendrimer contacts; no metal−metal contact occurs. This idea is consistent with the absorption data, as metal−metal contacts would simply produce larger nanoparticles, whose spectra would differ significantly from those of the aggregates. In addition, it can be seen from the TEM image in Figure 4 (see inset) that the nanoparticles in the aggregate are separated by light areas, which apparently represent organic domains. DLS experiments on PAMAM dendrimers in the absence of nanoparticles confirm that the dendrimers alone, while soluble at pH 5, aggregate in aqueous solution at high pH.

Although the pH-induced aggregation is temporarily reversible, solutions stored at elevated pH for extended periods are prone to *irreversible* flocculation and precipitation of black particles of Ag⁰.[29] At pH 7.5, this is manifested by a slow clouding of the brown solution followed by formation of perceptible black particles in the solution. The precipitation of black Ag metal, once started, can be accelerated by further increasing pH. The difference between the red and the black precipitates is clear. The red precipitate represents highly extended aggregates of the nanocomposites that grow too large to remain in solution. The black precipitate consists of fragments of Ag metal resulting from the agglomeration of the Ag nanoparticles (i.e., contact between the inorganic domains.)

The behavior of G6-OH/Ag(0) was qualitatively the same as G4-OH/Ag(0) in all respects except one. Although the G6-OH/Ag(0) system formed aggregates and a red precipitate at high pH, this system was never observed to yield the black metal precipitate. We hypothesize that the increased steric protection offered by the G6 PAMAM dendrimers prevents the metal particles from coming into direct contact with one another even under highly aggregated conditions.

An important question involves the mechanism for aggregation and de-aggregation. The formation of the red aggregate described above appears to depend on dendrimer-dendrimer

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interactions. The forces which favor aggregation of the dendrimers at high pH are probably dipole−dipole interactions, hydrogen bonds, and perhaps hydrophobic interactions between methylene units in the dendrimer arms. These interactions may not be any stronger than the solvation interactions (also dipole−dipole interactions and hydrogen bonds), but the dendrimers can form many of these bonds with one another. At low pH, the driving force for aggregation may be overcome by intense Coulombic repulsion caused by the multiple positive charges[30] residing on each dendrimer.

The interpretation proposed above is, on its face, at odds with the observation that addition of nitrate does not induce aggregation. If electrostatic forces are responsible for the dispersion of the dendrimer, then electrostatic shielding at high ionic strength should favor aggregation of the nanoparticles. However, as reported above, 0.01 M $NaNO_3$ has no apparent effect on aggregation at pH 5.5. It may be that even higher salt concentrations are necessary to produce aggregation and agglomeration. Unfortunately, much higher nitrate concentrations result in rapid reoxidation of the Ag. Many other types of anions also undergo strong interactions or reactions with Ag/Ag^+, confounding interpretation of the experimental results. The apparent contradiction here underlies the complexity of this system. Explanations can be proposed to reconcile the electrostatic dispersion hypothesis with the reported observations, but absent experimental data, any such explanation is speculative at best.

To test the ideas that pH controls the *dendrimer−dendrimer* interactions and that our observations are independent of the metal used, we prepared G4-OH/Cu(0) in the manner described by Crooks et al.[6] The Cu^{2+} starting solution was acidic and did not require the addition of any nitric acid. A stable solution of G4-OH/Cu(0) resulted. The reason for the acidity of the starting solution is well-known; di- and trivalent transition metals commonly yield acidic solutions due to complexation of OH^-. This behavior is not observed with Ag^+. When the G4-OH/Cu-(0) reduction was attempted at pH 9.3, a yellow-colored precipitate was formed that was similar to the red precipitate described above. No *bulk* Cu precipitate was observed in this solution, however. The absence of bulk Cu precipitation supports the notion that Cu−PAMAM interactions may be stronger than Ag−PAMAM interactions; however, when pH is controlled for, Ag^+ appears to be suitable for preparing dendrimer-protected nanocomposites analogous to the Cu, Pd, and Pt nanocomposites that have been previously reported. This conclusion is consistent with the report by Wang et al.[31] that dendrons can be used for the controlled growth and stabilization of nanoparticles even when the metal-dendron interactions are believed to be quite weak.

Conclusions

We have shown that pH is an important determinant of stability against aggregation and precipitation of dendrimer-protected metal nanoparticles. By controlling the pH of the reaction mixture, we have prepared stable, dendrimer-protected Ag nanoparticles by the Crooks method. Furthermore, pH can be used to control aggregation and precipitation in other metal-PAMAM systems as well. This new understanding may be applied in future efforts to prepare dendrimer-protected inorganic clusters. Additionally, the reversibility of the precipitation may be employed as one method for separation and/or recovery of PAMAM-containing nanocomposites following use as homogeneous catalysts.

Supporting Information Available: Details of calculation of nanoparticle absorption spectrum (1 pages). Ordering information is given on any current masthead page. This material is available free of charge via the Internet at http://pubs.acs.org.

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