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Title: Synthesis and Characterization of Dendrimer-Stabilized Nanoparticles

Department: Chemistry

Michael James Gabay
Printed Name

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Dr. Richard Crooks
Supervising Professor

_____ _____
Signature **Date**

Synthesis and Characterization of Dendrimer-Stabilized Nanoparticles

Presented by Michael James Gabay

In partial fulfillment of the requirements for graduation with the
Dean's Scholars Honor's Degree in Chemistry in the spring semester of 2008.

_____	_____
Supervising Professor	**Date**
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Honor's Advisor in Chemistry	**Date**

Abstract

It has been posited that different synthetic conditions, such as use of amine-terminated rather than hydroxyl-terminated PAMAM dendrimers, may result in the production of different species of nanoparticles, namely dendrimer stabilized nanoparticles (DSNs) rather than dendrimer encapsulated nanoparticles (DENs). Although DENs have been well studied, DSNs have received little attention because they are larger, less uniformly synthesized, and probably catalytically inactive. Nonetheless, we believe that an understanding of these nanoparticle systems requires knowledge of all possible species being formed. Moreover, we hope that better synthetic control of DSNs may allow for new applications. To this end, we have optimized literature procedures to form gold DSNs smaller than 2 nm. Two techniques, aqueous-organic extraction and transmission electron microscopy staining, were explored for their utility in differentiating the two species (DENs and DSNs). UV-Vis absorption spectroscopy indicates that extraction serves as a useful qualitative method for identifying the presence of a significant proportion of DSNs in a solution of nanoparticles. If reproducibility can be established, we believe this technique could be made quantitative. Perfection of a washing step to remove excess thiols would allow for corroboration of current evidence using transmission electron microscopy (TEM) images. Attempts to stain the dendrimer for TEM using uranyl acetate and phosphotungstic acid were unable to match the quality of currently published data, although some progress was made in removing prohibitive stain contamination. We outline several experimental changes that may result in this technique yet proving useful.

Background

Research on nanoparticles (NPs), objects with dimensions between 1-100 nm, has increased dramatically in recent years because of their interesting properties and applications, a result of the sometimes dramatic changes in properties as objects enter this small size regime. For example, bulk gold is very noble and will not easily undergo chemical reactions. However, particles of gold on the 2-3 nm size range are much more reactive and can even catalyze chemical reactions.[17] Some prominent applications currently under investigation include drug-delivery systems, medical devices and imaging technology, catalysis, information-storage devices, energy sources and storage, nanoelectronics, and chemical sensors.[10] The passage of the 21st Century Nanotechnology Research and Development Act by Congress in 2003 authorized roughly three and a half billion dollars for nanotechnology research funding, highlighting the current excitement in this area.

Many synthetic procedures have been developed to produce metal nanoparticles. However, to prevent aggregation of NPs into a bulk material, all syntheses must block them from combining with neighboring particles. A variety of molecules have been used in synthetic routes to accomplish this,[15,18-20] usually by physically attaching a ligand to surface of the particles, thus preventing the metal surfaces from contacting one another. Unfortunately, in addition to blocking aggregation, these surface capping methods also block catalytic reactions from occurring on the nanoparticle surface. One solution to this problem is the use of PAMAM dendrimers, large hollow sphere-like molecules, to encapsulate the nanoparticles. In effect, the nanoparticles are put into cages and cannot

contact other nanoparticles, but their surfaces are still available for chemical reactions. The peripheral functional groups of a dendrimer can be changed, allowing for solubility in a wide variety of solvents. Furthermore, they allow for tight synthetic control, yielding NPs in the 1-2 nm size range with high monodispersity or uniformity.[8,11,14] NPs formed using this method are called Dendrimer Encapsulated Nanoparticles, or DENs.

However, some reports have shown that, under certain conditions, particles too large to fit inside a dendrimer are formed,[1-3,21] implying that they are stabilized by dendrimers blocking their surfaces (dendrimer stabilized nanoparticles or DSNs), rather than simply being encapsulated by the dendrimers. We would expect the properties of the DSNs to be significantly different than DENs, meaning that they may have different utilities in terms of synthetic procedures. For example, it may be easier to produce core-shell nanoparticles with DSNs than with DENs. Therefore, it is useful to be able to distinguish between the two species experimentally so that better synthetic control can be developed for each species.

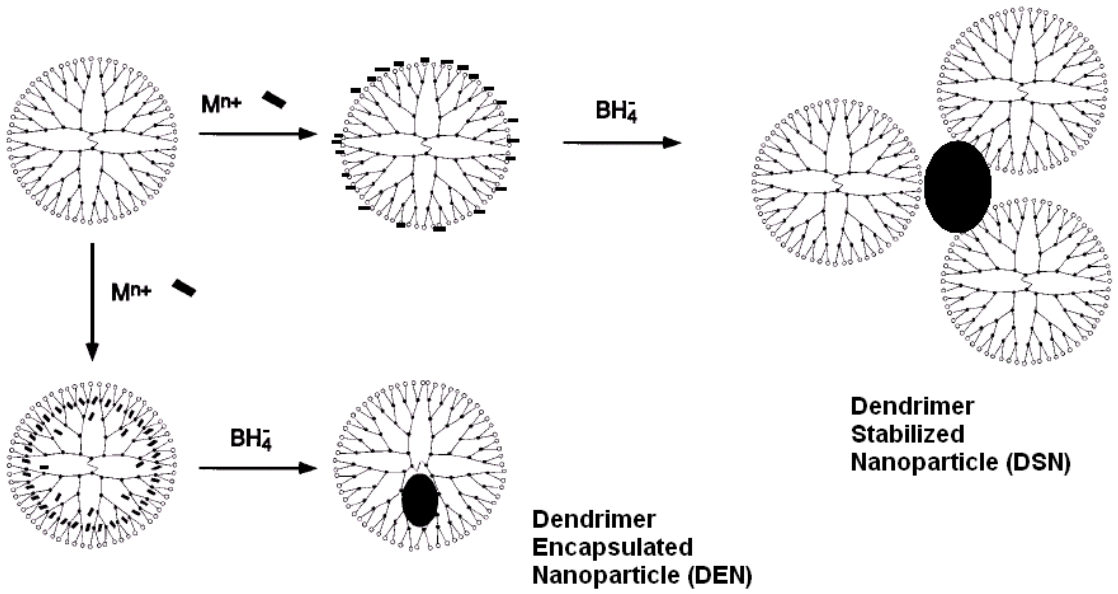

Figure 1: A comparison of the synthesis of DENs and DSNs. Depending upon the synthetic conditions, one route is believed to be strongly favored over the other. Scheme adapted from Garcia, et al.[3]

However, there are no current techniques available that can distinguish between the two species. It is unknown if DSNs and DENs are coformed using current synthetic methods and to what degree this may occur. The goal of this project has been to investigate possible methods of characterization. To do so, we began by investigating the synthesis of DSNs, with the aim of producing a more monodisperse system. This would allow us to more successfully characterize our nanoparticle solutions. Then, two characterization methods were investigated, namely extraction of the NPs from their dendrimer templates and transmission electron microscopy (TEM) staining.

Extraction of the NPs is a relatively simple process whereby short organic sulfur containing molecules, alkanethiols, are added in an organic solvent to the aqueous DENs

solution. The alkanethiols cap the NPs and remove them from the aqueous phase to the organic phase upon vigorous shaking, forming monolayer protected clusters or MPCs (**Figure 2**). Crooks has shown this process to proceed quantitatively.[4,5] Because DSNs are surface passivated, we expected extraction to fail for them. As a result, we hoped comparing the extraction success of solutions of NPs would allow us to determine if they were DSNs or DENs. This comparison was done using UV-Vis absorption spectra. We furthermore expected the strength of the absorbance in these spectra to be an indicator of the ratio of DSNs:DENs if they are coformed.

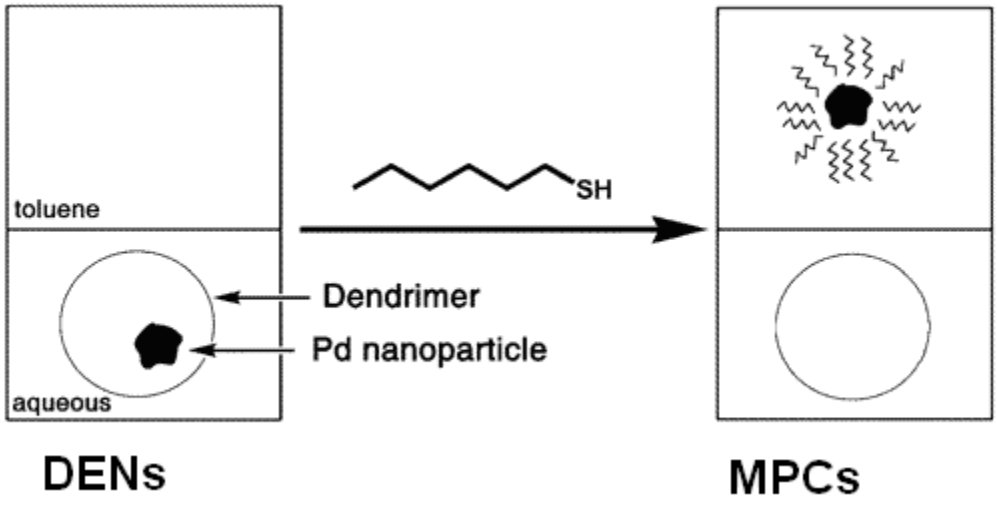

Figure 2: Scheme for extraction of NPs from dendrimers into alkanethiols, adapted from Crooks.[5]

We also predicted that TEM staining would allow us to differentiate between DENs and DSNs by visually comparing the relationship of the dendrimer to the nanoparticles in the micrograph images obtained. Tomalia[7,12,13,16] and Amis[6,7] worked on the problem of staining dendrimers with varying degrees of success, culminating in a report in

Macromolecules by Amis[6] where clear differentiation between the dendrimer and the nanoparticles was shown to be possible. We therefore expected to see a clear difference between a nanoparticle surrounded by multiple a (a DSN) and a nanoparticle encapsulated inside a single dendrimer (a DSN).

Results and Discussion:

Synthesis:

To characterize DSNs, we needed to have a reliable synthetic procedure to make them. To begin, we followed a procedure from Crooks[3] to make G2-$NH_2(Au_{1.4})$ NPs, signifying a ratio of 1.4 gold atoms to every 1 generation two amine-terminated PAMAM dendrimer. The procedure was followed exactly with the exception of scaling the volume down 20x due to the expense of the dendrimer. However, we were unable to reproduce their results initially. Upon adding the large excess of the reductant, sodium borohydride, the gold NPs quickly aggregated and precipitated. This was evidenced by a rapid change in solution color from orange to dark purple to clear with large black precipitate. In the UV-Vis spectrum, a large increase in the baseline was seen after reduction, as we expected due to the precipitate. Unlike the results of Crooks, we found no evidence of NPs, such as a monotonic increase in absorbance towards lower wavelengths or a Plasmon absorbance peak (see **Figure 3**).

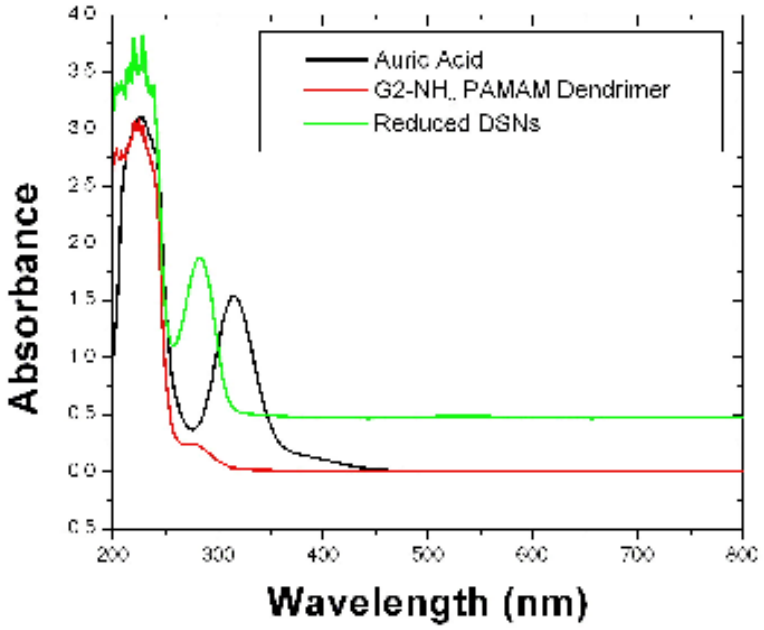

Figure 3: We see a large increase in the baseline absorption for the reduced nanoparticles, indicating the presence of precipitate, as we expected based on our observations. No evidence of NPs is present. We see that the source of our gold ions, auric acid, has no absorbance peak in the reduced species, indicating full reduction, as we would expect due to the presence of precipitate.

To discover the source of the discrepancy between our results and those previously published, we varied the complexation time of the dendrimer and metal ions, tried using fresher dendrimer stock solutions and different solvents for the dendrimer (we ended up using water for future experiments, to simplify our results), used lower concentrations, changed the order of addition of the dendrimer and metal salt, and varied the ratios of reductant to metal ion. All but the last showed no significant improvement, with precipitates occurring in all. Crooks had used a twenty molar excess of borohydride,

whereas we began using a simple molar equivalent of hydrides to gold ions. The amount of precipitate produced was drastically reduced. The solution was also stable for longer, retaining its purple color, characteristic of the nanoparticles, for a longer time. The UV-Vis spectrum, **Figure 4**, had smaller baseline absorption. A plasmon peak was observed at 517 nm, in rough agreement with the plasmon found by Crooks at 519 nm. Slight deviation was to be expected because our spectrum was measured in pure water solvent, whereas the previous data was measured in a water-methanol blend. Finally, a monotonically increase in absorption was observed towards smaller wavelengths, as in Crooks paper. All of these observations indicate a successful synthetic procedure.

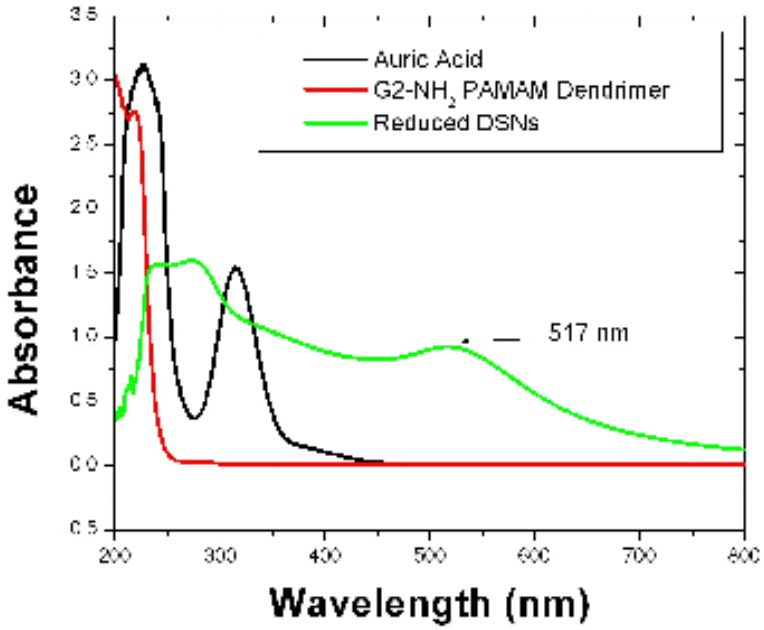

Figure 4: Lowering the ratio of reductant added yielded nanoparticles with a plasmon at 517 nm and a monotonically increasing absorption towards lower wavelengths, indicating

the presence of nanoparticles. The lowered baseline of the reduced species indicates decreased precipitate, as observed.

Moreover, transmission electron microscopy images confirmed that the particles were of roughly the same size as reported previously, with a comparable incidence of large aggregates present (**Figure 5**).

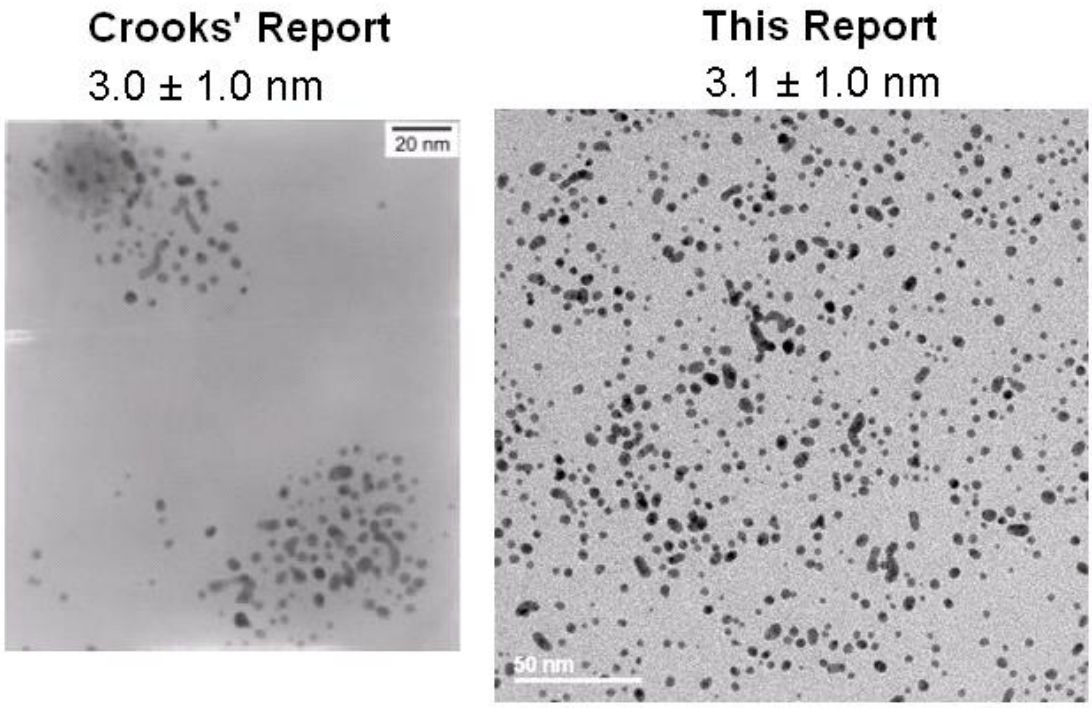

Figure 5: Transmission electron microscopy (TEM) images of G2-$NH_2(Au_{1.4})$ formed by Crooks compared to those synthesized in this report. An analysis of 100 nanoparticles in each image yielded sizes that agree within the standard deviation.

Unfortunately, some precipitate was still present as indicated by the slight increase in the baseline seen in **Figure 4** as well as its visibility to the naked eye. Because this would have complicated our proposed characterization methods, we began a series of

experiments to try and prevent the precipitation completely. We tweaked the reductant to metal ion ratio, lowered the overall solution concentrations, varied the speed at which reductant was added, and varied the time allowed for reduction before spectra were taken. Unfortunately, no improvements were seen for any of these experiments.

As a result, we switched gears and began working with a different synthetic procedure, adapted from Knecht and Wright.[9] After adjusting our concentrations and gold to dendrimer ratios, we found that we were able to produce, without precipitate, 10 uM G2-$NH_2(Au_5)$ and 10 uM G4-$NH_2(Au_{29.5})$. Although no plasmon is observed in the UV-Vis spectra, **Figure 6**, the monotonic increase in absorption as well as TEM micrographs demonstrate the presence of nanoparticles. The lack of plasmon is explained by the size of the nanoparticles which was less than 2 nm. For the NPs in the generation 4 dendrimer, this may indicate that DENs, rather than DSNs were formed, which would complicate characterization, as will soon be discussed. However, generation 2 dendrimers are not large enough to be spherical and cannot possibly encapsulate the nanoparticles, so we can be confident that the G2-$NH_2(Au_5)$ are DSNs, even if the G4-$NH_2(Au_{29.5})$ are actually DENs. Having done our best to improve the synthesis, we moved on to the characterization methods.

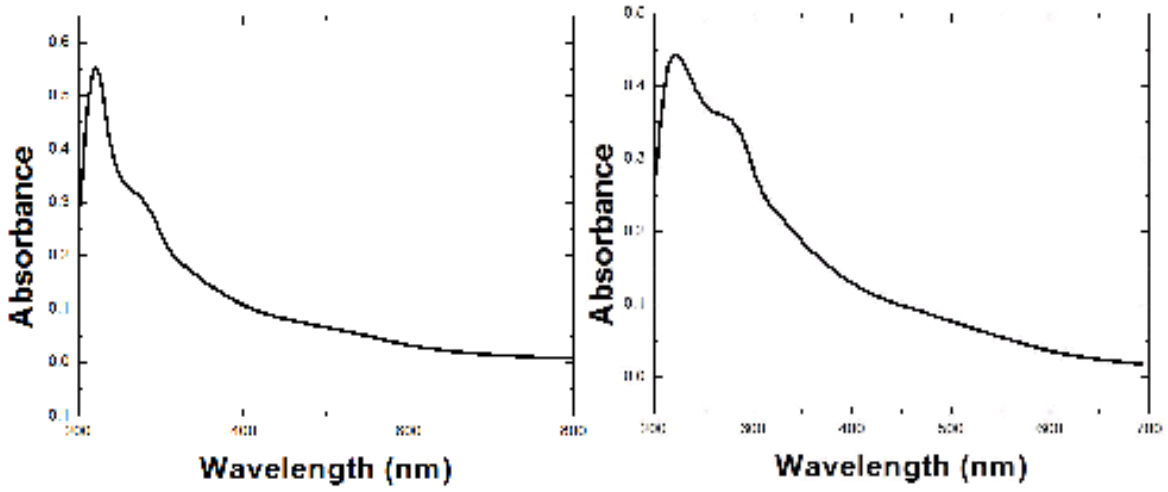

Figure 6: UV-Vis absorption spectra for 10 uM G2-$NH_2(Au_5)$ and 10 uM G4-$NH_2(Au_{29.5})$ on the left and right, respectively.

Extraction Work:

As a control experiment, extractions of G4-OH(Au_{55}) and G4-OH(Au_{147}) particles were performed according to literature procedures.[4,5] However, we found that increasing ionic strength using sodium borohydride caused further particle growth and eventually precipitation. According to Crooks, several other salts including sodium chloride yielded the same results because they merely serve to increase the ionic strength of the aqueous phase. This provides a stronger impetus for the MPCs to stay in organic phase. Once we switched to using sodium chloride, our results, **Figure 7**, matched those previously reported. The DENs are initially in the aqueous phase (black line), but after extraction, we see that the characteristic absorption of the nanoparticles can only be found in the organic phase (green line), implying complete transfer of the DENs from the aqueous to the organic phase.

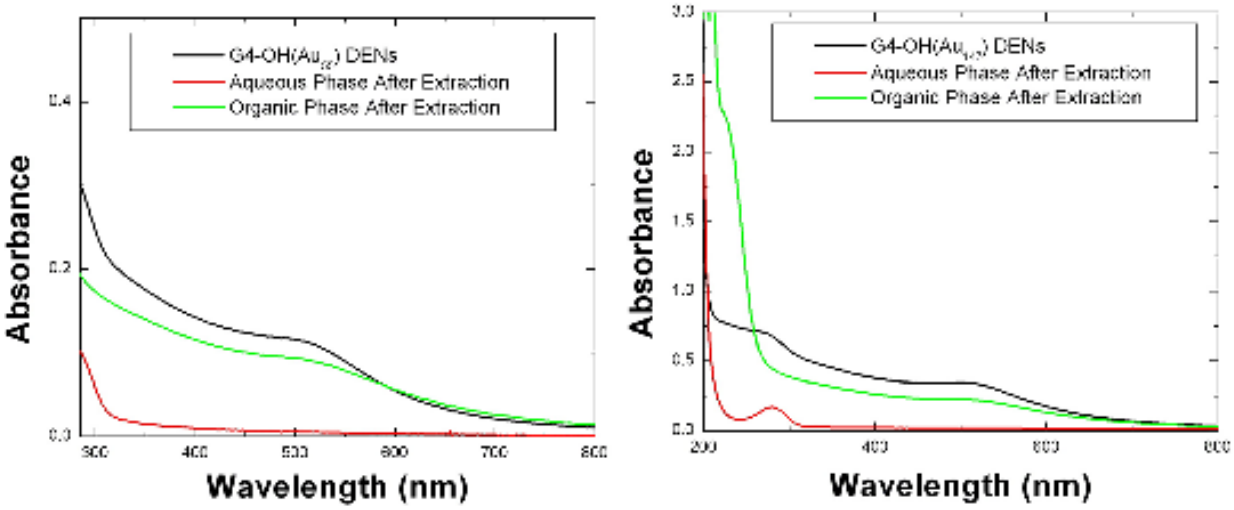

Figure 7: The UV-Vis absorption spectra of the extraction process for both larger and small DENs is shown to match literature results and demonstrate successful transfer of the nanoparticles from the aqueous phase initially (black line) to the organic phase after extraction (green line). Furthermore, no evidence of nanoparticles is present in the aqueous phase after extraction (red line).

Next, we wanted to compare these extractions with the extraction of DSNs. We used the synthetic procedure previously discussed to produce G4-NH_2($Au_{5.9}$) and G4-NH_2(Au_{118}). The use of amine-terminated dendrimers is believed to result in DSNs rather than DENs.[3] We did not expect the alkanethiols to be able to extract the DSNs into the organic phase this time because the surface of the nanoparticles is not exposed like in a DEN, but rather passivated by dendrimers. However, the UV-Vis absorption spectra of the extraction, **Figure 8**, indicated that nanoparticles were present in both the aqueous and organic phases after extraction. Thus, we conclude that either the extraction efficiency of DSNs is greatly reduced but does not go to zero or that our synthetic procedure produces a mix of

DSNs and DENs wherein only the DENs get extracted. In either case, we have shown that extraction can be used to identify solutions with a significant proportion of DSNs. We would have liked to use TEM evidence to corroborate these conclusions, but we found that the excess of thiols used results in burning of the TEM grid. Washing with ethanol would have ideally solved this problem, but we found that this step removed significant amounts of the nanoparticles along with the thiols. Given more time, it would have been useful to solve this problem so that TEM data could have been used here.

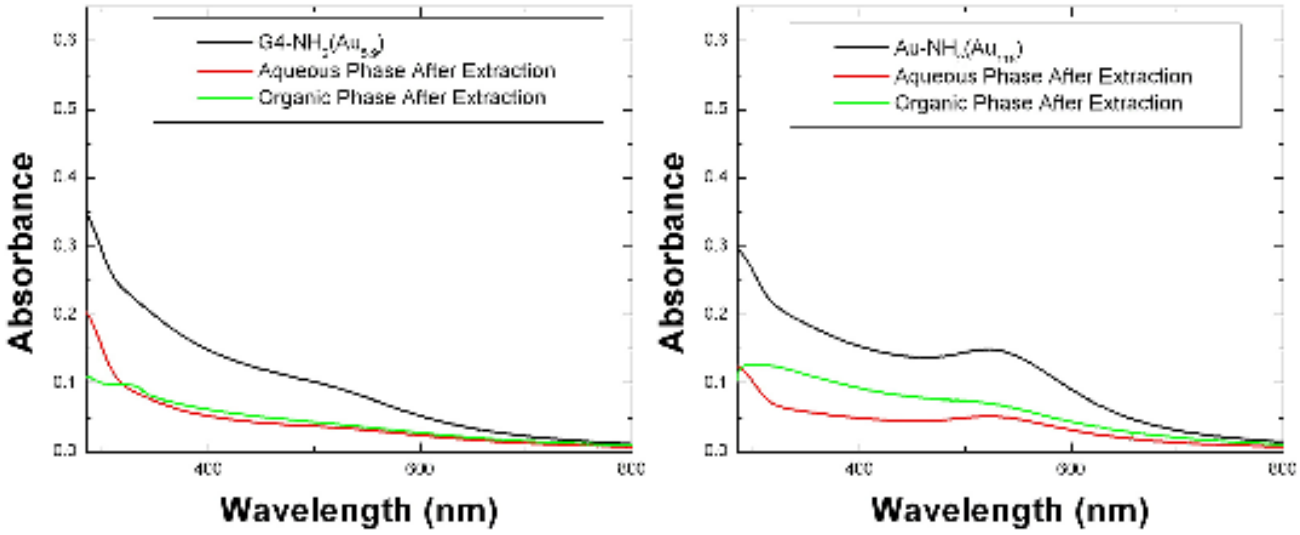

Figure 8: For nanoparticles we predict are DSNs, the extraction efficiency is greatly reduced. After extraction, we see that the characteristic absorbance of the nanoparticles is split between the aqueous and organic phases both for small and large particles. This indicates that extraction is only partially successful.

We expect that this technique is quantitative, but due to time constraints, we were unable to test the reproducibility of this technique for a variety of sizes of DENs and DSNs. Moreover, quantitative analysis is complicated by the lack of a standard. There is still a

question that any of our syntheses will result in pure DENs or pure DSNs, which would be useful for testing the accuracy of this technique.

Staining Work:

Staining the dendrimers was the other technique we hoped would allow for some differentiation between DENs and DSNs. Before we could compare these two species, we needed to be able to reproducibly stain the dendrimer. We began following the most successful paper for staining DENs by Amis[6] but were unable to reproduce his results. In general, we were only able to see large, micron-sized blobs of dried stain on the grid; nothing corresponded to the size or shape of a dendrimer. Different grid preparation techniques were compared to rectify this problem. We compared a "drop method" to a "tweezer method." In the former method, the TEM grid was floated upon a drop of dendrimer and then a drop of stain before it was allowed to dry and subsequently was measured. In the latter method, the grid was held in a pair of tweezers and drops of dendrimer and stain were added and then wicked away, using filter paper, before being allowed to dry and then measured. Less stain contamination was observed using the tweezer method, resulting in its subsequent adoption. Rinsing with ultrapure water before the drying step also resulted in some reduction of the stain contamination. However, no evidence of staining was being observed still.

Upon switching stains from phosphotungstic acid to uranyl acetate, **Figure 9**, we began to see some evidence of negative staining of the dendrimers. This means that rather than

directly staining the dendrimer, the area around the dendrimer was stained. As a result, uranyl acetate was adopted for future experiments.

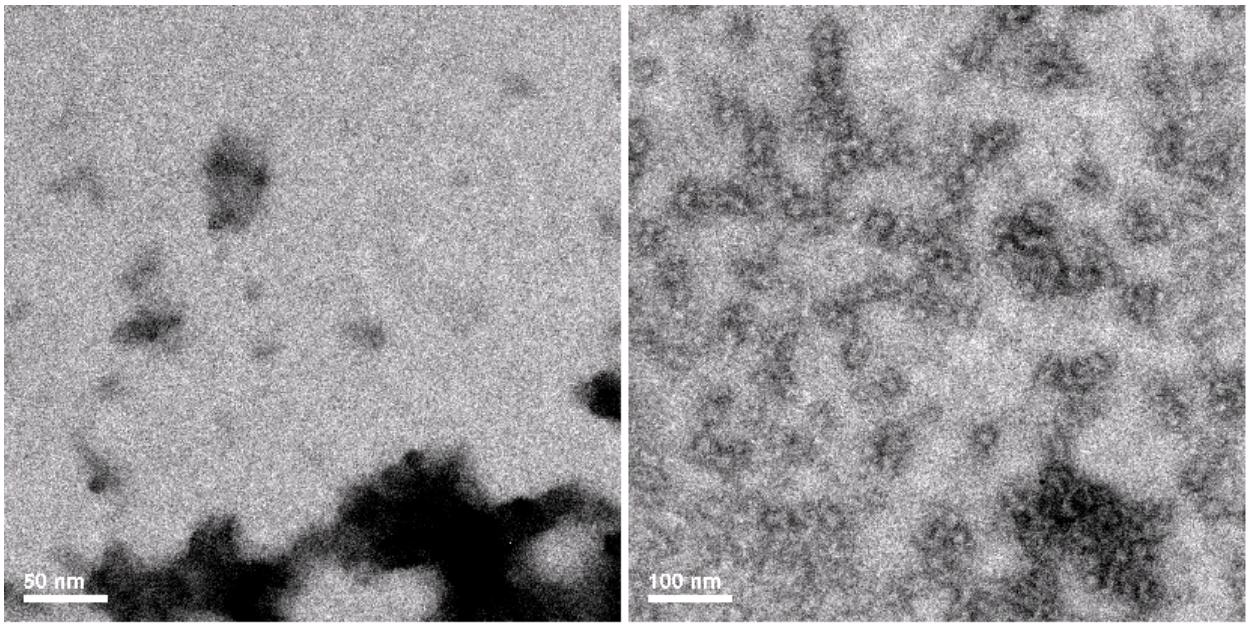

Figure 9: On the left, we see the results of staining G6-OH dendrimers with 2% phosphotungstic acid. On the right, we see the result of staining G6-OH dendrimers with 2% uranyl acetate. The latter appears to show some successful negative staining.

Given this encouraging result, we felt it appropriate to reduce the ever-present stain contamination by simply reducing the amount of stain used. As shown in **Figure 10**, this reduced the large scale contamination seen previously, but did not solve the problem entirely. In addition to this, we began to see some evidence of positive staining on roughly the same size as the generation six dendrimers, which ought to be 6.7 nm.[14] However, we did not observe the circular shapes we expected. This may be due to a combination of dendrimer-surface interactions and imperfections in the dendrimer, but it

may also have been that the stain observed did not correspond to dendrimers and was merely an artifact of the stain drying onto the grid.

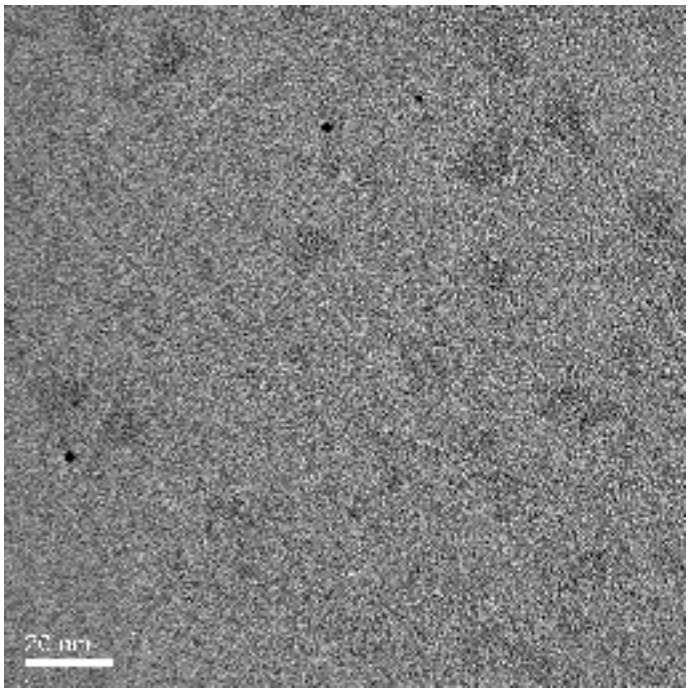

Figure 10: G6-OH dendrimer stained with uranyl acetate using the "tweezer method" described above. The ratio of stain to dendrimer was reduced from roughly 4000:1 to 1:1 for this image. It is unclear whether the blobs of stain correspond to dendrimers or not.

To ensure that we were observing dendrimers, we began spiking our samples with a solution of gold DENs. If these blobs corresponded to dendrimers, we would expect to see each blob have a nanoparticle sitting inside it. However, this did not appear to be the case, **Figure 11.**

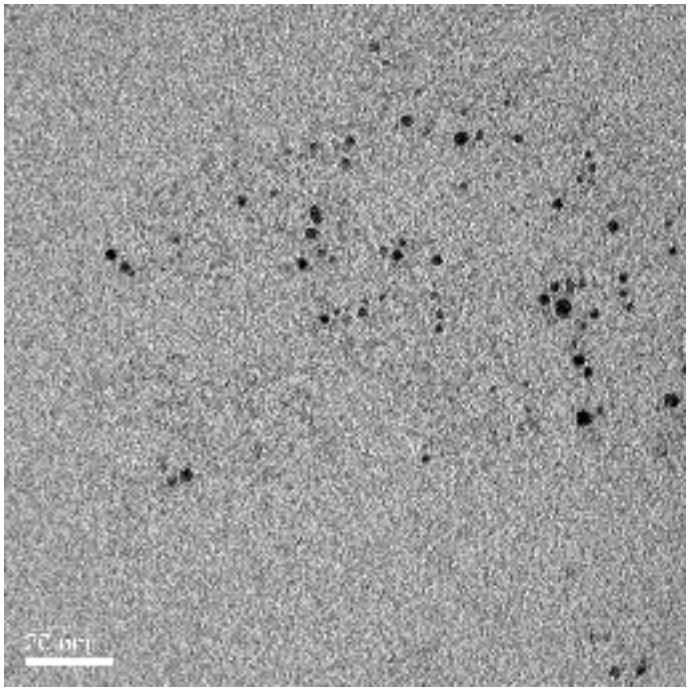

Figure 11: Same experimental set-up as in Figure 10 but with a spike of gold DENs to help identify dendrimers. We were unable to find anything that looked definitively like a dendrimer with a nanoparticle inside of it.

Furthermore, a control experiment was performed where a grid was stained as usual but without the addition of any dendrimer. The same stain blobs that we had supposed were dendrimer were still present, indicating that what we observed was not actually dendrimer. Attempts to increase the contrast of the dendrimer by using larger dendrimer sizes (G8-OH rather than G6-OH) showed no improvement.

Although success was not observed yet using this method of inquiry, its promise as a technique to distinguish DENs and DSNs merits further research. It may be helpful to try dissolving the stain in non-aqueous solvents such as ethanol or methanol, adjusting the pH of the stain solutions, trying another stain preparation method, or trying different

stains altogether, such as a double staining technique involving lead citrate alongside uranyl acetate.

Conclusion:

We predict that differentiation between DSNs and DENs will allow for better synthetic control of each and possibly for unique synthetic routes to produce novel nanoparticles. We have explored extraction and TEM staining as two methods to achieve this end. Although more work is needed for each technique, extraction has already shown itself capable of distinguishing the two species, at least qualitatively. Because UV-Vis spectroscopy lends itself to quantitative analysis, we expect that this will be possible using extraction if we can show that the extraction process is reproducible for various systems where we expect the proportion of DENs and DSNs synthesized to vary. At times it seemed that staining the dendrimers for TEM analysis was more art than science, significant improvements were made in later results compared to when we first started. We believe that subsequent experiments, as discussed in the previous section, should allow this technique to eventually be a useful one because Amis has already shown that successful staining is possible.

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