

GRAM QUANTITIES OF SILVER AND ALLOY NANOPARTICLES: SYNTHESIS
THROUGH DIGESTIVE RIPENING AND THE SOLVATED METAL ATOM DISPERSION
(SMAD) METHOD: ANTIMICROBIAL PROPERTIES, SUPERLATTIC SELF-
ASSEMBLY, AND OPTICAL PROPERTIES

by

ALEXANDER B. SMETANA

B.S., Illinois Wesleyan University, 2001

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Chemistry
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2006

ABSTRACT

This is an account of the synthesis of several drastically different forms of silver nanoparticles: Bare metal nanoparticles, dry nanoparticulate powders, aqueous soluble particles, and organic ligand coated monodisperse silver nanoparticles were all produced. The synthetic method was adapted from previous studies on gold nanoparticles and investigated to understand the optimal conditions for silver nanoparticle synthesis. Also the procedure for refinement of the nanoparticles was studied and applied to the formation of alloy nanoparticles. This extraordinary procedure produces beautifully colored colloids of spherical metal nanoparticles of the highest quality which under suitable conditions self-assemble into extensive three dimensional superlattice structures. The silver nanoparticle products were later tested against several biological pathogens to find dramatic increases in antimicrobial potency in comparison to commercially available silver preparations.

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Approved by:

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Kenneth J. Klabunde

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This is an account of the synthesis of several drastically different forms of silver nanoparticles: Bare metal nanoparticles, dry nanoparticulate powders, aqueous soluble particles, and organic ligand coated monodisperse silver nanoparticles were all produced. The synthetic method was adapted from previous studies on gold nanoparticles and investigated to understand the optimal conditions for silver nanoparticle synthesis. Also the procedure for refinement of the nanoparticles was studied and applied to the formation of alloy nanoparticles. This extraordinary procedure produces beautifully colored colloids of spherical metal nanoparticles of the highest quality which under suitable conditions self-assemble into extensive three dimensional superlattice structures. The silver nanoparticle products were later tested against several biological pathogens to find dramatic increases in antimicrobial potency in comparison to commercially available silver preparations.

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DEDICATION

This thesis is dedicated all my former teachers and student colleagues for their patience, knowledge, encouragement, and their enthusiasm without which this manuscript would not be remotely possible.

Chapter 1: Synthesis and Characterization of Noble Metal Nanoparticles

Introduction to metal nanoparticles

Nanotechnology is a discipline that has risen to a sort of scientific rumor that is being spread from culture to culture across the globe. It is vaguely described in most circles; the average conversations concerning it dwell on intangibles from innovative dreams to doomsday projections of tiny machines turning the entire world to a puddle of goo. In truth, nanoscience is simply the study of matter at the length scale of one billionth of a meter (10^{-9} m). The study of these materials is inherently difficult due to the small size of the particles and high reactivity that arises from their high surface area and unusual morphologies. Undaunted by this difficulty, exploration and creation of nano-sized structures is the result of scientists' never ending curiosity and constant probing of their universe to new extremes. This creative desire is epitomized by a challenge given to the scientific community by the brilliant physicist Richard Feynman who in 1959 charged scientists to find the means to write the encyclopedia on the head of a pin¹.

The hype for this area of science is not unfounded. Elements and compounds that researchers had previously probed took on new exciting properties when broken down to the physical dimensions of the nano length scale. The new nano-sized products garnered exponentially higher reactivities as a large portion of the atoms composing the material reside exposed on the surface of the sample. New features of materials were discovered that could not

be expressed by a few atoms or the infinite model of solid state physics, but exist only in this new area in-between at compositions of hundreds to thousands of atoms. Most interestingly, the properties and chemistry of the nano-materials were found to change as the size of the sample was changed, leading to adjustable features².

The study of nanoparticles in general and metal nanoparticles in particular is made possible only by the use of the latest technological discoveries in instrumental techniques. High resolution electron microscopy allows scientists in this field to probe the structure, size, and shape of the nanosized objects, with the latest instruments being able to resolve individual atoms and give information on the atomic structure of the crystals. Even more impressive are spectroscopy probes that can be used in situ in the electron microscope to provide insight into the elemental composition and even chemical nature of the atoms within the particles themselves.

Most solids, if the conditions are found, can be made into nano-materials. Much of the initial research in nanotechnology is concerned with synthesis of already known compounds into the nano size regime, and then subsequently probing the effects of manipulating the size of the material at this scale. This chapter is a review of the present state of research on metal nanoparticles, their synthesis, the techniques used to characterize them, and most importantly, a discussion of their applications to science and technology of the world at large. The synthesis of silver particles will be focused on here, however the techniques are applicable to many metals and occasionally other materials.

Characterization of nanoparticle materials

Microscopy

Nanoparticles were not first created in the last century, in fact many forms of materials have been of nanosize. However the technology to view and explore these materials was mainly lacking at the time leaving nanoparticles in a world of obscurity too small to have been noticed by science. The advent of imaging smaller materials has been possibly the greatest asset that has allowed contemporary scientists to fully engage the discipline of nanotechnology. This has provided a means to probe the size and morphology of these materials and to relate their size to their properties. There are three types of electron microscopes that are frequently reported in the literature to characterize nanoparticles, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM).

TEM

The Transmission Electron Microscope (TEM) is one of the primary tools of the materials chemist and is almost essential to accurately describe the size, and shape of metal nanoparticles. Images from the TEM allow scientists to obtain the equivalent of X-ray negatives of a sample; higher density material shows up dark with a high contrast supported on a low density thin carbon film. An incident beam of electrons is created by running a potential across a filament (often tungsten) that can vary from 50-300keV. The greater the voltage of the filament used the smaller the wavelength of the ejected electrons, and therefore a better resolution of the

sample can be obtained. This type of imaging can, in high resolution microscopes provide a magnification of over 500,000 times what can be seen by the naked eye³.

Samples are typically placed on a thin metal grid covered by a plastic or carbon film that supports the sample to be viewed. This grid is then placed in the electron beam between the filament and a phosphorous plate that lights up as electrons hit the surface. Electrons localized on atoms in the sample will block the passage of the electron beam and appear as dark areas on an otherwise illuminated image. Increasing atomic number of the element gives rise to greater contrast of the image, making heavy metals such as silver and gold to stand out against organic compounds. This allows for observation of the atomic arrangement within particles making possible the identification of single crystals, twinning, phase differences, and grain boundaries. The self-assembly of nanoscale materials is of vital importance to the application of metal particles to useful endeavors and will be discussed later. The assembly of the particles can be manipulated to suit a variety of needs; an electron microscope is pivotal in observing the details of which, an example is given in Figure 1 below.

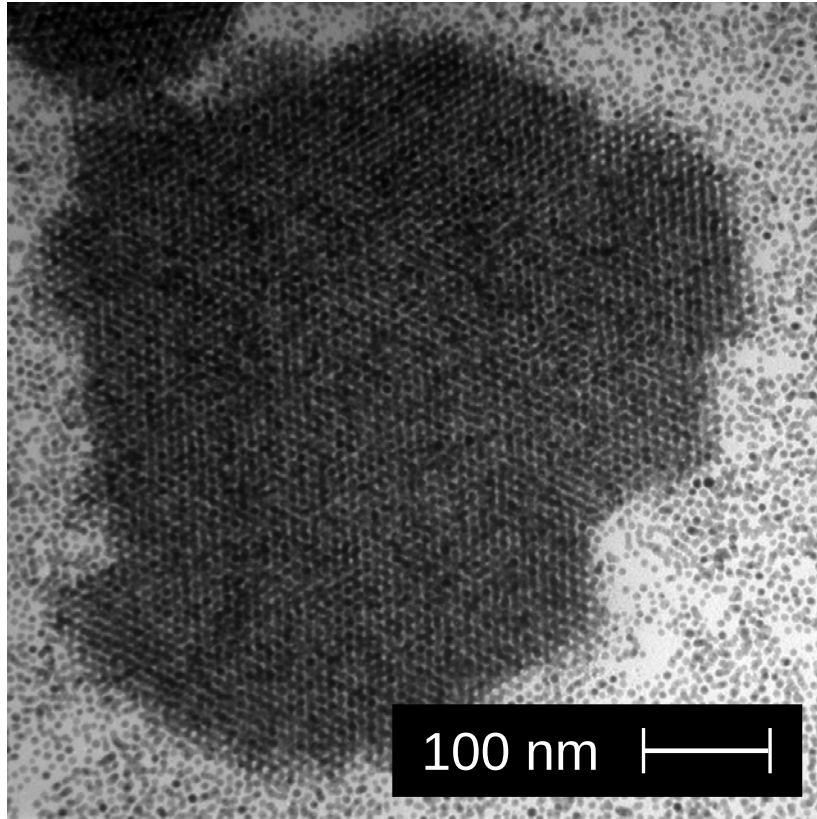


Figure 1 - Transmission Electron Microscope (TEM) image of gold nanoparticles that have self assembled into a well-ordered superlattice.

In addition to providing visual information about the sample, additional spectroscopic attachments can be used to provide elemental and chemical data about individual particles while being viewed in the microscope. The combination of these techniques can be a very powerful tool to characterize nanoparticle samples.

SEM

While the TEM can penetrate through samples, Scanning Electron Microscopy (SEM) scans a beam of electrons over a section of the sample and creates a topographical image of the surface of the particles. This instrument provides a 3-D image of the specimen and a clearer idea of the overall morphology of the sample. The resolution of an SEM is typically much more limited, with the best microscopes having the ability to resolve particles greater than ~5 nm in diameter. Most instruments however can only discern differences in a sample that are at least 20 nm apart. The SEM is amenable to most of the spectroscopic attachments of the TEM allowing for chemical as well as structural information.

TEM and SEM instruments rely on a high vacuum system that can be difficult to maintain. Excess gas will deflect electrons and drastically reduce the intensity and quality of spectra. This increases the cost of the instrument greatly, such that smaller institutes often will not have access to both microscopes, and often neither.

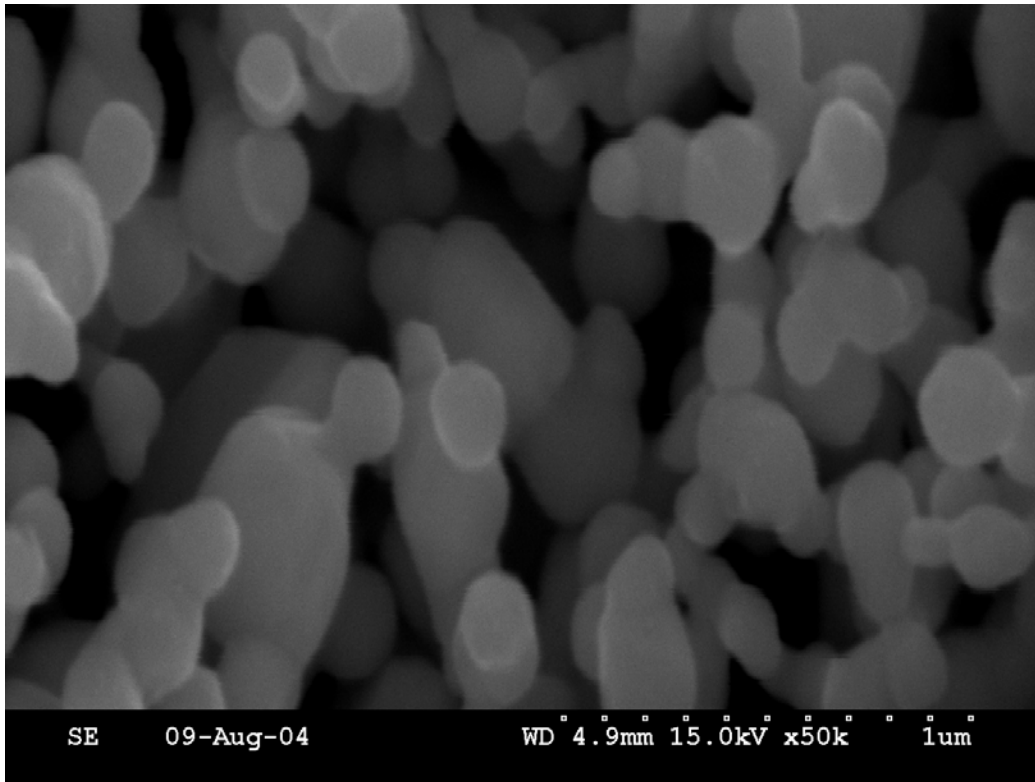


Figure 2 - SEM picture of silver nanosized powder displaying a very porous nature of the powder.

AFM

AFM instruments were first developed at the IBM laboratory in Zurich Switzerland. These instruments are typically far less expensive than TEM or SEM microscopes, but usually cannot provide as much sample information. AFM machines function by rastering the surface of a sample with a probe. The quality of the imaging is usually dependent on the sharpness of the probe itself (ideally ending in a single atom at the tip). The surface of the sample is constructed as a signal that is detected by electron tunneling across the space between the sample and the tip. By a feedback mechanism the tip can be set to a single potential and scanned across the sample

surface. The Z position of the tip is monitored and graphed as a 3-D topological map of the surface.

There are several benefits to AFM. Samples can be manipulated in a crude sense in the AFM by using the probe to physically push particles around or scratch the surface of the material, creating ordered structures mechanically on a length-scale of a few nanometers. It has no need of a high vacuum system as the samples can be tested in air, aqueous, or organic environments on the benchtop. This drives down the price of the instrument as a crude microscope can be constructed by students for ~\$10,000 compared to the million dollars needed to purchase a good TEM instrument.

Chemical Analysis

While being able to image samples in the nanosize regime is an important first step in being able to characterize materials, their chemical information helps us understand the nature of the sample. This information can provide insight to understanding the interactions that control their synthesis, and catalysis. Without this information the scientist can only infer the relation between structure and function of the novel nano-materials and speculate their composition. These methods can characterize nanoparticle samples, and can also aid in probing their properties.

Electron and X-Ray Diffraction

The identity of a crystalline sample can often be determined through analysis of its diffraction pattern. A diffraction pattern of a solid is dependent on the arrangement and size of the composite atoms which is unique for every compound. The pattern can then be compared to a large database of previously known samples and can identify a material. A diffraction pattern is obtained when a sample is penetrated with a monochromatic source of waves (either electrons or photons). The incident waves will be largely reflected by the internal atoms of the sample, destructively interfere, or otherwise lost. However there will always be a few paths in which the waves can travel through the sample and will constructively interfere with each other. An intense band will appear only where this condition is met with the remaining areas remaining dark according to the Bragg's law shown below. The band position is dependent on the arrangement of atoms within the solid and can be solved for the lattice spacings within the crystal⁴.

$$\text{Bragg's law: } n\lambda = 2d\sin\theta$$

This technique can be used within a TEM instrument using the electron beam as the wave source and is able to identify small amounts of sample this way, even single particles. A sharper spectrum is usually obtained with a separate X-ray diffractometer, but the sample size for

analysis must be much larger. The wave source for these applications is high energy X-ray photons generated by exciting metal filaments (typically Cu or Mo).

Amorphous samples have no internal order, resulting in no interference patterns for the sample and are thus impossible to analyze with this method. Interestingly, this technique can also detect the ordering and separation of the nanoparticles themselves in respect to one another in a regular array. This will be important if the nanoparticles self assemble into ordered structures. If this is the case the spectrum will give the identity of the particle composition superimposed on the diffraction pattern of the particle assembly.

Energy Dispersive X-ray Spectroscopy (EDX)

Energy dispersive X-ray analysis is a spectroscopic method by which the elemental composition of the sample can be determined. This method can be used in tandem with electron microscopes. The electron beam from the microscope will interact with the atoms of the sample causing some electrons from the inner atomic core to be ejected. An electron from the outer shell of this atom will quickly fall into this lower energy orbital and emit a photon of light in the X-ray spectrum to release excess energy. The photon's energy is very specific to the element it is emitted from and can indicate the presence of an element as low as 1%. The most advanced of these detectors are able to examine a particle with a resolution of 0.1 nm. An elemental map of the location of several atoms can then be constructed providing the identity and construction of a composite material. This is essential in determining the composition of nanosized electronic components. The intensity of the X-ray signal can be determined and a value for the percent

composition of each element in the sample can be calculated to aid in determining the nature of the material.

X-ray Photoelectron Spectroscopy (XPS)

One of the most powerful tools for probing the chemical composition of a sample is X-ray photoelectron spectroscopy (XPS). XPS is a spectroscopic method that can analyze the composition of any sample by measuring the energy of ejected electrons from the sample. Where EDX analyzes photons XPS analyzes the energy of the photoelectrons ejected from the sample itself. This method is much more accurate and will clearly show electrons from every orbital of the sample. Diffraction techniques can only view the bulk nature of crystals while XPS data can identify any substance amorphous or crystalline. XPS is also more of a surface technique; incident radiation only penetrates into the first 5-10 nm of the sample. This technique, in most cases, can identify not only the elements composing the sample but the oxidation state and bonding information of the atoms as well. The chemical information of the surface of nanoparticles is integral to the understanding of their function since that is the part of the sample that is in contact with its environment and is responsible for catalytic interactions.

UV-Visible Spectroscopy

Colloidal dispersions of noble metal nanoparticles, namely gold, silver, copper, and platinum were found to display vibrant colors in the visible spectrum. The position and shape of the absorbance is a reflection of the size, shape, and chemical environment of the particles⁵. This absorption is called a surface plasmon resonance (SPR), the origin of which will be discussed later in chapter 2. Spherical particles tend to give rise to a single absorption in monodisperse samples characteristic of the element while particles of lesser symmetry will often give rise to multiple peaks. The peak position has been observed to blue shift as the particle size decreases. The peak width will narrow as the size distribution of the sample decreases so with fewer types of particles contribute to the spectrum. This phenomenon allows for a quick analysis of the product, and is often used to study the kinetics of nanoparticle reactions. Unfortunately this phenomenon has only been observed for a very small number of elements.

The relationship between the changing shape and size of the nanoparticles with their physical properties can allow scientists to design synthetic materials with specific applications in mind. Depending on the nanoparticle composition other forms of analysis may be included in their characterization. These analyses are typically specific to the materials. Use of a Superconducting quantum Interference Devices (SQUID) is used to probe the magnetic properties of ferromagnetic materials. Fluorescence studies are typically conducted on semiconductor nanoparticles or systems in which fluorescent labels have been attached to particles. Various electronic techniques including Cyclic Voltammetry have been used to examine the oxidation potentials of nanoparticles compared to their bulk counterparts.

Synthesis methods for spherical metal nanoparticles

Nanotechnology is slowly emerging from its infant stages. Applications from this area of science are only just beginning to form and only in a rudimentary fashion. In order for this discipline of science to grow new nanoparticle samples need to be available in large supply for study and integration into other materials. To make this into reality synthetic routes must be found to make nanoparticles from all types of materials. Control of the size of each type of particle will lead to the possibility for integration in new devices, and allow resourceful probing of their capabilities so they can become agents of technological advancement.

Spherical noble metal nanoparticles are an ideal material for a study of this scope. A sphere is identical in all aspects in space, when the size of these particles is changed only one dimension changes, and therefore a direct correlation between size and behavior can be determined. Geometrical triangles, rods, wires, and irregular particles contain more than one variable to account for and insinuations must be made. Moreover, noble metal nanoparticles are suitably stable for analysis and carry the potential to be involved in several exciting applications.

Many approaches to the synthesis of metal nanoparticles have been taken each with its own advantages and disadvantages. This ranges from the first documented formation of nanoparticles by reducing gold salts in solution to elaborate chemical vapor deposition apparatus that stabilize particles in inert gases. Unique and wonderful new nanoparticles often also call for extreme synthetic preparation methods. Scientists have gone to enormous lengths using many powerful techniques available including lasers, microwaves, and sonication to induce metal nanoparticle formation and produce high quality products. Other nanoparticle formation

strategies developed have included photo-induced reduction of metal ions, bio-reduction, and use of versatile highly branched dendrimer macromolecules as nano-reactors.

Michael Faraday and the first colloidal gold solution

Modern metal nanoparticle synthesis is most often traced back to Michael Faraday who discovered a method to reduce gold salts with phosphorous into a colloidal suspension, and delved into the reasons behind the red color of glass impregnated with small amounts of gold. While several chemists and alchemists had previously formed gold colloids, Faraday suggested that the color change was due to the small size of the clusters of gold particles absorbing the light⁶. This idea was expounded upon by Mie who solved the Maxwell equations of electricity and magnetism for small metal nanoparticles⁷. This work related the optical properties of the metal nanoparticles to their environmental condition giving credence to the usefulness of such colloids.

Turkevich, Brust, and the wet chemical reduction of nanoparticles

In 1951 Turkevich and Stevenson made a fascinating study of the preparations of gold nanoparticles known at the time⁸. Reproducing the experiments themselves, the researchers compared the products on an early TEM. The pair also developed the optimal conditions for their own preparative scheme involving the citrate reduction of gold ions which proved not only

the simplest method of the techniques investigated but yielded the best quality of particles as well. The citrate method is a very cheap and easy method of creating metal nanoparticles, and variations of this procedure are still in use today. The system has no waste products as the citrate molecules act as a reductant and complexing agent in solution, eliminating the need for purification of the particles. Particles formed from this method are typically a little larger at 15-20nm in diameter and have only a fair size distribution⁸. The method has been applied successfully to many methods such as silver, however since it is an aqueous system it is not amenable for use with some of the more reactive metals. Other variations on this method were later introduced in an attempt to manipulate the size of the particles.

Interest in metal colloids waned for many years until the 1990's when the applications for nanoparticles began to drive an explosion of scientific interest that is currently thriving. Many other synthetic approaches similar to the Turkevich method were found that relied on reduction of metal salts in solution. The benefits of other reducing agents such as sodium borohydride^{9,10,11} hydrazine¹², Hydroxylamine hydrochloride¹³, glucose¹⁴, hydrogen gas, ethylene glycol¹⁵, and long chain amines^{16,17} were studied extensively. The power of the reducing agent had a direct dependence on the products, that were unique in size, shape, quantity, or quality of the colloid produced. Some methods favor products that can be dispersed in water, vs. organic solvents, while others could provide a reliable synthesis in which purification of the particles is not required.

The most famous of these techniques is the Brust method which utilizes a two-phase system and produces particles of 1-4nm in diameter¹⁹. The Brust synthesis relies on a phase transfer agent tetraoctylammonium bromide (TOAB) to bring the unreduced gold salt AuCl_4^- from the aqueous to the organic phase. The salt was then reduced by addition of sodium

borohydride quickly forming nanoparticles in solution. These particles are protected from one another rapidly due to the immediate presence of dodecane thiol in the organic phase that stabilizes the particles from aggregation much like citrate ions in the Turkevich method. Dodecanethiol, however displays a much stronger binding to the metal center as the sulfur atom is a softer base and has a better interaction with the soft noble metal atoms than the acid moiety. This ligand is the standard molecule used in any new synthesis of metal nanoparticles today. The Brust synthesis provided the means to metal nanocrystals that were stable enough to isolate by precipitation from solution and study extensively, helping to drive the interest in this field of science.

More sophisticated syntheses were developed to control the immediate environment of the metal atoms as they are first reduced. One very successful and interesting wet chemical synthetic preparation is the creation of a microemulsion system, or inverse micelle liquid crystal within the solution to perform the reduction. By instituting a delicate balance between a surfactant and two solvents of drastically different polarities surfactant molecules order themselves much like a cell membrane into spherical pockets separating the aqueous and organic phases. The area inside each micelle is limited which encourages small particles. The separation between micelles prevents aggregation between particles when the reducing agent is introduced to the solution. This process has been shown to form particles with a very narrow size distribution demonstrated by Stoeva, and can be applied to many different metals¹⁹. Variations of this technique were applied with other solvents including an aqueous/ supercritical carbon dioxide system in which the micelles contained water but the solvent was CO₂. This also proved to be a successful synthetic method²⁰. Unfortunately, the wet chemical reduction methods

require purification of the nanoparticle product as the solution contains many excess ions that interfere with some of the particles properties.

Physical Vapor Deposition (PVD)

The previous discussion has shown that the synthesis of metal nanoparticles via wet chemical methods is very reliable, quick, and at times can produce very monodisperse products with a fair degree of control over experimental conditions. However the amount of nanoparticles that can typically be made through this method is rather small, while requiring vast amounts of solvents. Also the presence of excess ions in solution may be problematic and can take away from the applicability of the product in many devices. Vapor deposition methods can bypass some of these problems. This synthesis typically involves the evaporation of a source material into a high vacuum and collecting the product after it has reacted with a stabilizing agent²¹. The experimental equipment required for this type of synthesis can often be elaborate, requiring very low pressures and high voltages. High purity products can be obtained through this process however, and the amount of nanomaterials produced can be increased greatly over solution chemistry approaches²¹.

Physical Vapor Deposition (PVD) is typically used to produce thin films and coatings for the creation of many electrical components, antireflective coatings on optical equipment, or hard coatings on mechanical tools. Nanoparticulate metal powders can be created in this way as well. In fact almost any material can be atomized in such a reactor from many different forms (powder, rod, wire, ect.). Although there are several ways to evaporate the source material, thermally heating the sample from a tungsten crucible is the most common. The evaporated

metal atoms or clusters can be cooled by a carrier gas such as helium to reduce reactivity, and then deposited on a cold finger. When the reaction is completed the powder can be scraped from the cold finger and can be composed of crystallites as small as 5-15 nm in diameter²¹. Where coatings are desired a free path to the substrate is optimal. PVD has the drawback in that if composite materials are desired the evaporation is not likely to be uniform due to different boiling points. Using lasers to evaporate the metal can overcome this deficiency, however it adds complexity to the reactors. Many composite materials can be produced if the reactor is slightly modified and gases such as O₂, N₂, or hydrogen are allowed to react with the metal vapor to produce oxides, nitrides, and hydrides respectively through a process called Chemical Vapor Deposition (CVD).

An interesting variation of this method is the Solvated Metal Atom Dispersion (SMAD) method developed by Klabunde *et al*^{22,23}. This procedure involves co-evaporating a metal source along with a stabilizing solvent that co-precipitate on a cold finger. If the solvent is in great enough excess to the material then the reactive atoms are stabilized and can be recovered by a controlled warming process where the atoms aggregate only slightly. The product can be polydisperse but is able to be refined by a process they developed termed digestive ripening²⁴. Vapor deposition methods are difficult to set up, however the products obtained from them are often of very high quality, and can provide in situ information on the formation of the nanoparticles if spectroscopic attachments are incorporated into the reactor.

Synthesis of metal nanoparticles through Sonication

Recently scientists have discovered that ultrasonication can be used to create extreme reaction conditions in liquid environments. The irradiation of liquids with sound waves produces

acoustic cavitation where in localized, small areas of the solution enormous temperatures and pressures can be reached that can drive chemical reactions in unique ways²⁵. This technique has been applied to the synthesis of metal nanoparticles as well as the creation of nanoparticle oxides, sulfides, and unique polymer surfaces. Li and coworkers studied the effects of low-power ultrasonication to nanoparticle synthesis. During a typical AOT/isooctane inverse micelle reduction of silver nanoparticles by NaBH_4 it was found that in the absence of sound waves the micelles created spherical reaction chambers. Reduction of silver ions in these micelles produced spherical silver nanoparticles. When the reaction vessel was sonicated during synthesis the shape of the micelle itself became ellipsoidal and caused aggregation of the particles inside the micelles. The morphology of the particles was shown to be dependent of the sonication time as nanorods and nanowires were formed with increasing sonication time respectively. The structure of the inverse micelles was followed through small-angle X-ray scattering studies²⁵.

The laboratories of Nagata expanded sono-chemical techniques to prepare bimetallic nanoparticles namely Au/Pd catalytic particles²⁶. Their proposed reaction scheme involves the reduction of gold and palladium salts by radicals formed from the effects of sonication on the solution. In between the collapsing cavitation bubbles they suggest that hydrogen radicals form, which react with sodium dodecyl sulfate, the radicals of which reduce the metal. Core-Shell structures were found to form due to the lower reduction potential of gold vs. palladium.

Synthesis of metal nanoparticles through microwave irradiation

The use of microwaves has been a recent advancement in chemical synthesis beginning in 1986²⁷. Similar to the use of sonication, microwave synthesis induces unique and extreme

reaction conditions on the sample. Microwave irradiation causes rapid volumetric heating and has been found to drastically increase reaction rates. Kamarneni et al has adapted an efficient synthesis of silver nanoparticles using a two-phase liquid synthesis driven by microwave heating²⁷. Their synthesis involves a toluene/ethylene glycol system with dodecanethiol added to the toluene layer. The synthesis is conducted in a Teflon container where the microwave irradiation sustains the reaction at 170 °C for 3 hrs.

The nanoparticles obtained through this method were very monodisperse at roughly 10 nm in size, and displayed a high propensity to self-assemble. The synthesis through this method was simple, and allowed a relatively large amount of silver to be processed at once at 0.15g AgNO₃. Many products could be achieved with this method, which is applicable to production of silver sulfide nanocrystals by adding thiourea to the reaction system. Nanocubes were even obtained by reducing the amount of dodecanethiol in solution with respect to the silver ion²⁷.

Gedanken applied microwave synthesis to the creation of bimetallic core-shell Au/Pd particles using the polyol synthesis²⁸. The formation of gold is again attributed to the more favorable reduction of gold thermodynamically. The experimental setup of microwave reactions is simple, merely consisting of a microwave fixed with a water cooled condenser to prevent solvent loss during reaction²⁸.

The use of Dendrimers to stabilize metal nanoparticles

A remarkable recent discovery in science are large, branched dendrimer molecules²⁹. Dendrimers are macromolecules composed of a molecular core with large branching polymer chains extending out from the core into solution³⁰. These molecules have a great deal of flexibility and can be engineered to have very different characteristics on the surface or interior

of the dendrimer. Materials scientists have recently attempted the application of these macromolecules as nanoreactors where metal salts can be reduced to form very small nanoparticles. The most common dendrimer used is poly-(amidoamine) or PAMAM. This molecule is created by repeating units of β -alanine and is thus very bio-friendly and water soluble. PAMAM can allow for transport of drugs, and in some cases nanoparticles through cellular membranes in living organisms³¹.

Synthesis of silver metal nanoparticles can be accomplished simply by allowing silver nitrate salt to infiltrate the dendrimer, and subsequent reduction by NaBH_4 ³¹. Particles from this synthetic route can be made with a narrow size distribution and are very small, typically 1-3nm in diameter. The authors attributed the small size of the nanoparticles to the templating effect of the dendrimer, its high amount of permanent charges in the branched molecular chains discouraging aggregation, and the use of proper ratios of metal to dendrimer active sites³². These “magic numbers” of reagents correspond to particularly stable formations of metal nanoparticles of 55, and 140 metal atoms arranged in a sphere. The use of these ratios was found to cause a great reduction in the size distribution of the product²⁹. The use of dendrimers as synthetic templates has also been shown to be amenable to the formation of other metals, as well as alloy, and core-shell structures³².

Biological formation of nanoparticles

Many organisms find exposure to heavy metals quite toxic. Their response to high levels of metal ions in solution is for the organism to reduce the ions and transport them out of the cells. This behavior can be exploited to synthesize nanoparticles and provides a green chemistry route

to formation of nanoparticles. There are several living species that can be used for the reduction process. Klaus *et al* reported a synthesis driven by the bacteria *Pseudomonas stutzeri* Ag259³³. This bacterium is somewhat unique because silver is highly lethal to most single celled organisms, but this organism can be recovered from deep within silver mines. When placed in a solution of AgNO₃ the bacteria become nanofactories as the survival mechanism of the cell is to reduce the ion, gather the reduced metal in a central place and export them from the cell. The reduction of the silver ion in this case resulted in silver nanoparticles as spheres, triangles and hexagonal platelets.

Sastry and coworkers found that a similar effect can be found for the fungus *Verticillium*³⁴. With the fungus, the nanoparticles were much more monodisperse and formed almost exclusively 25nm spherical particles. TEM images show that the reduction process occurs at the membrane of the cells and the resultant particles remain attached to the fungal cells in solution. During studies of environmental remediation Dokken *et al* discovered that alfalfa plants are able to uptake several heavy metals and reduce them into nanoparticle form. The nanoparticles can then be separated from the biomass and may be viable method of cleaning up contaminated water supplies as well as a legitimate method of processing nanoparticles³³.

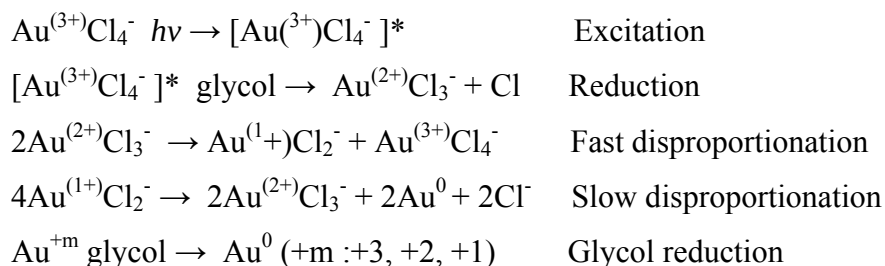
Photo reduction

In an attempt to find a controllable procedure for a reproducible synthesis of metal nanoparticles some research groups have attempted the photochemical reduction of metal salts. Mallick *et al.* found that by irradiation of gold chloride in a solution of Triton-X they could reproducibly prepare a solution of seed nanoparticles³⁴. This precursor could then be used to

create uniform colloids of larger particles by adjusting the amount of additional metal salt to nanoparticle seeds in solution. By this method the group was able to form particles of 35-40 nm, 55-65 nm, and 65-85 nm in diameter with the addition of greater amounts of free metal salt.

The mechanism of reduction is proposed to be an initial homolytic splitting of H_2O into hydrogen and hydroxide radicals by UV light. The radicals could either create a radical of Triton-X or reduce the metal in solution independently. The formation of a solution of seed crystals was found to be vital to production of quality colloids since the reduction potential of metal ions to metal atoms in the ground state is so much higher than the potential to reduce metal ions onto metal clusters. From this seed solution more metal atoms could be slowly deposited upon the surface of the crystallites with milder reducing agents, thus allowing for more controlled growth. The authors claim that synthetic methods that involve creation of seed crystals and growth simultaneously, are much more apt to result in polydisperse solutions, as new seeds are constantly being created in solution and rapid agglomeration occurs from the use of harsh reducing chemicals. The authors have been able to apply this method to the synthesis of copper and silver metal particles as well as gold³⁴.

Work completed in El-Sayed's laboratory has also led to a synthetic procedure to create metal nanoparticles photochemically³⁵. Their method involves the use of ethylene glycol as a reducing agent and poly(vinylpyrrolidone) (PVP) as a capping agent. Similar experiments have been conducted without UV irradiation, however they require temperatures of 160 - 280 °C in order for the ethylene glycol to reduce the metal salts. By irradiation of 250-400nm wavelength light the reaction can be driven at room temperature. From earlier observations and through studying the reaction kinetics (by UV-Vis absorption) with respect to the ethylene glycol mole fraction to gold salt they proposed the following mechanism for the photoreduction of gold salt:



The use of UV light has drastically cut down on the energy demands for the preparation of nanoparticles by this procedure, and forms a quality solution of nanoparticles.

Under a different set of experimental conditions, irradiation of pre-formed spherical nanoparticles by low energy light sources was found to cause the growth of the particles into flat triangular nanoplates³⁶. This reaction was observed to be caused by 350-700nm wavelength laser light. Attempts at carrying out the reaction in the dark or with less energetic light resulted in no reaction. The formation of spheres into prisms is an interesting result that is most likely caused by the use of the surfactant Bis(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium salt (BSPP) and may be aided by the presence of citrate ions in solution. While the reaction is driven or initiated by the light energy, the shape is more likely a result of the combination of surfactant molecules in solution. The synthesis of nanoparticles of various shapes and sizes has given researchers access to materials with further enhanced properties albeit with more complex and less predictable results.

Laser Induced Synthesis of Nanoparticles

Most synthetic methods described above are focused on a bottom-up approach to the formation of nanoparticles. This is usually brought about by the growth of seed particles from metal ion precursors, reduced in solution. One of the few very successful top-down approaches to the synthesis of nanoparticles, other than the SMAD approach is laser ablation of bulk precursors. In this method bulk material is first placed into a solution environment. This can either be an aqueous or organic environment and typically will contain dissolved capping agents available to coat the particles once formed. A laser is used to excite the bulk material and provide a reactive species that agglomerate and form nanoparticles³⁷.

There are a few different mechanisms proposed as to how the zero valent metal atoms/clusters are created in this procedure. The group of Kondow believes that the laser pulse ejects a large number of silver metal atoms that quickly agglomerate, since metal-metal binding is very favorable in solution³⁸. As the number of available metal atoms decrease locally, competition with ligands in solution then slows and finally terminates the growth of the particles. This process is repeated with another blast of the laser. Kamat believes that excitation with the laser causes electrons to eject from the metal, and in the case of a powder this severe charging of the material can cause the material to explode and thereby provide the metal atoms needed for nanoparticle formation³⁹.

Laser ablation in general is very versatile in that many products can be formed by introducing different starting materials into solution from metals, to semiconductors. Mixed elemental systems can also be formed as demonstrated for Au-Ag alloys by placing the bulk alloy into solution followed by laser irradiation⁴⁰. The size of the particles can even be adjusted

somewhat by varying the amount of ligands in solution or reducing the power of the laser to provide lesser amounts of reactive atom precursors³⁷.

Another bottom-up approach using laser synthesis has also been devised by Bjerneld *et al*⁴¹. This group used the laser energy to initiate the reduction of metal ions in solution by citrate ions. Using this method they were able to dictate the growth of nanostructured metal surfaces on glass. The size of the metal deposition and the exact location of the deposition of the metal atoms could be controlled very easily by a well focused laser. The utility of this method is demonstrated by their creation of SERS sensors inside of glass capillaries of microfluidic systems for ease of sensing small amounts of analytes for lab-on-a-chip devices⁴¹.

Applications of Nanoparticles in the World

Nanoparticles are quickly going to find their way into our everyday lives. Metal nanoparticles in particular have a wonderful potential to improve the quality of life, and boost technology to a new level of excellence. One of the most apparent needs is the integration of nanoparticles into electrical components, further extending the power of today's computer systems. Nanoparticles are also being designed for sensors of unprecedented sensitivity unreachable from yesterday's micron sized devices. The potential of nanomaterials as catalysts and reactive centers is greatly amplified by the vast increase in the number of surface atoms in comparison to bulk counterparts. An example of this is the increased biological activity of silver metal nanoparticles against bacteria vs. the bulk metal⁴²⁻⁵⁴.

Nano-Electronics

Despite their small size, above 1nm the products metal nanoparticles are composed of enough atoms to still behave as metals. The most exploitable property of this is that the particles have a plethora of electrons all at a very similar energy to one another and can be easily conducted or stored on a particle. In conjunction with the nanoparticles' incredibly small size this allows for the possibility of creating incredibly small electronic components. Quinn and coworkers have demonstrated the versatility of a nanoparticle as an electron storage center by observing 15 different oxidation states on a gold nanoparticle as electrons are stored or removed from the metal dot⁴². The most common preparation for nanoparticles leaves the metal sphere protected by a monolayer of a dodecane thiol molecule. This layer is insulating making each particle intrinsically a nanocapacitor where a small amount of energy can be stored.

The great difficulty has been found to not be the formation of these incredibly small electrical components, but in connecting them and their communication with one another. One ingenious method is the use of double stranded DNA to crosslink the nanoparticles by functionalizing the nanoparticle itself with oligonucleotides that will selectively attach the particle to specific parts of the DNA strand. If impregnated with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ the DNA strand provides a tunneling route for electrical currents⁴³. An alternative method of creating a planned electronic circuit was the use of aryl dithiols and dinitriles to connect metal nanoparticles to one another in a 2-D array. In this way the Andres group measured the conductance of several metal clusters with organic linkers with various lengths⁴⁴.

Metal nanoparticles can be integrated into media storage devices in ways other than by storage of electrons as well. Magnetic particles can also be created using some of the synthetic

methods mentioned above. For example Ono *et al* created Mn-Pt ferromagnetic alloys for this purpose⁴⁵. The major limitation of these materials is the ability to orient the nanoparticle to a certain value (N or S) and have it remain at that value at room temperature. Oftentimes even at this temperature there is enough energy for the particles to rotate and move freely of their own. Another interesting route to a high memory density material, however read only, can be obtained by utilizing the bright optical absorption of the nanoparticles. Hodak formed core-shell alloys of gold/silver nanoparticles with a complex absorption⁴⁶. These particles can quickly and easily be converted to alloys by irradiation of a laser. Careful writing on a 2D superlattice of these core shell particles could lead to huge information storage in exceptionally small areas⁴⁷.

Surface Enhanced Raman Spectroscopy (SERS)

A very interesting application has arisen recently from noble metal nanoparticles. This arises from a phenomenon discovered by Martin Fleischmann In 1974. He noticed an unusually large Raman signal from a pyridine molecule absorbed on a roughened silver electrode⁴⁸. Attempts to study and enhance this behavior led scientists to find that this SERS effect only occurs near noble metal surfaces that have nanosized features. It is still not completely understood why, but the surface plasmon resonance effect displayed by the nanoparticles has an enormous enhancement on Raman signals. The increase is large enough to boost the sensitivity of the signal to 10^{16} times its normal operational limit. In fact single molecule detection has been observed for certain dye molecules absorbed on specially created silver surfaces⁴⁹. New sensors based on this type of SERS signals offer unbelievable increases in sensitivity for applications that including homeland security, as explosives, and toxins can be detected at miniscule levels.

SERS sensors have made Raman spectroscopy a true compliment for IR spectroscopy allowing analysis of smaller quantities of materials.

Metal nanoparticles as biosensors

In a more straightforward use of the sensitivity of the optical response of the nanoparticles in relation to their environment is the use of silver nanoparticles as optical biosensors. By coating silver nanotriangles with biotin, Van Duyne and coworkers were able to detect very small amounts of streptavidin which would complex with the biotin and cause the electronic contribution of the ligand to the nanoparticle to change⁵⁰. Again the small size of these materials and large response to small amounts of stimuli make metal nanoparticles ideal sensors. There have been many studies into the use of nanoparticles as biosensors. Gold is typically the material chosen as it is the most chemically stable product available, and it can be functionalized with relative ease by thiol containing molecules. El-Sayed's group tried to create cancer cell detectors by coating gold nanoparticles with a receptor for a protein called an epidermal growth factor receptor⁵⁰. Cancerous cells were found to display this protein much more extensively than normal cells. By observing the scattering intensity from a mixture of cells the cancerous cells are easily discernable by the high absorbance of the particles.

In a process first conducted by Chad Mirkin at Northwestern University, the color change of gold colloid between soluble particles and agglomerations is a very strong red to blue change. Gold nanoparticles coated with DNA base pairs will reversibly agglomerate when the complimentary DNA strand is entered into solution. This simple method has been shown to have amazing sensitivity and selectivity often recognizing the difference between DNA with a single

different base pair⁵¹. This has even been carried out to the extent where colorimetric biosensors have been made to detect the presence of lead in ultra low concentrations⁵².

Antimicrobial action of silver nanoparticles

Several research groups have looked into the potential for silver nanoparticles to be used as antimicrobial agents. Silver has long been known for its lethality to single celled organisms. It is hoped that along with increased surface area nanoparticles of silver can be found to have increased action to pathogens. NURCRYST has already produced a burn wound dressing that is impregnated with silver nanoparticles that have proven superior in its ability to keep a burn wound clean and decreases the time to heal the wound itself⁵³. Nanoparticulate silver may indeed find its way into a great many applications as the need for sterile surfaces arise. The Institute for New Materials in Germany has already added silver to its nonstick coating on hearing aids to cut down on the risk of infection.

Catalysts

The potential for nanomaterials as catalysts is possibly more tremendous than any physical property of the materials. The incredibly small size of the nanoparticles creates a high number of surface atoms, in fact up to 50% of the atoms may reside on the surface of the particle available for reaction. When coated with ligand molecules the surfaces that previously needed to be used as heterogeneous catalysts can now be incorporated into homogeneous systems that can

then be precipitated from solution. Use of metal nanoparticles as a catalyst has already been tested for aiding solution phase chemistry, as shown by Yeh's group who reported large advances for the Heck reaction with a Au-Ag-Pt trimetallic particle⁵⁴. Metal nanoparticles involved as catalysts for the cleavage of oxygen and hydrogen gas have been incorporated into electrodes for fuel cells, a scientific technology that may drastically change the way the world consumes energy.

Summary

Nanotechnology is predicted to be involved in the next great technological revolution. Some of the first applications of which are discussed above, but the potential of these materials is just being realized. The reproducible synthesis and control of the properties of metal nanoparticles is providing an initial supply of products that are being probed by scientists to their most fundamental constituents in an attempt to understand the process of formation and origins of their unique and enhanced properties. As shown above there have been many different approaches to the formation of metal nanoparticles each with their own advantages and disadvantages. As these methods are refined and understood the next quantum leap in technology becomes all the more real.

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Chapter 2: Synthesis of Silver Nanoparticles: A Study of How their Size, UV-Vis Absorption, and Self-Assembly Properties Change with Stabilizing Agent

Introduction to SMAD synthesis

The first step of the integration of nano-size products into modern technology is the synthesis of large quantities of these exceptionally small entities. The Solvated Metal Atom Dispersion (SMAD) synthesis technique is a batch process that allows for gram quantities of nanoparticles to be produced in a single experiment. The Klabunde group has utilized this technique for an assortment of materials including metal, semiconductor, and organometallic products, a testament to the versatility of this technique^{1,2,3,4,5}. This chapter will detail the application of this technique to silver metal and the adaptation of experimental technique specifically for silver in order to produce high quality organic ligand-coated silver nanoparticles.

In order for meaningful data to be obtained on a nanosized product either the methods used must be capable to measure the properties of a single particle, or the product must be homogeneous. Single particle measurements are exceedingly difficult, and require an excessive amount of ingenuity and technology to make possible. These observations are typically made in tandem with an electron microscope of some kind to ensure data is collected on a single specimen. Much more common is the characterization of a large quantity of nanoparticles in solution that all have identical properties and can be measured by more conventional means akin

to molecular compounds. For this to be possible the particles must be very uniform with respect to one another since at the nano-size regime the properties of a particle vary considerably with small changes in size (a direct reflection of the number of atoms composing the particle), shape, and local environment of the particle.

There is, however a great difficulty in creating large amounts of sample that is highly uniform due to the reactive nature of the particles themselves. Most compounds, once they are separated into nano-sized dimensions, their reactivity is so high that they have a strong tendency to aggregate and fuse together attempting to reduce their surface area and surface potential. This aggregation must be prevented if the particles are to remain small and uniform. This is especially true for metallic nanoparticles. In the bulk state metals prefer to have binding between a central atom and its 12 neighboring atoms. As the size of the particles is reduced the number of surface atoms increases markedly at the nano length scale resulting in a large number of atoms that will have significantly fewer neighboring atoms to bond with. This will cause dangling bonds of higher energy to be present on the surface of the particle.

One method of stabilizing the particles is to coat them with an organic molecule that buffers the particles from one another and prevents metal-metal contact. This dates as far back as 1951 with Turkevich using citrate ions to coat gold nanoparticles⁶. Different synthetic procedures have been found to have varying degrees of success at obtaining narrow size distributions, many of which are described in detail in chapter 1.

Once the particles are stable in solution then they must be refined through some procedure to create a monodisperse sample. Particles can be subjected to size selective precipitation, filtered, or refined some other manner⁷. The use of ligands does have the drawback of blocking the surface of the particle available for interaction with other species. They may also

change the chemical nature of the particle themselves and interfere with catalysis on the surface of the particle. The large upside of protecting nanoparticles with organic ligands, is that the properties of the particle can be drastically different when the nature of the ligand is changed. This chapter describes the synthesis of four silver nanoparticle colloids utilizing different protecting ligands created via the SMAD procedure coupled with digestive ripening. Studies were then conducted to determine the influence of the ligand on the particle size, size dispersity, light absorption, and superlattice ordering of each colloid.

Detailed SMAD Process

Components:

The preparation for this reaction is non-trivial especially when first assembling the reaction apparatus. The driving theory behind the synthesis is the evaporation of a metal to individual atoms/clusters, separation of the atoms from one another by solvent molecules, and halting their aggregation as soon as possible. All glassware is treated for 24 hours in a base bath of saturated KOH in isopropanol. After washing, the glassware is further treated in an acid bath containing a ratio of 3:1 distilled water to sulfuric acid for an additional 24 hours, rinsed, and dried overnight in a heating cabinet. The metal is evaporated from an aluminum oxide coated tungsten crucible.

The crucibles are purchased as tungsten wire baskets from Johnson and Matthey of a size large enough to hold the reactant silver (typically ½” diameter). These wire baskets were painted with an aluminum oxide cement purchased from Zircar ceramics until a thick insulating

basket is formed. This alumina coating helps focus the heat into the center of the crucible to encourage evaporation of the metal. When applying the alumina cement the crucibles are dried after every coating at 60 °C and at 200 °C every 10th coating to prevent cracking when used in the reaction chamber. A total of 30 coatings are typically used, occasionally more if a higher boiling metal is to be used in the reactor. Copper wire is then wound around the contact points of the tungsten wire where they will be secured to the reactor electrodes for a better electrical contact. Additional insulation, alumina mat from Zircar, is wrapped around the basket of the crucible to again minimize the loss of heat to the reactor. This is typically fixed to the crucible with chromium wire. The preparation stages of the crucible are shown in below in figure 3. The crucible is then placed into the reaction vessel and degassed to remove any trapped volatile components in the cement. This is accomplished by heating to ~1400 °C under vacuum. The desired temperature for degassing of the crucible is 15-20% hotter than that required for evaporation of the reactant metal to ensure a high vacuum during the reaction process, and to minimize contaminants.



Figure 3 - Preparation of crucibles for metal evaporation. A wire basket is purchased for use, coated with aluminum oxide cement, contact points are wrapped in copper wire, and finally insulation with alumina mat is secured around the outside of the crucible.

The reactor itself is composed of a 3000 mL elongated glass vessel with $\frac{1}{2}$ " thick walls. The head of the reactor has 5 outlets, four of which are ground glass 24/40 joints, while the fifth outlet is an O-ring joint. One outlet is connected to a high vacuum system that can reduce the pressure of the vessel to a minimum of 1×10^{-3} Torr. At pressures higher than this the temperature required to evaporate silver is so high, that the organic solvent used in the synthesis decomposes on the hot crucible and catastrophically raises the pressure of the reaction vessel halting evaporation of the metal.

Through two opposite outlets water cooled copper electrodes are introduced into the reaction chamber and sealed with Teflon O-rings. The temperature of the crucible is controlled by adjusting the current flow through the electrodes. This is accomplished through a potentiometer, and a power transformer that converts voltage into current. The center outlet is

fitted with a long glass tube sealed on the reactor end and placed directly over the crucible. This end has approximately thirty 1 mm holes in it designed to disperse the solvent evenly throughout the reactor. The other end is fitted with a ground glass joint that attaches well to a Schlenk tube containing the desired solvent for reaction. The solvent shower is typically placed 3.5 - 4.0 cm above the crucible for optimal evaporation. Lowering the solvent shower with respect to the crucible will cause it to be coated with excess metal atoms, while raising it higher than 4 cm leads to poor coverage of the solvent on the bottom of the reactor.

The fifth outlet, which uses an O-ring seal, is used to introduce a heating element for the solvent shower. It is composed of two copper wires that can supply a current to a chrome wire which is then led from one copper lead, around the solvent shower, to the second copper lead. Heating the solvent shower is necessary to prevent the solvent gas from condensing into a liquid in the shower head. If this happens a drop of the solvent will form at the tip of the shower, drip into the crucible, and violently disintegrate, causing the liquid metal to jump out of the crucible terminating the reaction.

Reaction preparation:

Silver shot, 0.3 g (1 eq.) is then placed into the crucible while the capping agent (20 eq.), and 60 ml of 4-tert-butyl toluene is added to the bottom of the reactor flask with a stir bar. The higher boiling point of 4-tert-butyltoluene proved to produce nanoparticles with a tighter size distribution than the chemically similar, but lower boiling toluene used in previous studies². Both organic liquids were previously dried over molecular sieves and bubbled with argon for a minimum of 1hr prior to use. This system was sealed to vacuum and the liquids in the reactor were degassed by the freeze-thaw method twice. The reactor was surrounded by liquid nitrogen,

and evacuated for 2 hours typically reaching pressures of 1×10^{-3} Torr. A schematic of the reaction chamber is shown in figure 4.

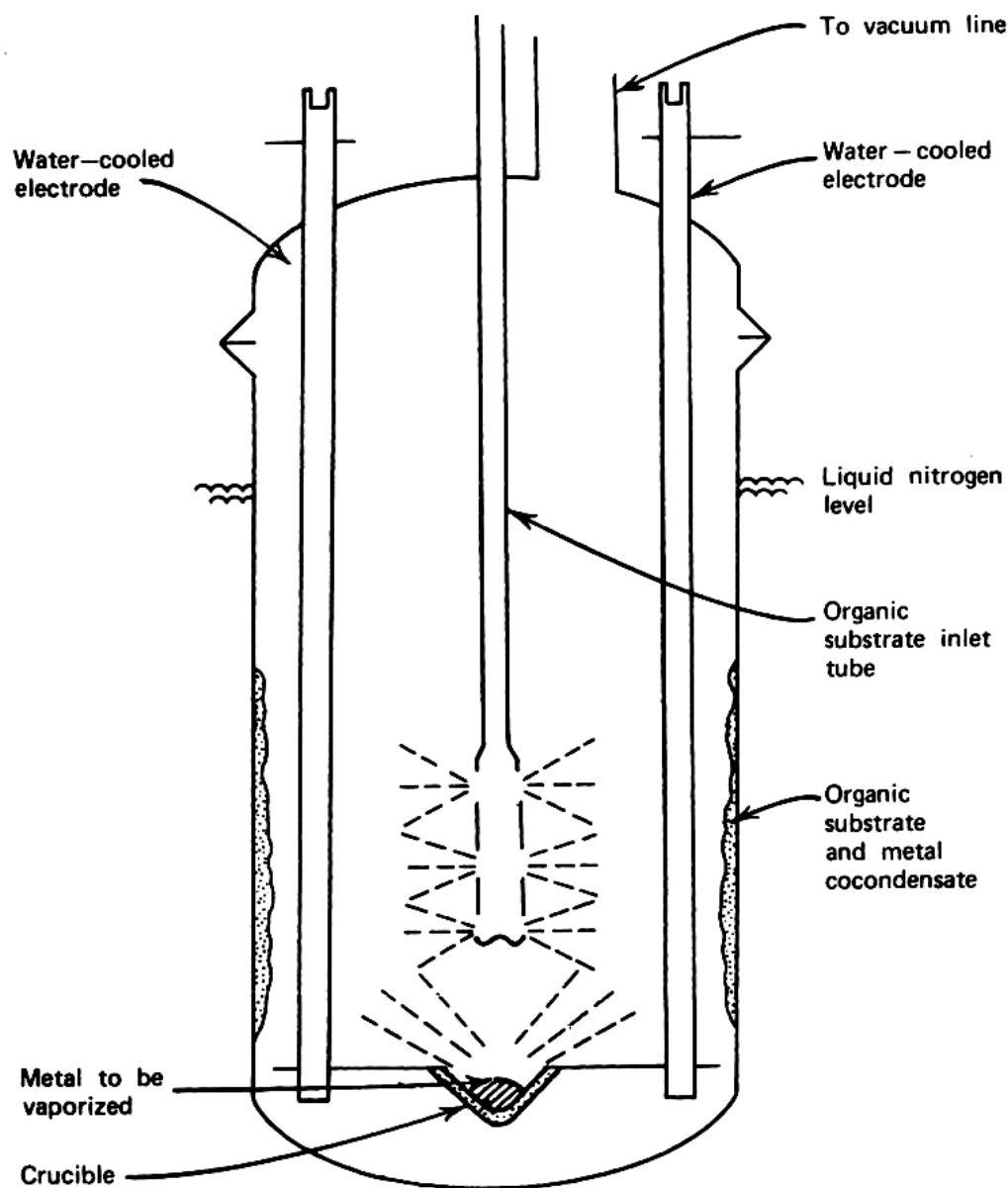


Figure 4 - Schematic assembly of SMAD reactor.

The solvent used for the SMAD synthesis of silver in this chapter is 2-butanone. This solvent provided the most stability for the metal atoms in comparison to pentane, toluene, THF, and acetone. Each solvent was tested in a reaction without stabilizing ligands and the size of the particles was used to gauge the ability of the solvent to prevent aggregation (see chapter 4). A new and unopened bottle of solvent was dried over freshly activated type 4A molecular sieves for 12 hours. It was then placed into a schlenk tube and degassed via the freeze-thaw method⁸ a minimum of 5 times, or until no further gas appears to be leaving the solvent. The heating element for the solvent shower was connected to a potentiometer and applied a 10% voltage for the duration of the experiment.

Reaction Procedure

After the above apparatus is has been assembled it is placed under vacuum for a minimum of 2 hours 40 ml of 2-butanone was slowly (roughly 1 hr) deposited on the walls of the reactor flask forming a bed of frozen solvent. If this evaporation is conducted too quickly a poor matrix will form closer to the consistency of ice and does not allow for an even layering of further solvent molecules. During this time the crucible was slowly heated to the evaporation temperature of Ag (~1200 °C at reaction conditions). This is typically slightly higher than the melting point of the metal itself. The flow of 2-butanone was slightly increased and the crucible temperature was maintained throughout the reaction.

Once the silver begins to evaporate a yellow color appears in the frozen matrix which slowly turns to brown as the concentration of the metal increases in the solvent bed. If at any point the concentration of metal atoms appears too high, the temperature of the crucible is

lowered as a thick layer of solvent is applied to the reactor walls. Conversely, if evaporation during the reaction does not occur due to a poor vacuum, the deposition of metal and solvent can be alternated, however this is not ideal.

Once evaporation has ceased, ideally when no further metal remains in the crucible, an additional 40 ml of 2-butanone was evaporated on top of the metal-solvent matrix. The liquid nitrogen was removed at this stage and the vessel was allowed to warm under a static vacuum. At this stage in the reaction the metal is in the form of highly reactive zero valent atoms or clusters dispersed in the solvent matrix. Agglomeration is only slowed by the presence of the polar solvent which loosely binds the atoms at the low temperatures used. Due to this it is imperative that the reactive metal come into contact with the capping ligand as quickly as possible. To facilitate this, the frozen ligand in solvent at the bottom of the reactor can be warmed without heating the solvated metal by careful use of a heating gun (or a vigilantly watched water bath). When the melting ligand first bumps in the chamber a small amount of argon is applied to the reaction vessel to prevent loss of product on crucible insulation and heating is stopped. This prevents further bumping of the product. Stirring of the product was begun as soon as the stir bar was free of the frozen mixture on the bottom of the flask.

At this point the product became a black solution that matures to a brown color as it warms to room temperature and interacts with the ligand in solution. Argon was introduced into the vessel to above atmospheric pressure and the mixture was siphoned out into a Schlenk flask for storage. The storage flask was repeatedly placed under vacuum and flushed with argon before transfer. Additionally argon was flowing at greater than atmospheric pressure out of the reaction flask and out of the siphon tubing when attaching the transfer attachment to ensure air would not come into contact with the product. Exposure of air to the chamber can be limited to

1-3 seconds with a positive flow of argon essentially preventing all but the smallest traces of oxygen from entering the reaction chamber.

The flask containing the product is then flushed 4 times alternating argon gas and vacuum to remove any traces of oxygen that might have entered during transfer. Methyl ethyl ketone (typically ~120 ml) was slowly evaporated under vacuum from the as prepared product mixture overnight, leaving a dispersion containing only t-butyl toluene, silver, and capping agent. As shown below in figure 5 the particles are relatively small but are non-uniform in shape and size. In a normal lab scale experiment 0.5 g of silver can easily be converted to nanoparticles in this manner. The process is amenable to scale up to by a factor of 100 or even 1000 with access to larger facilities. The as prepared product at this stage is not of sufficient quality to study with any accuracy due to the large difference in properties such a wide range of particles would have.

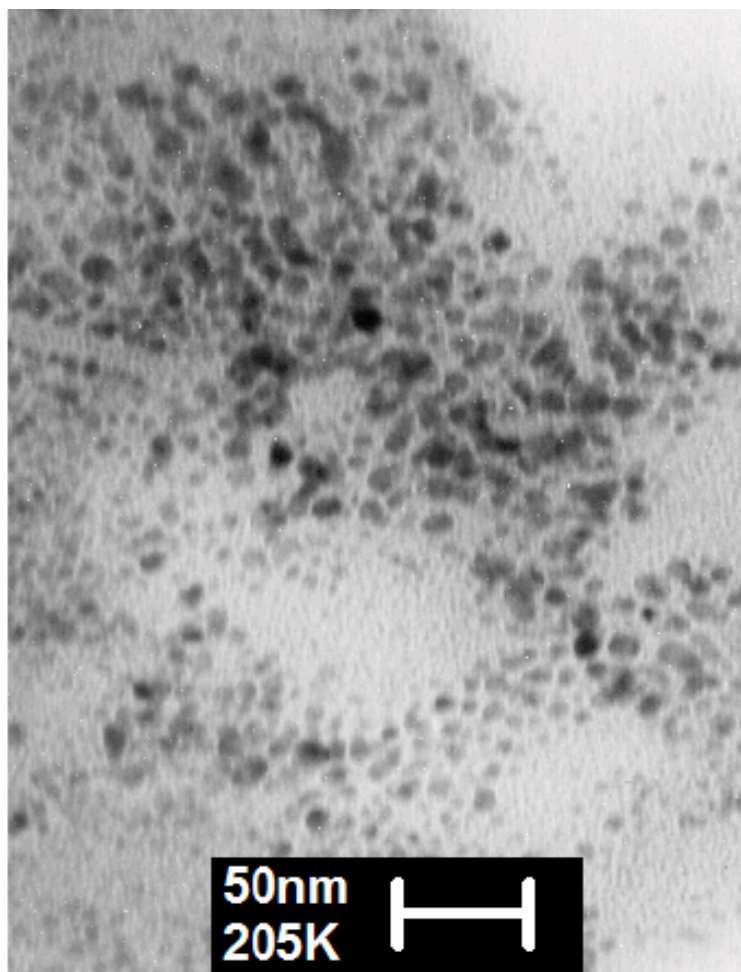


Figure 5 TEM photograph of as prepared SMAD colloid. Silver particles are protected with dodecane thiol and are a small size, however the particles do not have a very uniform size distribution.

Digestive Ripening

Digestive Ripening is a term used to describe the refinement process of nanoparticles in solution through heating, first described by Lin and coworkers⁹. During this technique the atoms and ligands are in flux with the environment and the particles are able to exchange atoms until an equilibrium size is obtained. After heating the colloids for a few hours to a few days the

polydisperse as prepared SMAD colloid becomes a uniform solution of spherical particles seen in figure 6.

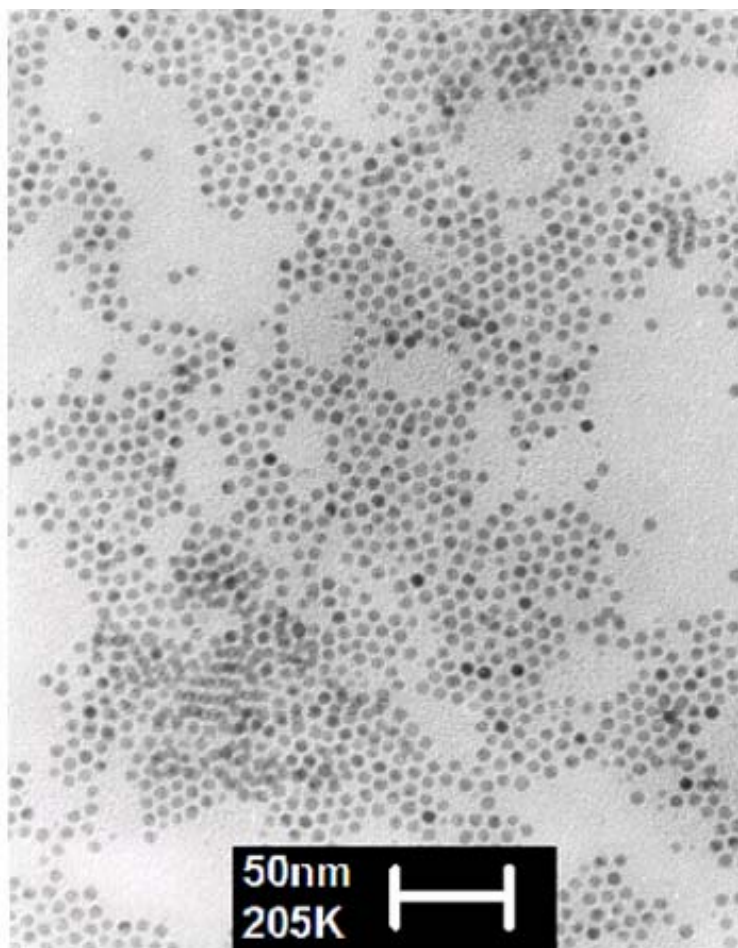


Figure 6 Silver nanoparticles made by the SMAD process then refined by digestive ripening. The particles display a very narrow size distribution and are quite homogeneous.

The procedure for digestive ripening is simple. A container that will exclude air during heating is integral to the success of the reaction. To accomplish this, an adapter with a rubber septum is fitted on the Schlenk tube, a process that again is conducted under positive argon pressure and can be fitted in 1-2 seconds. To this an inlet for argon is introduced through a

needle and the gas outlet is passed through a bubbler to prevent backflow of air. A constant flow of argon over the reaction seems beneficial to the quality of the final product.

The product from the SMAD preparation was heated in the original Schlenk flask under a flow of argon to reflux at 190 °C. The nanoparticle dispersion was refluxed for times varying from 90 minutes to 2 days under an inert atmosphere. The resulting colloid is a dark solution which is a vibrant yellow color when diluted. As can be observed in figure 4 the particles have reached an equilibrium size. In separate experiments the digestive ripening procedure has been carried out in the absence of light, to prevent any photo effects with the silver metal but no noticeable difference was observed in the product. The entire synthetic process is illustrated in figure 7.

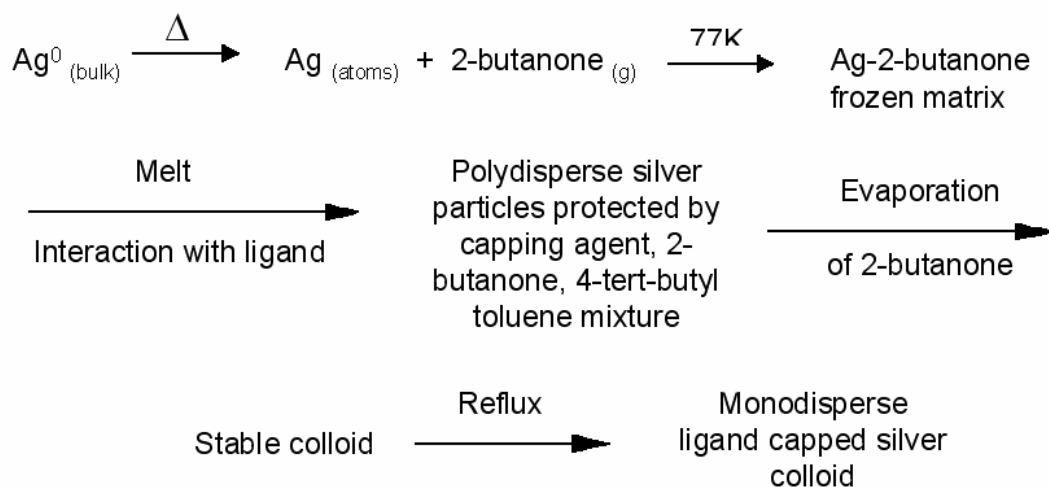


Figure 7 - Reaction pathway of SMAD synthesis and digestive ripening process.

Optical Properties of Metal Nanoparticles

The optical response of noble metal nanoparticles is a very unique phenomenon. It is confined to a select number of elements that display free electron behavior in particles under 100 nm in size. Despite these restrictions this property has been utilized for hundreds of years as the coloration of decorative glasses. Scientific study of this phenomenon was first accomplished by Michael Faraday at the turn of the 19th century as he attributed this optical response to small gold clusters in solution¹⁰. Mie was the first to investigate the origin of these colors mathematically as he solved Maxwell's equations of light for small spherical particles of metal¹¹. These calculations are widely used today to predict the absorbance features of various metal particles, and mimics experimental observations very closely.

The absorption of light in this instance is typically termed a surface plasmon resonance (SPR). The phenomenon is described as the response of the electron gas (free electrons in a metal particle) to the presence of an electromagnetic wave in the vicinity of the particles¹¹⁻¹⁴. This wave of light causes an oscillation of the free electrons. The frequency of oscillation will be determined by the density of electrons, the effective electron mass, and the shape and size of the charge distribution. This translates into a very product specific property. The absorption will be dependent on the specific characteristics and the local environment of the particle.

As the size of the particle is increased a greater number of electrons are involved, and will have lesser displacement from the nuclear framework. If the shape of the particle is altered from spherical there will be separate responses of the electrons for different directions of electron

oscillation. When the composition of a metal particle is altered, once again the free electrons will have a different affinity for the particle and will also cause a shift the position of λ -max. Likewise for particles with heterogeneous compositions (more than one metal domain), a complex spectra will be observed¹².

The optical response of the particles is also very sensitive to its surroundings. The absorbance is highly dependent on the refractive index of the solution, which is amplified for molecules very near, or bonded to the surface of the metal particle. Exciting new sensor devices have been developed using this property allowing detection of numerous entities including protein recognition. Binding of molecules to the nanoparticles will have an added effect based on the electron donation/withdrawing properties of the bonded functional group affecting the conduction electrons¹⁴.

Experimentally the SPR is found to be dependent on the size, shape, and environment of the particles. It can therefore be used as a gauge of the size dispersity of the particles in solution. A strong narrow absorption is representative of very similar particles, while a broad, weaker band will be the result of the contribution of many particles over a large size distribution. The SPR may also be used as an indicator of the size of the particles, as long as the composition of the nanoparticle is held constant. A blue shift of the peak maximum generally denotes a smaller particle size.

Figure 8 displays a UV-Vis spectrum showing a comparison of the SMAD as prepared sample and a digestively ripened silver colloid with dodecanethiol as the capping agent. The UV-Vis peak is considerably narrower after digestion, a reflection of the greatly reduced size distribution that occurs during this process (figure 5). Also the peak maximum is shifted from

445 nm to 420 nm. This is due to the fact that a much smaller particle is dominant in solution which absorbs light at a higher energy.

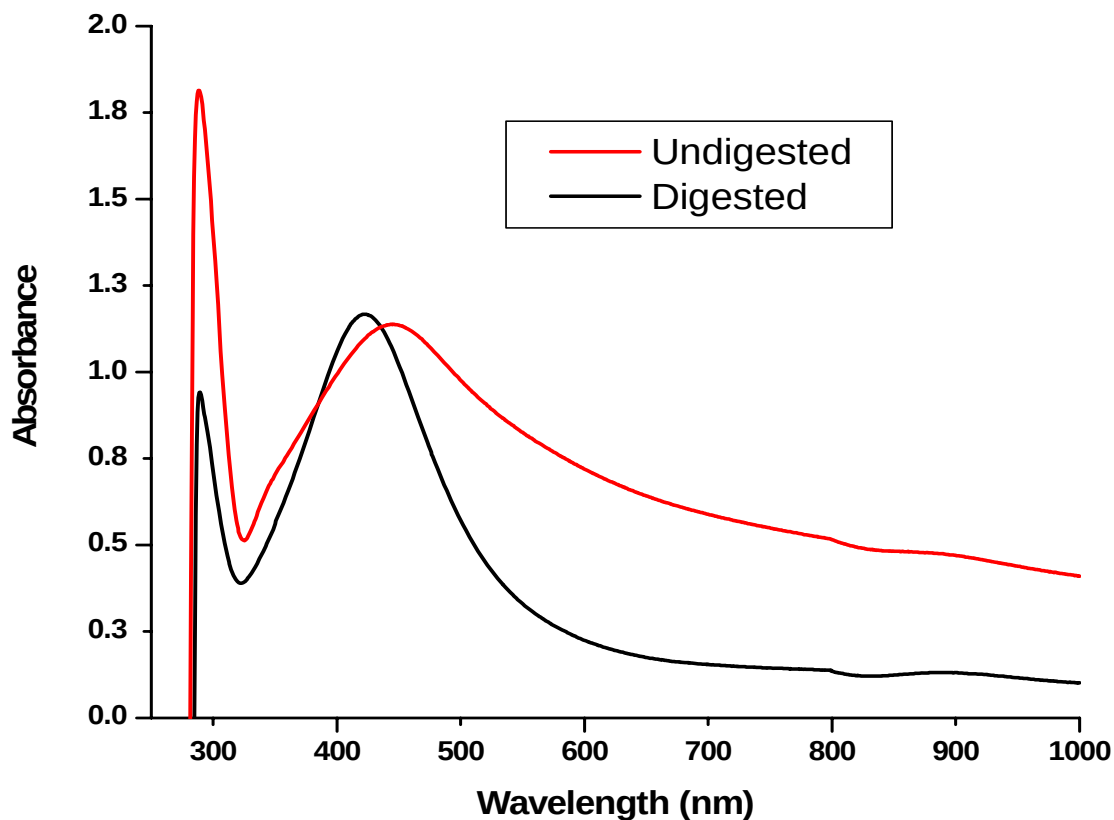


Figure 8 - UV-Vis spectrum of silver nanoparticles capped with dodecane thiol before digestive ripening (red) and after (black).

Superlattice Formation

The spontaneous ordering of nano-materials is becoming a huge area of research recently. The synthesis of nanoparticles that display self-assembly is absolutely essential for the easy fabrication of practical devices incorporating small particles. Constructing these arrays by

individual particle placement would be impossible considering the size needed for advanced materials. This is especially true for spherical nanoparticles as the formation of superlattices is an important precursor to the application of nanoparticles as novel optical and electrical materials¹⁵.

It is fascinating that the nanoparticles construct the exact same packing features as their composite atoms. Nanoparticle superlattice ordering is merely an order of magnitude larger in spatial dimensions. The ordering of both the atoms in the particles and the nanoparticles themselves is cubic close packed. This packing style allows for a maximum amount of nearest neighbors in a hard sphere model, with components of a similar size. Superlattice formation is somewhat curious in this respect because the particles are by no means perfect spheres. The particles typically have many flat faces to them, and are surrounded by ligands that protrude at an angle from these surfaces.

The ligands facilitate the superlattice formation of the particles by not only molding a roughly spherical shape, but also the long aliphatic chains interdigitate between one another on neighboring particles and direct the ordering of the superlattice. The particles seem to encourage growth along a preferential axis as the crystal evolves as will be seen in the results below.

The growth of superlattices appears to be dependent on the elemental composition of the particle and the shape of the protecting ligand. This detail will be discussed further in the next section. Few research groups have managed to grow large 3-D superlattices, work is currently being conducted in many laboratories to encourage the growth of these structures. A tight size distribution is essential, and careful control must be taken over drying conditions.

Property Changes with Ligand Head Group

Four colloids were produced to determine the influence of the ligand molecule on the silver nanoparticle properties. The experimental details for each colloid are very similar. The purification of the ligands before introduction into the SMAD reactor is slightly different, but once dry the capping agent is placed on the bottom of the reaction flask, and dissolved in 4-tert-butyltoluene. Analysis of the particles before and after digestive ripening was conducted by TEM, and UV-Vis spectroscopy to probe the size, and morphology of the particles, and their response to incident light.

The ligands used in this study consisted of dodecane thiol, trioctyl phosphine, dodecyl amine, and dodecyl alcohol. The ligands were made as similar to one another as possible in the aliphatic chain so as to focus on the effect of changing the polar head group on the final product. Each chemical functionality is expected to have a different affinity for the particles predicted by the bond energies between silver and the ligating atom listed below. Also the amount of electron donation to the particle by the bonded atom will make an impact in the position of the absorbance peak in the UV-Vis spectrum.

List of bond energies for silver and gold diatoms¹⁶

Ag-Ag	159.2 KJ/mol
Ag-O	221 KJ/mol
Ag-S	217 KJ/mol
Ag-Br	293 KJ/mol
Au-S	418 KJ/mol
Au-O	223 KJ/mol

Dodecane Thiol protected particles

Dodecane thiol is truly the industry standard for protecting silver and gold nanoparticles. Nearly every research group has used it for protecting their particles due to its suitability as a stabilizing ligand, some of which is evidenced in chapter 1. The sulfur head group is a soft electron donor that binds extremely well to the soft silver atoms surrounding the nanoparticle. Unfortunately this bond is not nearly as strong for silver as it is for gold and the sample must be kept free from exposure to oxygen for successful digestive ripening. This is evidenced by the nearly identical binding energies for Ag-S and Ag-O bonds.

The twelve unit carbon chain of dodecane thiol provides a sufficient insulating buffer such that when a particle is saturated with this molecule, collisions between particles in solution do not lead to aggregation. The length of this chain can be adjusted easily, and will alter the properties of the colloid. As an example, specific spatial distances between particles in a superlattice can be engineered through careful selection of the chain length. Control over this parameter allows the user to tune the optical or electrical properties of the structure, essential for preparing superlattices for sensor purposes. The chain length also has a significant impact on the solubility of the particles. Shorter chain thiols reduce the solubility of the particles in solution. This encourages precipitation of the particles and superlattice growth even at room temperature. Conversely longer apolar chains greatly increases the solubility of these particles in organic solvents. A fine example of this is discussed by Prasad et al. in the use of hexadecane thiol to retard superlattice growth of gold nanoparticles¹⁷.

Colloids produced with dodecane thiol through the SMAD method and digestive ripening formed spherical particles with a mean diameter of 6.6 +/- 1nm. The product is homogeneous and is of very high quality. The particles display a slightly broad UV-Vis absorption at 420 nm

that is very consistent with the literature data¹⁸. The broadness of the peak suggests some preliminary formation of superlattices at room temperature. The peak does narrow noticeably upon heating the sample while in the spectrometer as the superlattices dissolve back into solution.

The most remarkable feature of these particles is their ability to self-assemble into large three dimensionally ordered superlattices. This can be achieved through careful evaporation of the solvent. The concentration of the particles in solution to used to create the superlattices is also very important. The images below were produced by evaporating a 3 μ L drop of colloid in 4-tertbutyltoluene for 4 days in a dry box of inert atmosphere. The concentration of the sample is approximately 0.025 M. These particles have the strange tendency to order into three dimensional triangular structures when they precipitate out of solution as displayed in figure 9. This geometry is repeated throughout the TEM grid seen in the low magnification images. This feature must arise from preferential growth directed by the underlying shape of the particles brought about by the dodecane thiol ligands. Similar triangular superlattices have been found to form in only two other groups, that of Pelini¹⁹ and of Wang²⁰ who also use dodecane thiol as a protecting agent and must constitute very similar particle surfaces. Remarkably Wang's particles are 2 nm larger in diameter, due to different synthetic preparations, yet still have the same crystallization pattern.

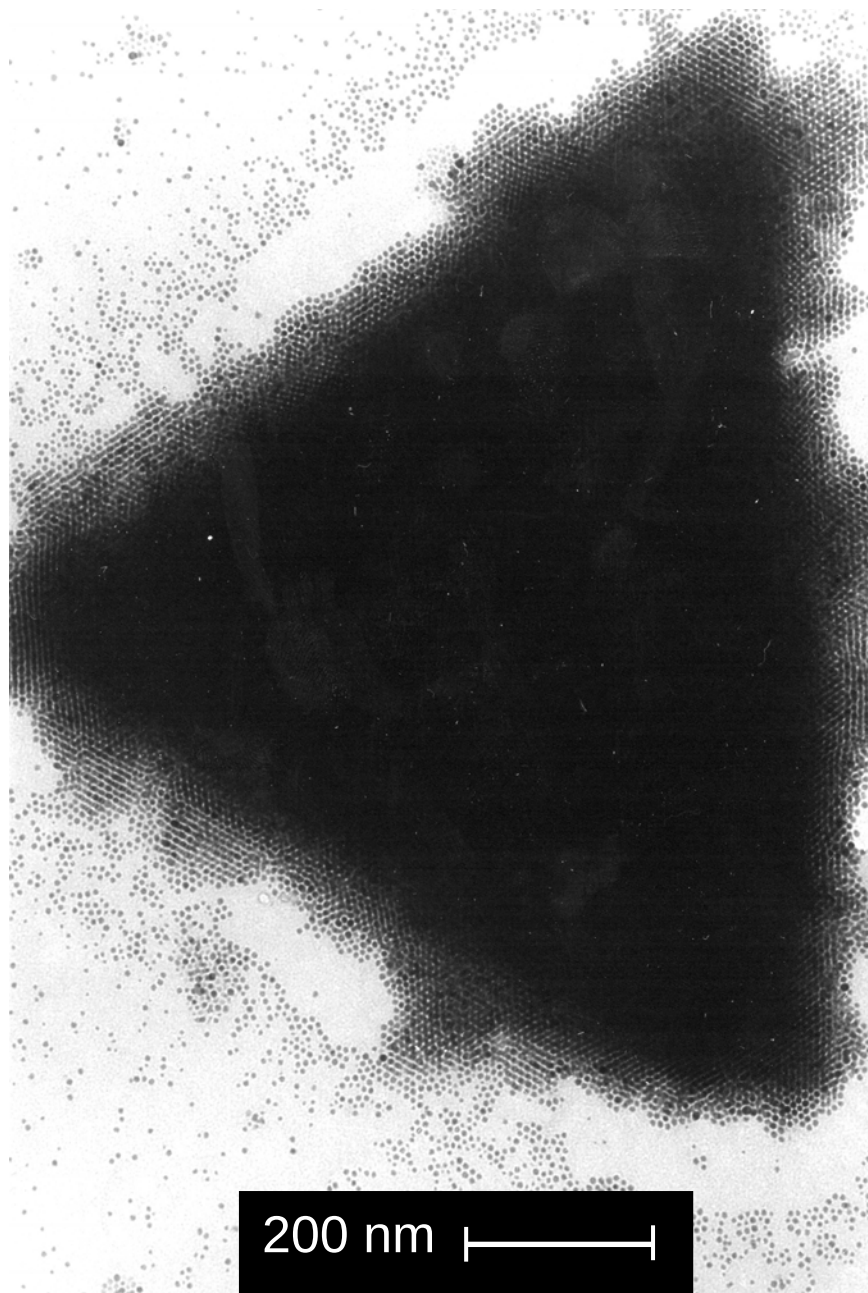


Figure 9 - Triangular superlattice of dodecane thiol protected silver nanoparticles.

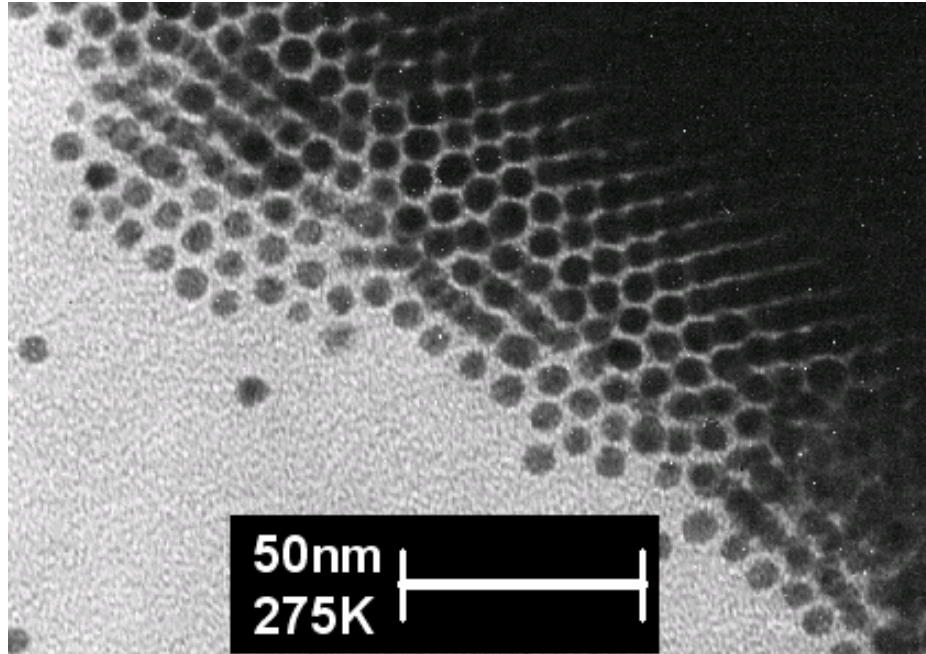


Figure 10 - High magnification image of superlattice in figure 9. The image highlights the precision ordering of the self-assembled structure.

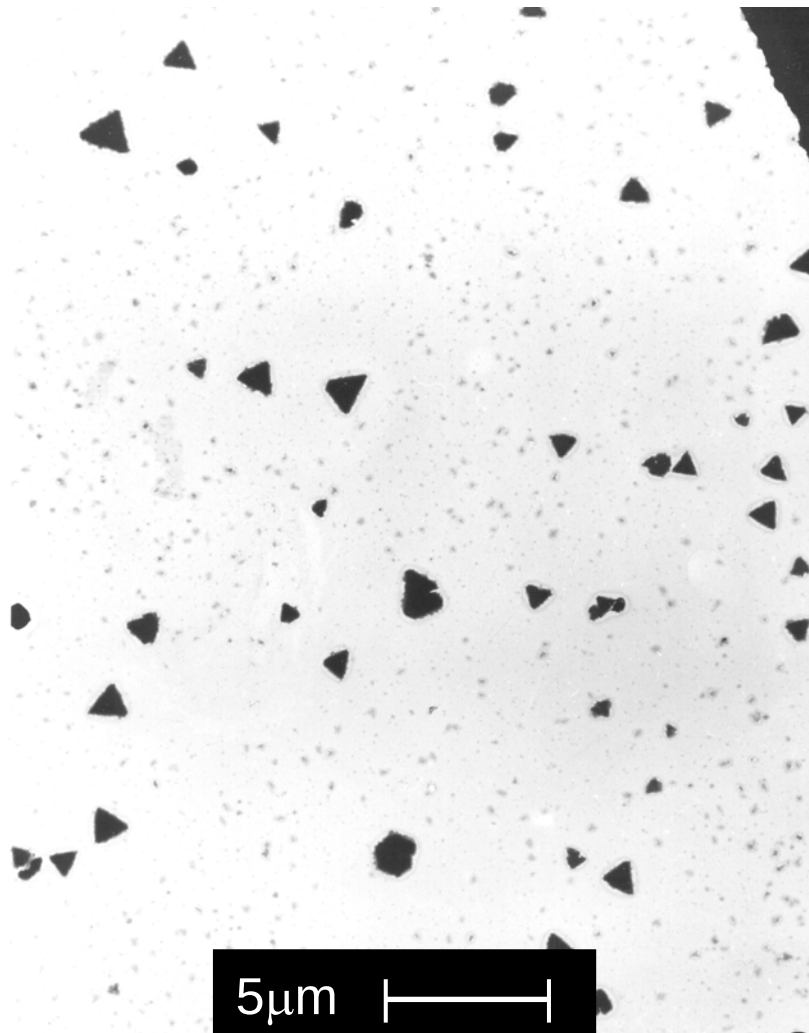


Figure 11 - Low magnification image of TEM grid with several triangular superlattices of dodecanethiol protected silver nanoparticles.

Two dimensional superlattices as shown in figure 12 and 13 are much more common in the literature. Figure 12 shows a hexagonal arrangement of nanoparticles formed over an extremely long length scale. As the shades of the picture suggest there are three layers of particles stacked on top of one another in this sample.

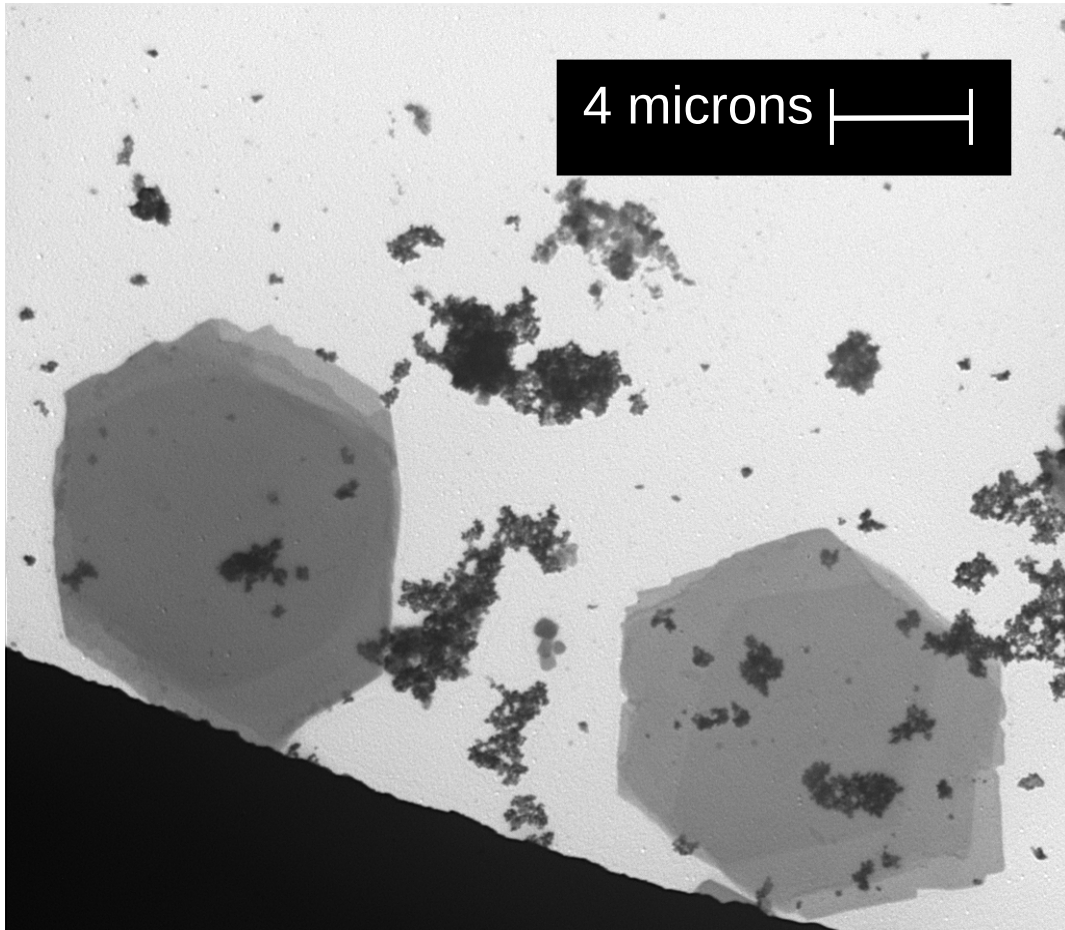


Figure 12 - The hexagonal 2-D superlattices of dodecane thiol coated silver particles.

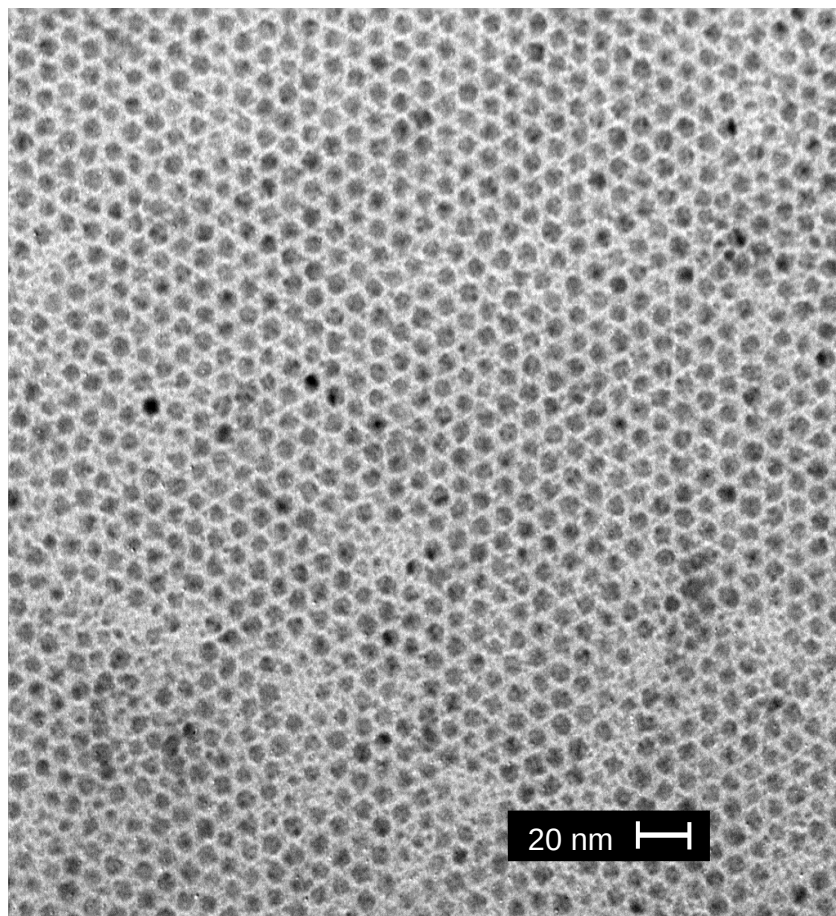


Figure 13 - Two dimensional superlattice of dodecane thiol particles in a very well-ordered packing arrangement.

Trioctyl Phosphine

Nanoparticles coated with trioctyl phosphine (TOP) were found to form very nice spherical particles with a diameter of 6.0 ± 2 nm. The particles display a very sharp plasmon absorption peak at 414 nm seen below in figure 14. The difference in peak width between trioctyl phosphine and dodecane thiol is most likely due to the greater solubility of the trioctyl phosphine particles. As particles aggregate in solution the SPR peak tends to broaden. This occurs much more readily for dodecane thiol particles. The presence of three aliphatic chains

opposed to the single chain of dodecane thiol most likely increases the particles solubility despite the shorter chain length. Upon digestive ripening the size distribution of the TOP ligated particles considerably narrows and the UV-Vis spectrum is dominated by a single peak representing very similar particles.

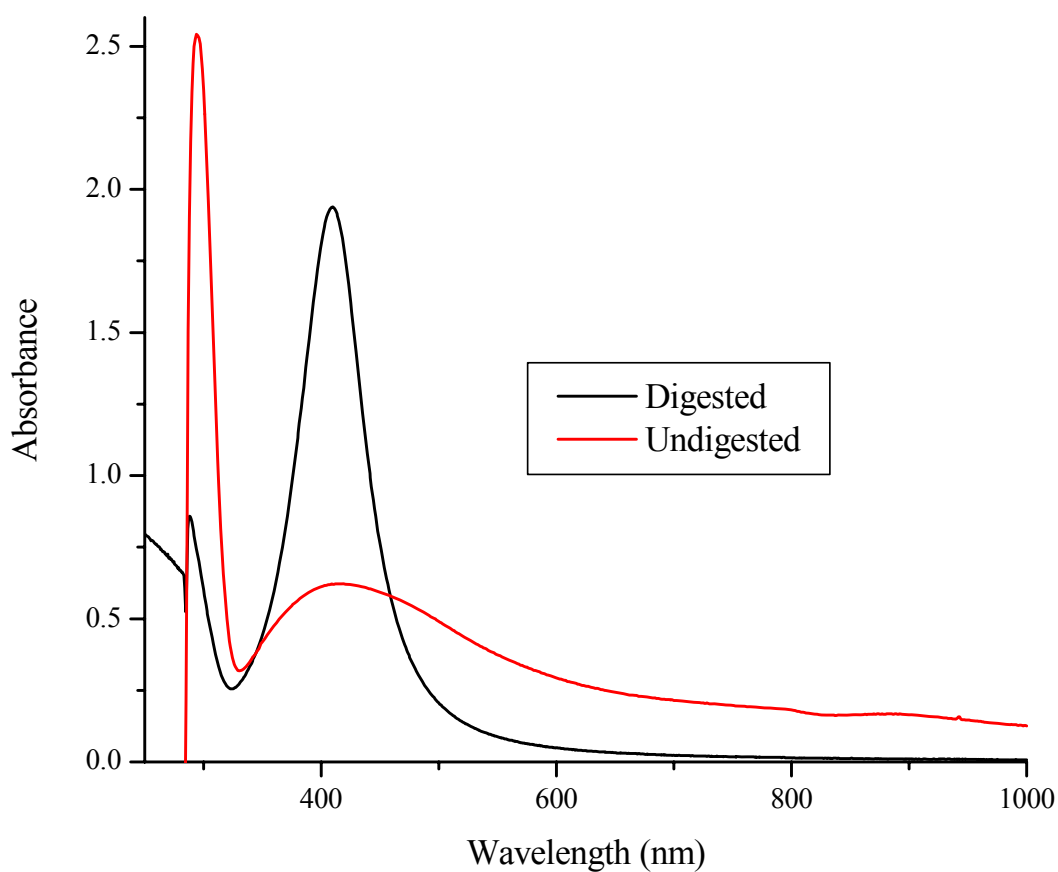


Figure 14 - Surface plasmon absorption resonance peak for trioctyl phosphine coated silver nanoparticles. The red line represents an as prepared SMAD colloid while the black line represents particles that have been through the digestive ripening process.

The crystallization characteristics of trioctyl phosphine particles are very unique. Instead of forming well ordered two dimensional superlattices or triangular 3-D superlattices like dodecane thiol they form circular structures on the TEM grid as seen in figures 15 and 16. This is almost definitely the result of the different shape of the ligand used compared to the single straight chain dodecane thiol. When dodecane thiol binds to the surface of the particles a regular ordered shape can easily be imagined that can facilitate interdigitation. Conversely trioctyl phosphine has three ligands that must face out into solution at an angle much smaller than 90° . Also the presence of three hydrocarbon chains must inhibit an ordered packing as seen in the case of dodecane thiol. Surprisingly these particles still crystallize into a geometrical shape that is consistent throughout the sample. This type of ordering is unique our knowledge.

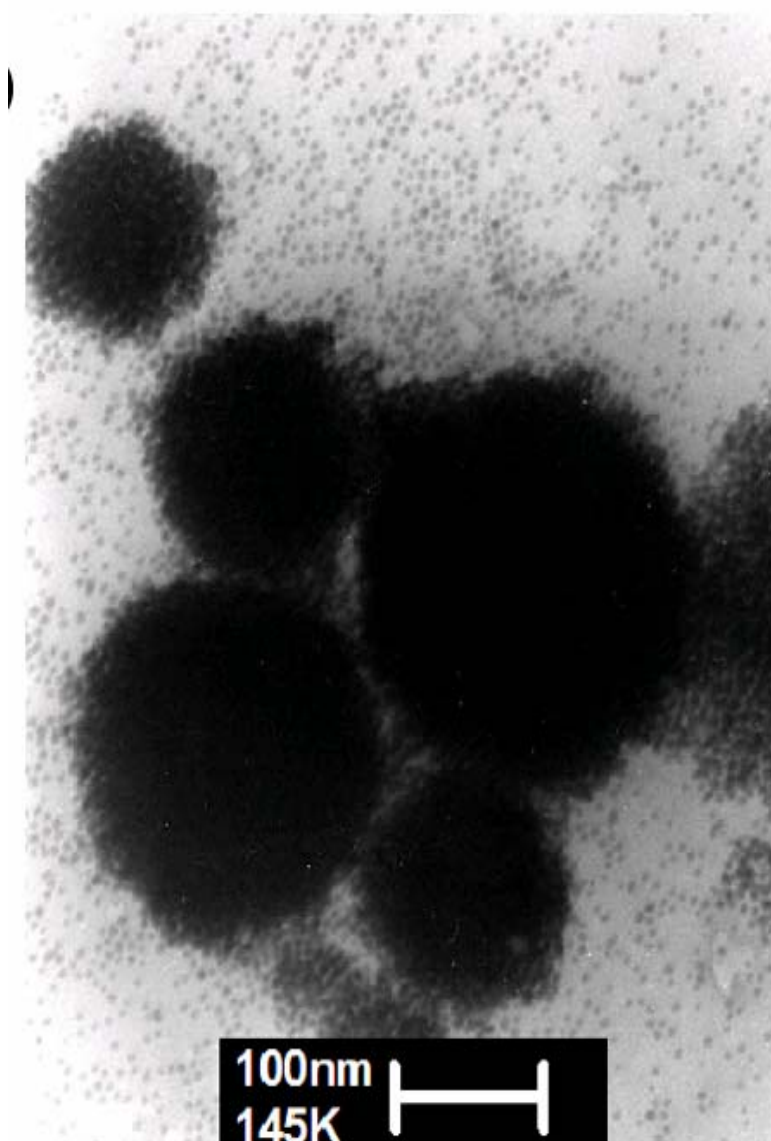


Figure 15 - Circular 3-D superlattices of trioctyl phosphine protected particles. The difference in superlattice morphology is thought to be due to the different number of alkane chains on the ligand. In the case of dodecane thiol only one chain is present and may allow for easier, or more ordered packing.

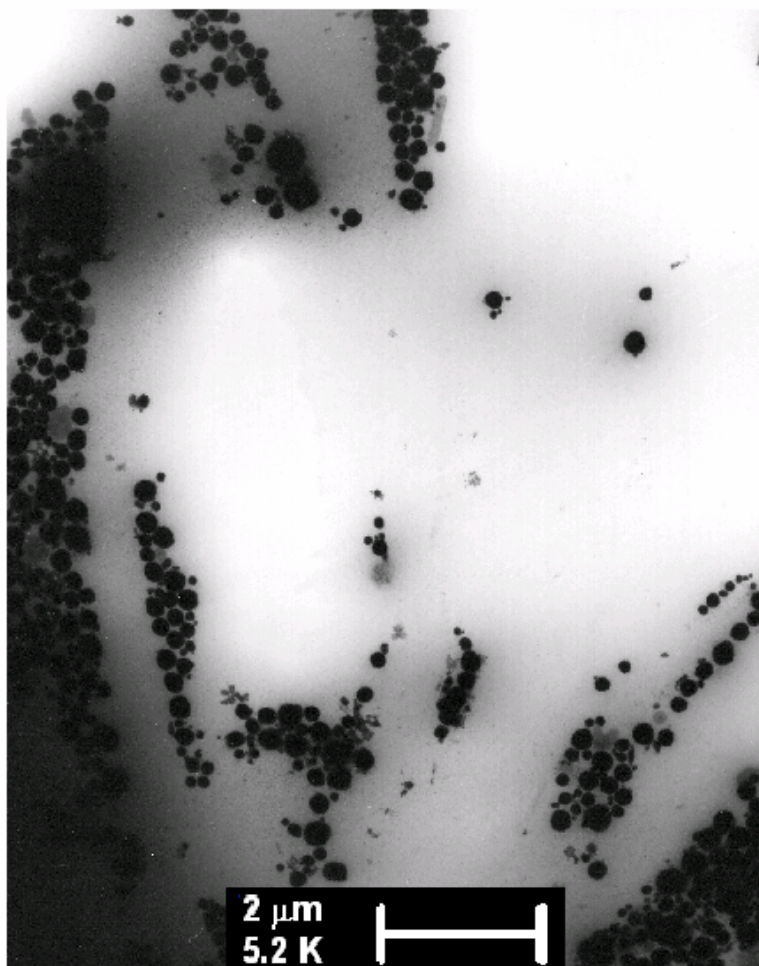


Figure 16 - Low magnification image of TOP ligated silver particle superlattices.

Dodecyl Amine

Dodecyl amine did not prove to be able to stabilize all the particles in solution. A population of the particles was of a very similar size and morphology of 14 nm spherical particles. The particle size for gold nanoparticles was also seen to increase when dodecyl amine ligands were used to protect the particles. This result is unfortunate, however nitrogen is a harder base than sulfur and would not be expected to bind as well to the silver surface which may explain the difficulty in creating a homogeneous sample.

Some two dimensional ordering was found for these particles but mainly the system was rather polydisperse. The UV-Vis spectrum shows a strong peak at 412 nm that most likely represents the 14 nm particles that comprise a majority of the product. Despite the larger particle size the optical absorption peak is blue shifted with respect to the dodecane thiol particles. This is most likely due to different ability of the amine to donate electrons to the silver particle compared to the sulfur as it is more electronegative and will pull some of the electron density away from the particle and cause it to be more difficult to displace the electron gas.

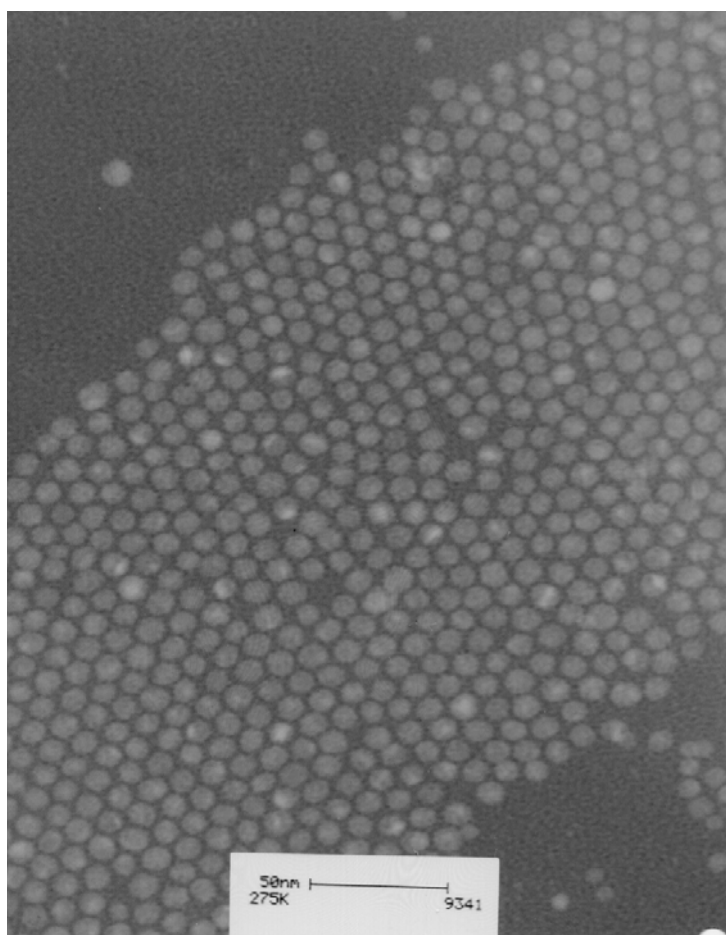


Figure 17 - Dodecyl amine stabilized silver nanoparticles. Particles are much larger on average, and are not as homogeneous as with dodecanethiol or trioctyl phosphine.

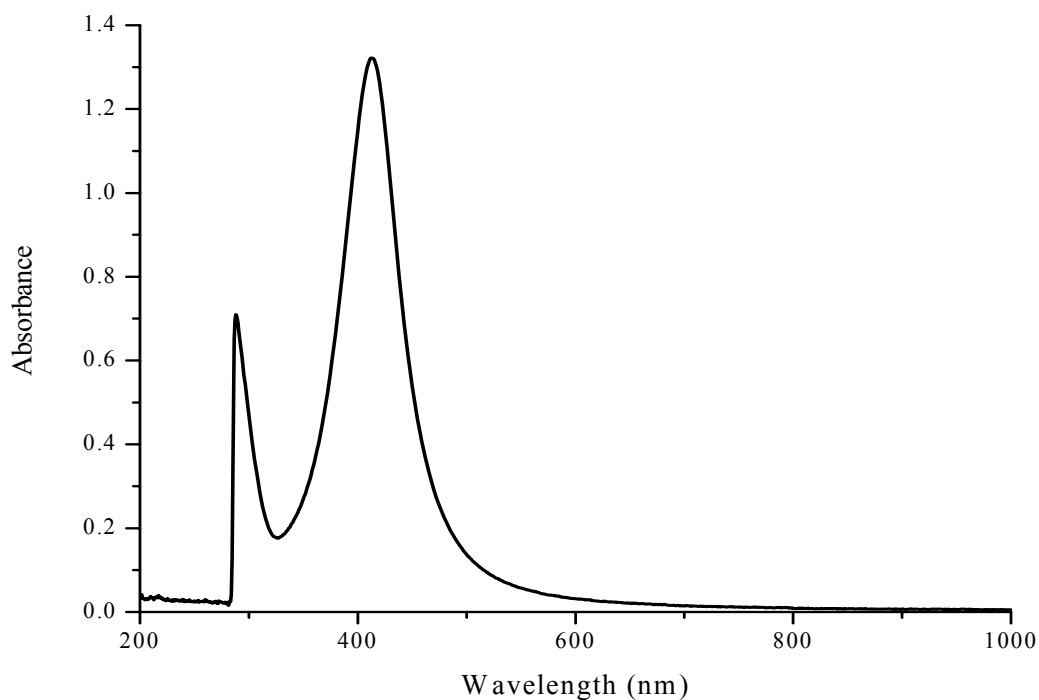


Figure 18 - UV-Vis spectra for dodecyl amine stabilized silver nanoparticles with a peak at 412 nm.

Dodecyl Alcohol

The alcohol group displays only a slight ability to bind the silver particles as most forms of silver in the sample are large, irregular, and undistinguishable from unprotected particles. This is emphasized by the UV-Vis spectrum that shows no surface plasmon features that are characteristic of small particles. In fact it displays no features in respect to SMAD particles produced without any capping agent at all. The difference shows that even butanone is a better protecting agent than the dodecyl alcohol. The TEM data confirms this, as the particle size for this sample is very large and suggest poor, if any coordination to the particle surface (figure 20).

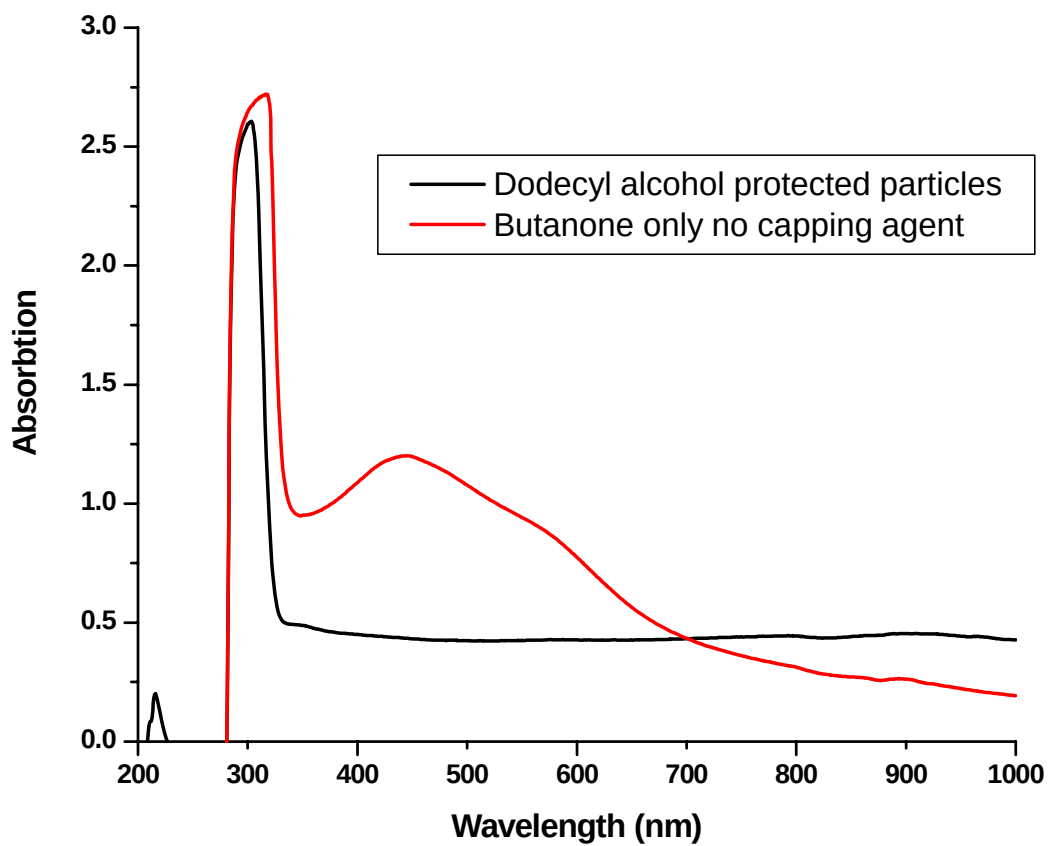


Figure 19 - UV-Vis spectrum comparing silver nanoparticles formed without the presence of ligands in red, and particles synthesized in the presence of dodecyl alcohol.

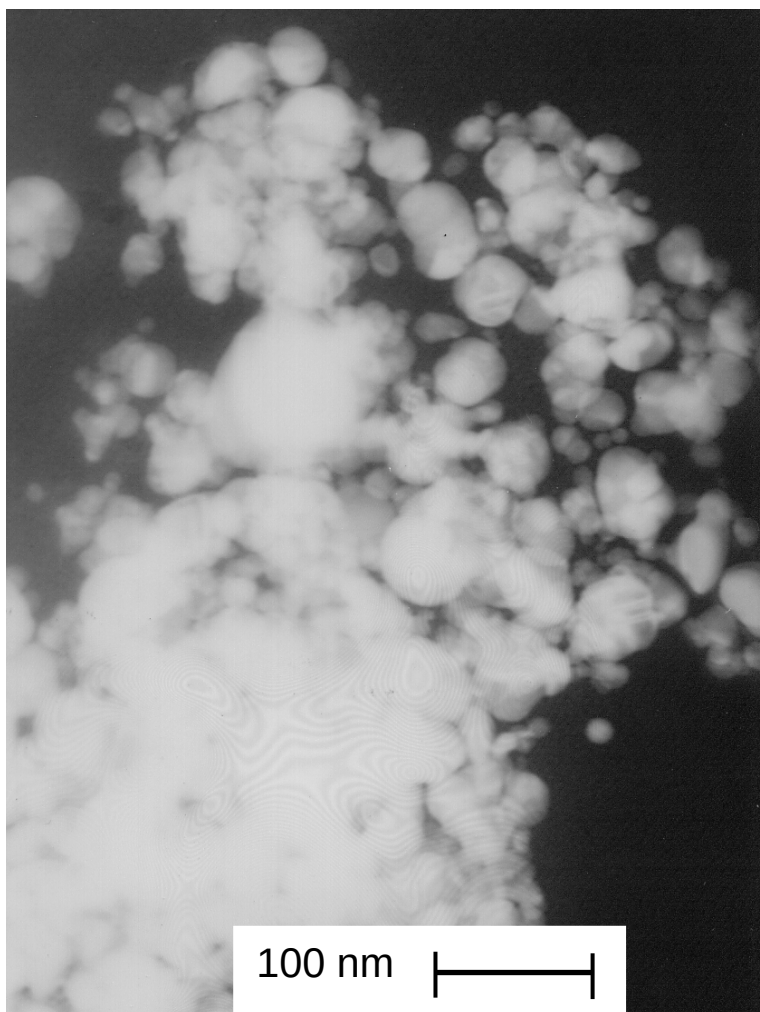


Figure 20 - TEM micrograph of dodecyl alcohol protected particles. The particles are very large and are a testament to the poor binding ability of the ligand to the silver particles.

Summary

The SMAD synthesis provides a means to achieve nanoscale products rapidly and with large quantity. The properties of the nanoparticles themselves were found to be highly dependent on the ligand used to coat them. These ligands are designed to stabilize the reactive metal particles and allow them to be soluble in solution. It was shown that dodecane thiol

molecules are the most efficient at protecting the metal particles, while trioctyl phosphine also does a nice job. Dodecyl amine cannot completely stabilize the metal particles from one another in this system, although at least a portion of the sample is monodisperse. Dodecyl alcohol had little effect on keeping the particles from sintering. The optical absorbance of the nanoparticles in solution, known as the surface plasmon resonance absorption, was seen to shift by as much as 15 nm depending on the specific environmental conditions of the particles.

The superlattice structure proved to be drastically affected by the molecular geometry of the protecting ligand. The dodecane thiol capped particles produced some remarkable large 3-D triangular superlattices. Even when the ligand molecules took on an abnormal shape, as in the case of trioctyl phosphine, a regular superlattice pattern emerged, yet in a spherical structure. The size of the particles was also found to be affected by the capping agent as the particle diameter was 6.6 nm for dodecane thiol, 6.0 nm for trioctyl phosphine, 14 nm for dodecyl amine, and a polydisperse solution for the dodecyl alcohol particles.

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Chapter 3: Synthesis of Nobel Metal Alloys: A Mechanistic Study of Digestive Ripening

Introduction: Digestive Ripening

For the incorporation of nanoparticles into technology it is imperative that a large supply of monodisperse particles is available for investigation and integration into useable devices. It is ironic that one of the greatest assets of nanoscience is also a major restriction. Since the properties of the individual particles change so drastically with small variations in size and shape, the quality and homogeneity of the particles must be exceptional in order to take advantage of these new attributes. Hence in order to have predictable nanoparticle properties all the particles must be quite similar in size. Digestive ripening, as discussed briefly in chapter 2, is a process whereby a product of this quality is obtainable in a facile and reproducible way.

Digestive Ripening is a process of self-refinement of nanoparticles. It is a phenomenon by which the very components of the nanoparticles themselves become interchangeable and exchange with other nanoparticles in solution. With the expenditure of enough time, the colloid reaches an equilibrium form in which all the nanoparticles are very similar in size and properties. This method was first described by Xiao Min Lin in the laboratories of Klabunde and Sorensen with respect to gold nanoparticles¹. The conditions for this procedure are extremely simple as a colloid of nanoparticles only needs to be heated anaerobically in an excess of ligand for this phenomenon to take place.

There is an even more remarkable finding from this procedure than the attainment of an equilibrium size of a monometallic nanoparticle system. Digestive ripening of separate metals

results in a homogeneous alloy in a ratio akin to the quantities of starting materials used². When conducted on nanoparticles that exhibit distinctly separate surface plasmon resonances (SPR) this alloying phenomenon can be tracked easily by observing the change in optical properties of the colloid with time. This feature gives us a tool to monitor the progress of the reaction. More importantly it allows us to investigate the mechanism of digestive ripening itself, a remarkable process that appears to involve transfer of individual atoms in solution between metal nanoparticles.

Metal nanoparticle Alloys

Alloys of nanoparticles open up a greater toolkit of nanotechnology reagents for chemists and engineers. Not only can the particle characteristics be changed by manipulating the size and shape of the particles, but by controlling the composition as well. Catalytic effects, among others, can be greatly enhanced by utilizing these alloys. An example of this is the use of Au/Ag nanoparticle alloys on mesoporous aluminosilicate supports in the oxidation of CO³. Many of the reduction techniques lead to mixed metal nanoparticles, typically core-shell type structures. These particles form as the most reactive metal is reduced first creating small particles that are then coated with the less stable metal. These particles have some interesting applications including more complex optical absorbance, but are generally used as an intermediate to homogenous alloys, or hollow particles^{4,5}.

Synthesis

The reagent colloids used were prepared by a metal vapor-solvent co-condensation method (SMAD), which is described in detail in chapter 2 and in the literature^{6,7}. These colloids are composed of polydisperse metal nanoparticles with a surfactant (dodecane thiol) to metal ratio of 20:1, and are dispersed in a 4-tert-butyltoluene solvent. The as prepared SMAD colloids are subsequently digestively ripened such that the reagent colloids used are spherical and of a similar size. In a typical experiment, 0.2 g of metal is suspended in 60 ml 4-tert-butyltoluene.

Separate colloids of single element nanoparticles are physically mixed in an inert atmosphere and heated to reflux (198°C) until the reaction is deemed complete by optical spectroscopy. A constant flow of argon is provided over the sample mixture to ensure isolation from air. A typical alloying experiment will be completed in 17 hours for Au/Ag. The alloying process through digestive ripening is much longer than for mere ripening of a single metal colloid which can be as short as 3 hours, and this difference is discussed later.

The Au-Ag alloying experiment was carried out at several different ratios of silver nanoparticles to gold nanoparticles producing alloys of a number of different compositions. Au-Cu alloying experiments were also carried out in an attempt to produce alloys of a completely different material. The reaction temperature and nanoparticle concentration were altered in several experiments in an attempt to investigate the kinetics of the reaction. The yield of monodisperse particles from this reaction is essentially 100%, however, some mirroring of the walls of the glass vessel used for reflux takes place after time.

Instrumentation: UV-Vis spectra were recorded on a Cary 500 UV-Vis-NIR spectrophotometer at 2% of the initial concentration of the initial colloid concentration against a background of 4-tert-butyltoluene in a separate cell. Low magnification TEM photographs and electron diffraction measurements were taken on a FEI CM100 transmission electron microscope. The samples were prepared by depositing 3 μ l of nanoparticle colloid on a carbon coated copper grid. HRTEM (high resolution transmission electron microscopy) pictures and energy dispersive X-ray (EDX) analysis were performed on a Joel JEM-2010 F TEM/SEM microscope. The HRTEM samples were precipitated with ethanol from the 4-tert-butyltoluene solution and excess dodecane thiol, centrifuged, and washed twice more with ethanol before re-dispersion in hexanes and deposition on a holey carbon nickel grid.

Characterization

TEM

The alloy nanoparticles produced are of a spherical shape as were the parent colloids. The particles size distribution was determined from a population of approximately 200 particles and is displayed for the relevant particles below. The particles are very nearly monodisperse, and are slightly smaller than the nanoparticle precursors. From the law of conservation of mass if the particle size is reduced, in order to have the same amount of metal atoms more particles must be produced by digestive ripening. This leads us to believe that some nucleating species must be produced during the reaction.

A tribute to the narrow size distribution of the alloy is the excellent ability of the colloids to produce large two and three dimensional superlattices of self-assembled particles formed by the alloy particles. The self assembly process is important in that the materials will be able to be incorporated into novel electronic and optical devices quite easily.

Table 1 - Particle size of reagents and alloy products

Ag	6.6 +/- 1.0 nm
Au	6.7 +/- 0.9 nm
Cu	6.0 +/- 0.6 nm
Ag/Au	5.6 +/- 0.5 nm
Cu/Au	4.8 +/- 1.0 nm

High resolution TEM images of the particles seen in figure 21 show no evidence of core-shell particles but of homogeneous metal mixtures. Core-shell particles would appear as dense regions (gold) surrounded by lighter regions or vice versa⁴. This would be especially apparent in the case of the Au/Cu alloy. The importance of this result will be discussed later on.

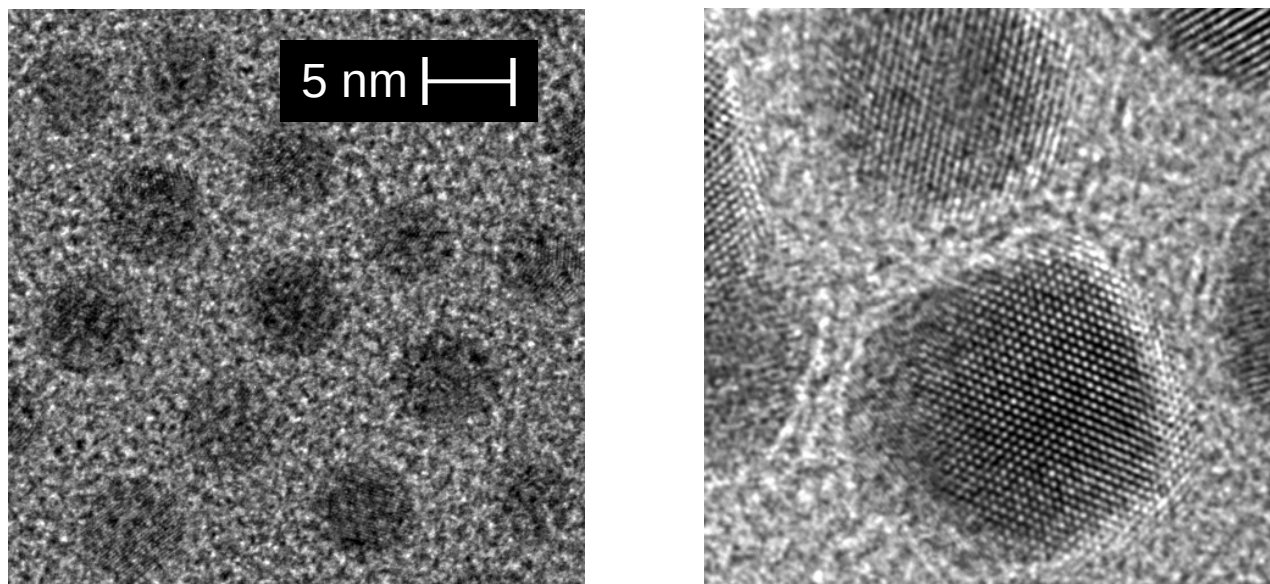


Figure 21 - HRTEM pictures of a) Ag/Au alloy nanoparticles and b) Cu/Au alloy nanoparticles.

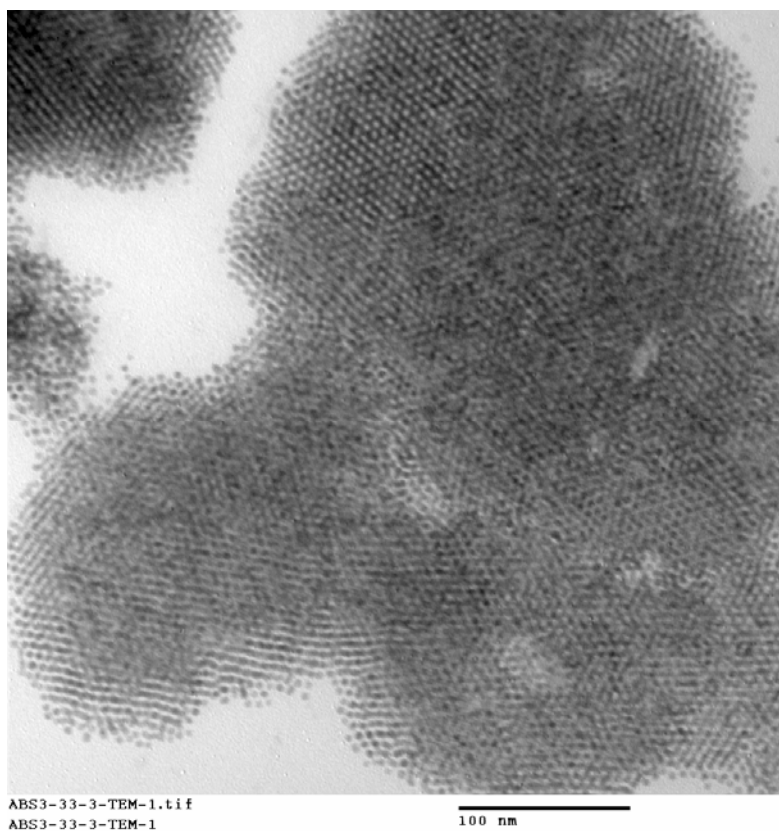


Figure 22- TEM image of Au/Cu alloy nanoparticles self-assembled into a 3-D superlattice.

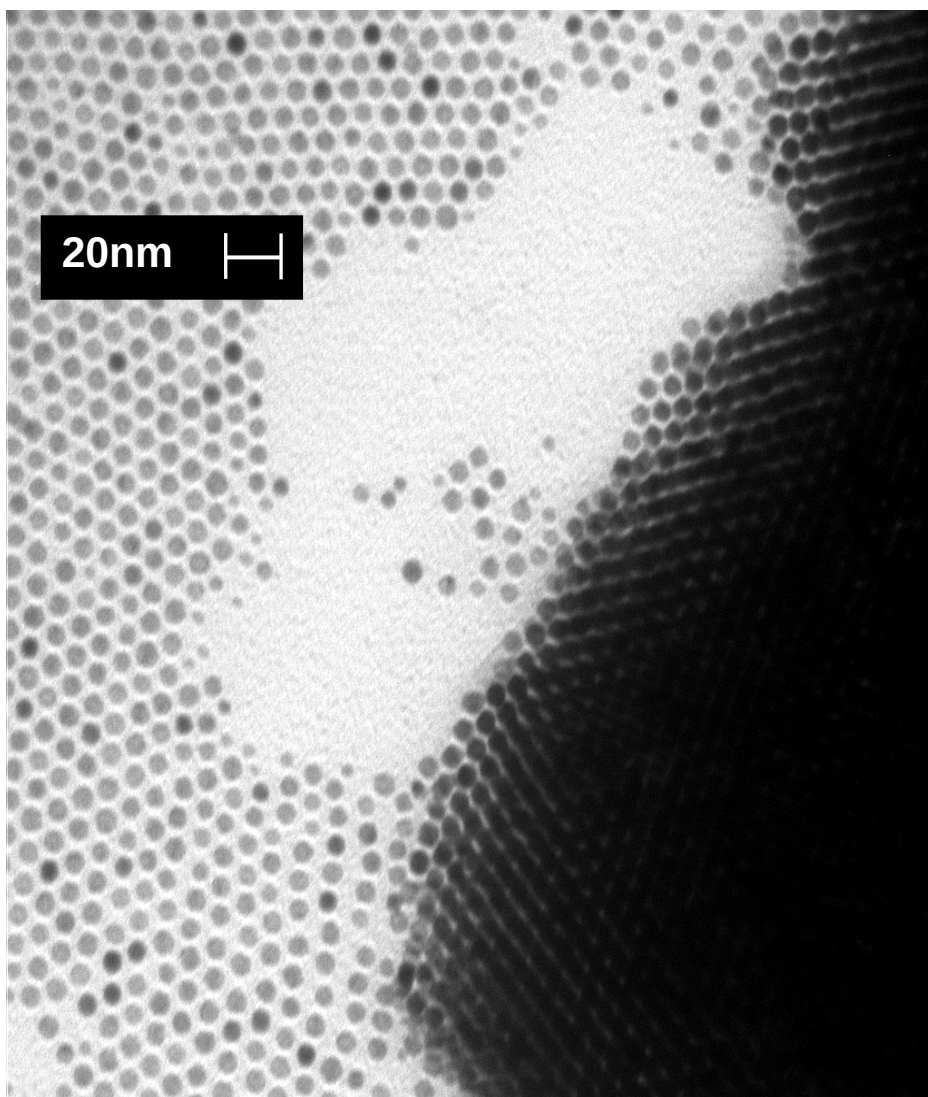


Figure 23- TEM image of Au/Ag alloy nanoparticles in a 2-D and 3-D ordered superlattice.

UV-Vis

Au / Ag alloy optical absorption

The progress of the Au/Ag alloy formation can be conveniently followed by optical spectroscopy. A 0.1 ml aliquot of the sample was removed from the reaction and the peak position was monitored in the visible region. The discrete gold and silver surface plasmon resonance peaks are very distinctive at the beginning of the reaction. As the metal nanoparticles interact, these peaks shift towards each other and slowly sharpen into a single symmetrical peak. The reaction is deemed complete when no further narrowing of the peak takes place, suggesting the particle size and composition has come to equilibrium, as seen in figure 24 below.

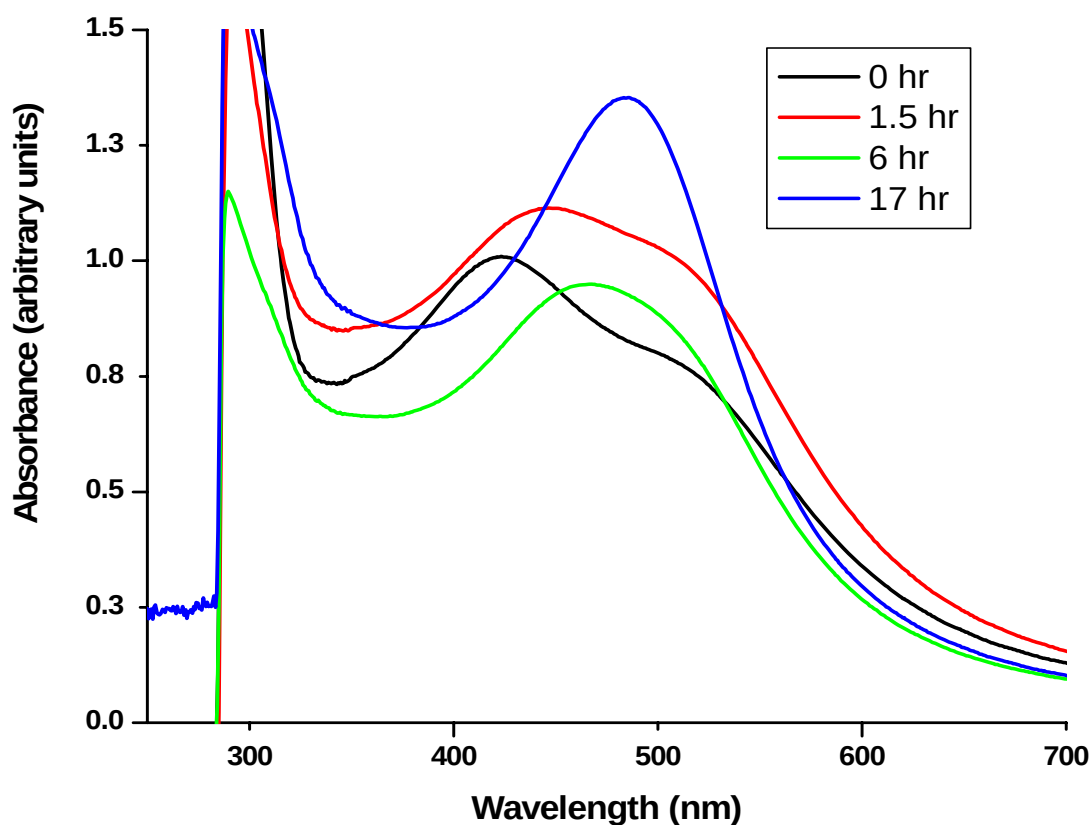


Figure 24 - UV-Vis spectra taken over the period during which an alloy formed between gold and silver. The individual peaks seen at the beginning of the reaction move towards each other and form a single symmetrical peak at the end denoting a homogeneous alloy particle.

Gold Copper alloy absorption

Unfortunately, in the case of our dodecane thiol coated copper nanoparticles there is not much of an optical absorbance. The final product can be distinguished from pure gold however as a deep purple color emerges, distinctly different from the red precursor as the gold plasmon peak is red shifted to 545 nm which is very strong evidence of copper alloying with gold.

Similar results for a Au/Cu alloy have been seen by Kim et al⁸. As in the Ag/Au case the reaction is determined to be complete when no further change in the size or position of the peak is noted in the spectrum between aliquots.

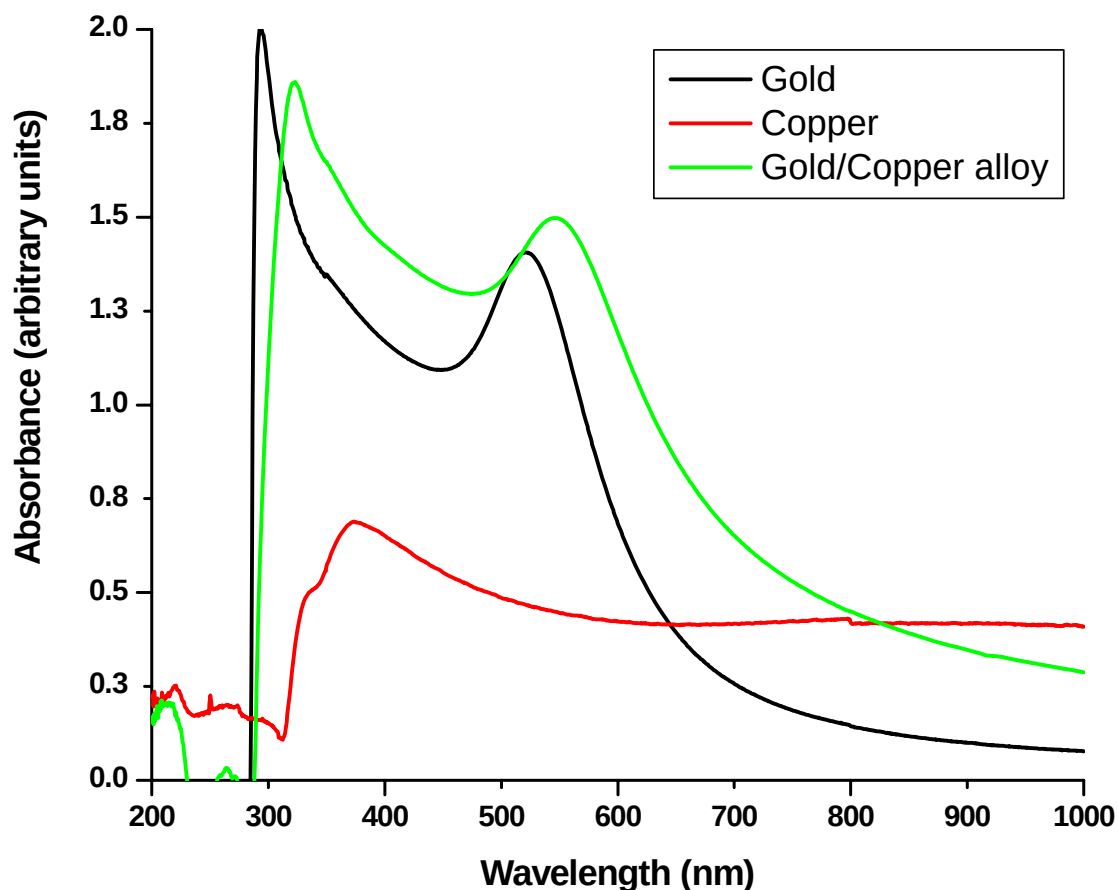


Figure 25 - Optical spectrum of Au, Cu, and the Au/Cu alloy after digestive ripening. The alloy is seen to be red shifted from the sharp gold plasmon peak.

Peak Position

The peak position can be tailored in the case of gold and silver by manipulating the ratio of gold and silver reagent colloids before the alloy reaction begins. One goal of synthetic nanoparticle scientists is to be able to have complete control over the size of nanoparticles. This event seems unlikely at this stage because of especially stable sizes of nanoparticles. It is possible however, to tailor composition of the nanoparticles in a linear fashion. The optical absorption of a gold silver alloy can be adjusted to any point between 420nm and 530nm in this way. Several examples of this from our work are seen in figure 26 below. Similar results have been observed by other groups using different synthetic procedures^{9,10}. It was found in our digestive ripening process that there was some difficulty in obtaining alloys through this method that are highly enriched in silver, these colloids tend to resist alloy formation until more gold is present. Some reactions were successful as evidenced by the 455 and 460 nm peaks in figure 6. This experiment is important in that any alloy can be synthesized through this reaction when gold and silver are used. This allows one to specifically engineer the optical properties of the colloid to any wavelength desired.

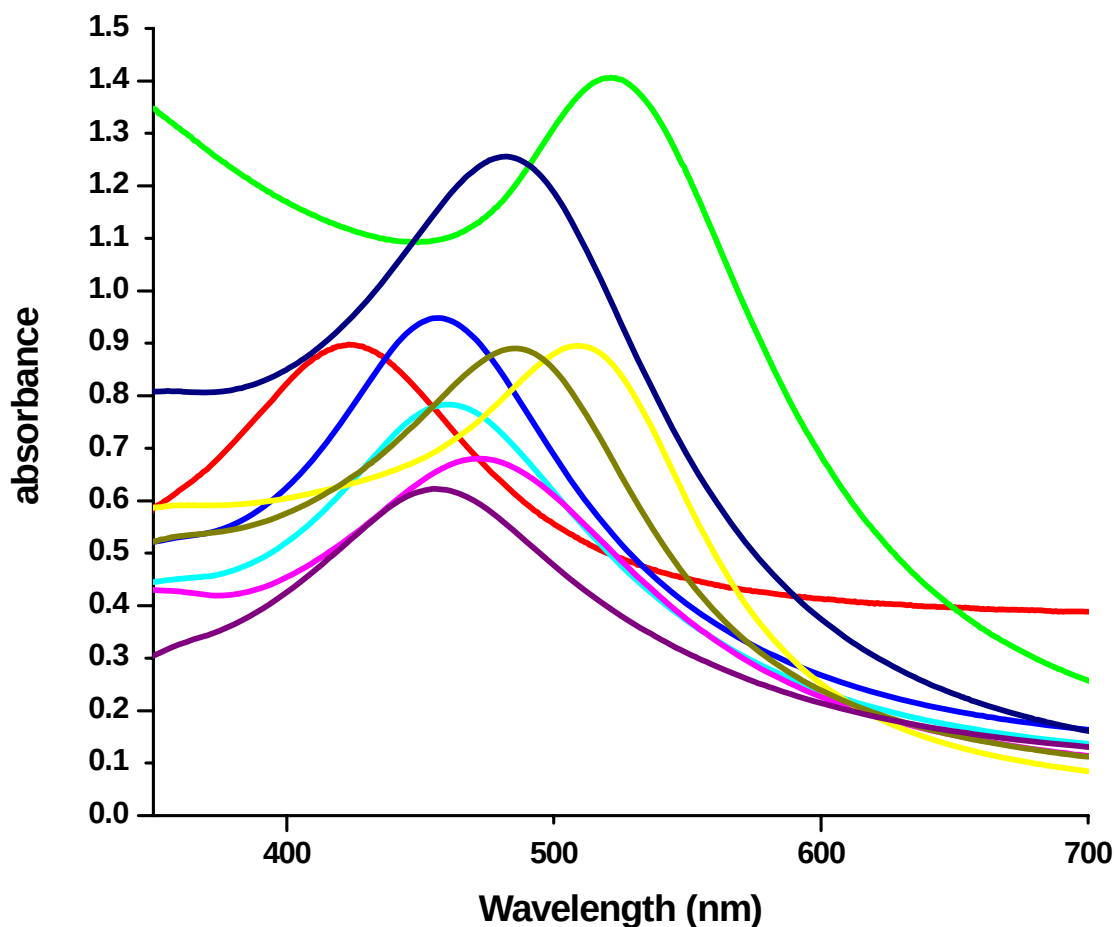


Figure 26 - Several different alloys of gold and silver showing the versatility of the digestive ripening method to produce many alloys by merely changing the concentration of metal nanoparticle precursors.

An experiment was conducted to see whether a similar phenomenon would occur for different mixtures of gold and copper metals. Small amounts of gold nanoparticles were added sequentially to a colloid of pure copper nanoparticles. Strangely, only one peak continued to arise in the spectrum at 542nm suggesting that only one form of Au/Cu alloy was stable in nanoparticle form.

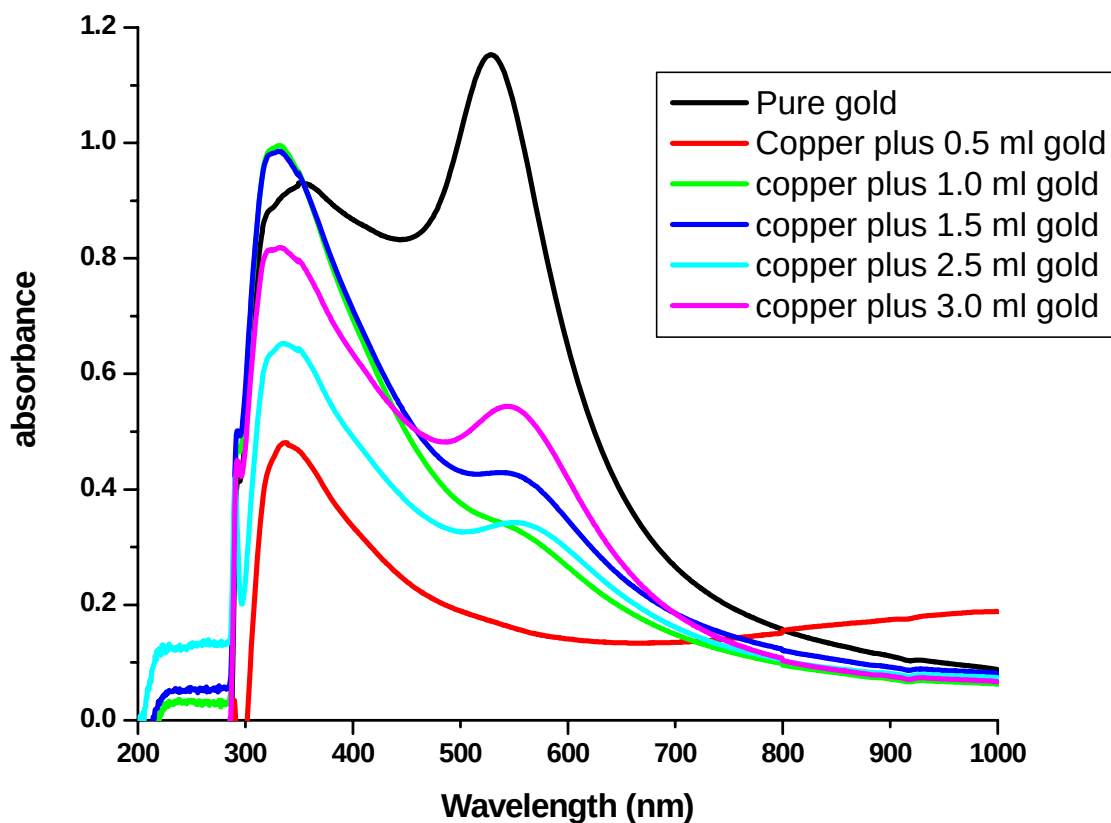


Figure 27 - UV-Vis spectrum of copper nanoparticles reacting with increasing amounts of gold nanoparticles. Only one peak appears, and gains intensity suggesting one stoichiometric product, unlike the case of silver and gold.

Crystal Structure

An attempt was made to characterize the nanoparticle alloys by obtaining diffraction patterns by two methods; either an electron diffractogram viewed in the electron microscope, or from a powder XRD spectrum.

To obtain an XRD spectrum of these particles the samples needed to be precipitated on the surface of a smooth plastic sample holder. The colloids tested were initially made insoluble by adding 5 ml of ethanol/ 1 ml of sample, shaken, and then centrifuged. The supernatant was removed and the remaining particles were re-dispersed in a minimal amount of hexanes. This solution was placed drop-wise on the sample holder, and the hexanes were allowed to evaporate between every drop. This had the effect of growing some extensive superlattices on the surface of the sample holder. These were analyzed by the major lines that came through the spectrum representing the distances between nanoparticles in the lattice.

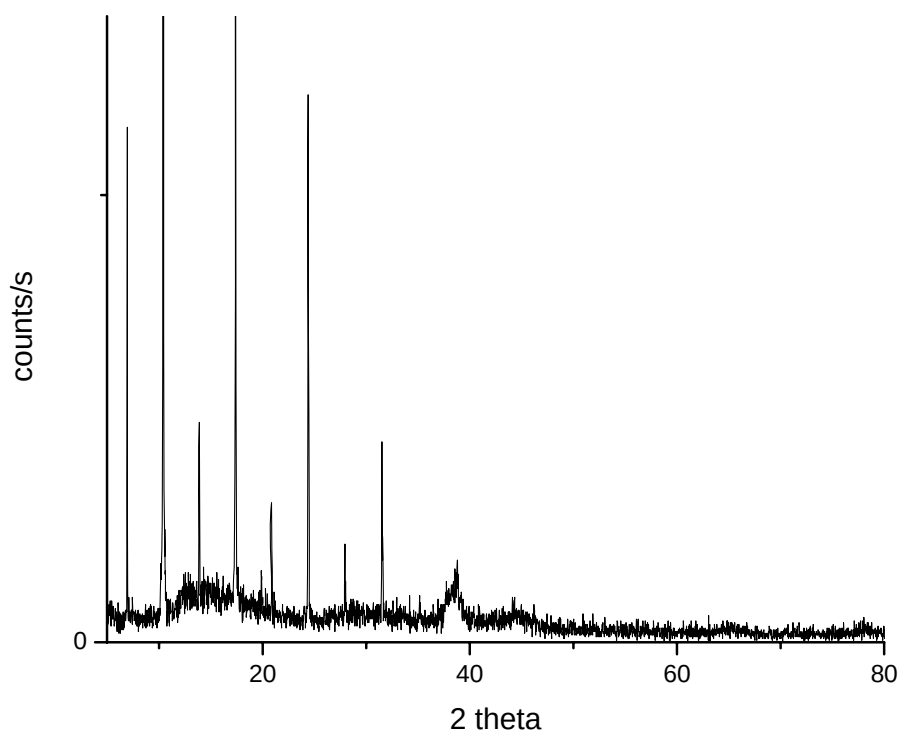


Figure 28 - XRD spectra of Au/Cu alloy nanoparticles. The sharp low angle peaks are from superlattice spacings, while broad peaks are from alloy nanoparticles.

Unfortunately gold, silver, and their alloys are extremely similar to one another in their atomic structure. All of the metals display the same fcc packing. To make things more complicated their atomic radii are nearly identical, despite being in different rows of the periodic table, at a covalent radii of 144pm. This led to indistinguishable spectra as the values of the nanoparticles are within the instrument's limits of detection. Copper, on the other hand, does have a distinctly different value for the atomic spacings as do several of its stable alloys with gold. However, to our surprise, the major diffraction structural studies of the Au/Cu system were very similar to the major lines of gold itself, but also matching the intermetallic species Au₃Cu.

The UV-Vis data confirms the complete reaction of gold nanoparticles in solution leading us to believe the only product to be Au₃Cu. There are some additional minor peaks in the Au₃Cu alloy that it was hoped would appear in the powder XRD spectrum. Unfortunately, since the sample is in nanoparticle form there is a great deal of line broadening, as is common for nanoparticle samples, rendering the minor peaks impossible to differentiate from the background. This spectrum is displayed in figure 28. The only gold/copper alloy to have these peaks is in a ratio of 3:1 and would suggest this composition of the alloy particles.

Table 2 - d-spacings for metal samples

Au	1.23	1.40	2.01	2.38
Au/Cu alloy	1.25	1.44	2.05	2.41
Au ₃ Cu *	-	1.45	2.05	2.36
Cu *	1.28	1.81	2.08	-

* Values obtained from the database of inorganic compounds¹¹

EDX

Due to the failure of the crystal structure to clearly identify the alloy stoichiometry, the samples were analyzed by EDX with a machine that could perform an elemental analysis on individual alloy particles. Due to the higher energy of the electron beam used in this microscope, the particles needed to be washed free of any excess ligands and heavy solvent. To accomplish this, the particles were precipitated with ethanol and washed twice with ethanol before redispersion in hexanes and shipment. Then the colloids suspended in hexanes were deposited on a carbon coated nickel grid. Carbon and Ni compose the unlabeled peaks in the spectrum below. Two particles from separate areas were analyzed by the EDX spectrum, one of which suggested an alloy with a ratio of Au_3Cu , supporting the evidence of the crystal structure above. The other particle showed a slightly higher amount of copper in the particle at Au_3Cu_2 .

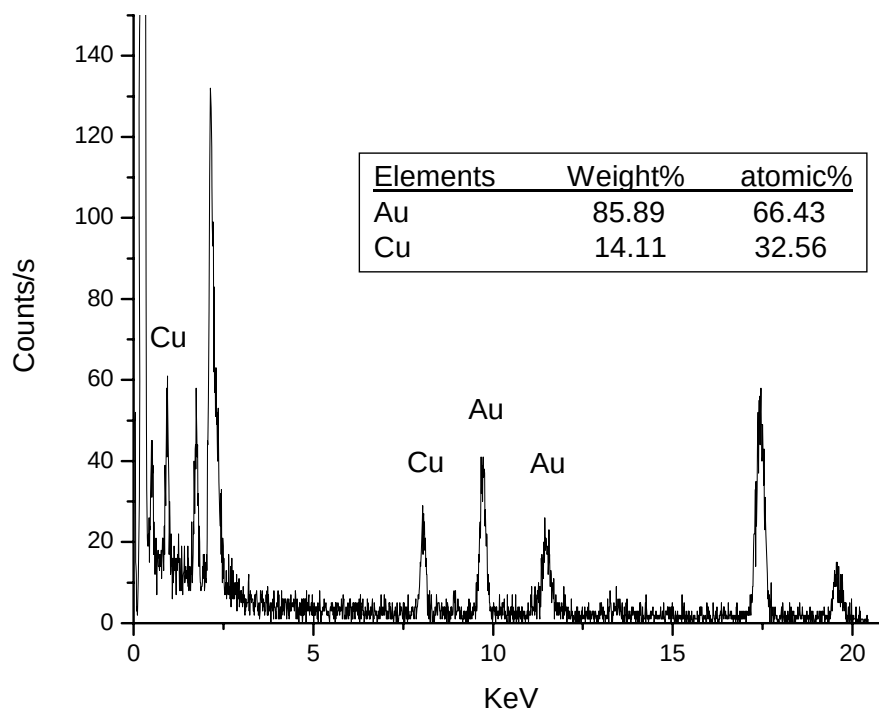


Figure 29 - EDX spectrum of a single particle of our Au/Cu alloy. Peaks for both metals are present in the particle at a ratio of 2:1 gold to copper metal.

EDX analysis was also performed on the Ag/Au particles in order to confirm the presence of both metals in a single particle. The presence of both metals was found in each particle analyzed, although during analysis some growth occurred, probably due to particle fusion. Nevertheless, the results support the general concept of digestive ripening since each particle examined contained both gold and silver.

Kinetics

In order to gain some insight about the reaction pathway during digestive ripening, the experimental conditions were held constant while the concentration or temperature was altered. To increase the temperature of the reaction t-butyl toluene solvent (b.p. 198 °C) was replaced by octyl ether (b.p. 300 °C). This was accomplished by placing the colloid under high vacuum overnight at 40 °C to remove the t-butyltoluene solvent, followed by addition of octyl ether. The excess dodecane thiol was not removed under these conditions, nor was there any movement of the initial peaks from this treatment in the UV-Vis spectra. At the boiling temperature of octyl ether, the alloying reaction occurs remarkably fast and the reaction time shortens from 17 hours to 60 minutes. However, under these conditions a significant portion of the metal mirrors out on the glass reactor walls and lowers the overall yield of the reaction. In an attempt to prevent this, a carbon coating was sprayed on the glass walls before introducing the colloids, but failed to prevent mirroring of metals on the reactor walls.

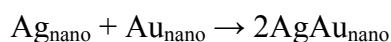
A mixture of silver and gold colloids in 4-tert-butyltoluene was placed in a water bath at 90 °C to determine if the alloying reaction would proceed at lower temperatures. After 15 hours the individual plasmon peaks slowly began to shift towards one another, but even after 90 hours the plasmon peak due to alloy was not observed. Some atom transfer did take place between nanoparticles, but most likely only on the surface, and true alloying does not take place. The implications of this are discussed in detail in the next section

For several Au/Ag alloy reactions in 4-tert-butyltoluene the concentration of the colloids was altered to determine the effect on the reaction time. To accomplish this two mL of silver colloid and two mL of gold colloid were mixed and evaporated until only 0.5 mL of liquid remained, and then one mL of 4-tert-butyltoluene was added, thus producing a sample 2.6 times more concentrated. This reaction was completed in the short time of 3 hours. Similarly, in

another experiment 0.5 ml silver colloid was mixed with 0.5 ml gold colloid and added to 9 mL of 4-tert-butyltoluene for a 10 fold dilution of the initial reaction conditions. This reaction did not reach completion until 35 hours of digestive ripening.

From the experiments above a rough estimation of the activation energy for the digestive ripening process can be obtained.

The chemical equation for digestive ripening is assumed to be the irreversible reaction



The rate of reaction v is defined by the change in the concentration with the change in time expressed mathematically as:

$$v = -\frac{1}{v_a} \frac{d[\text{Ag}_{\text{nano}}]}{dt}$$

$1/v_a$ is the coefficient of the reaction from above

The concentration of the reactants is estimated by an 80% yield of the parent SMAD reaction.

The rate is related to the rate constant k for the reaction by:

$$v = k[\text{Ag}_{\text{nano}}][\text{Au}_{\text{nano}}]$$

From these parameters the activation energy of the reaction can be determined through the Arrhenius equation¹².

$$k = Ae^{-E_a/RT}$$

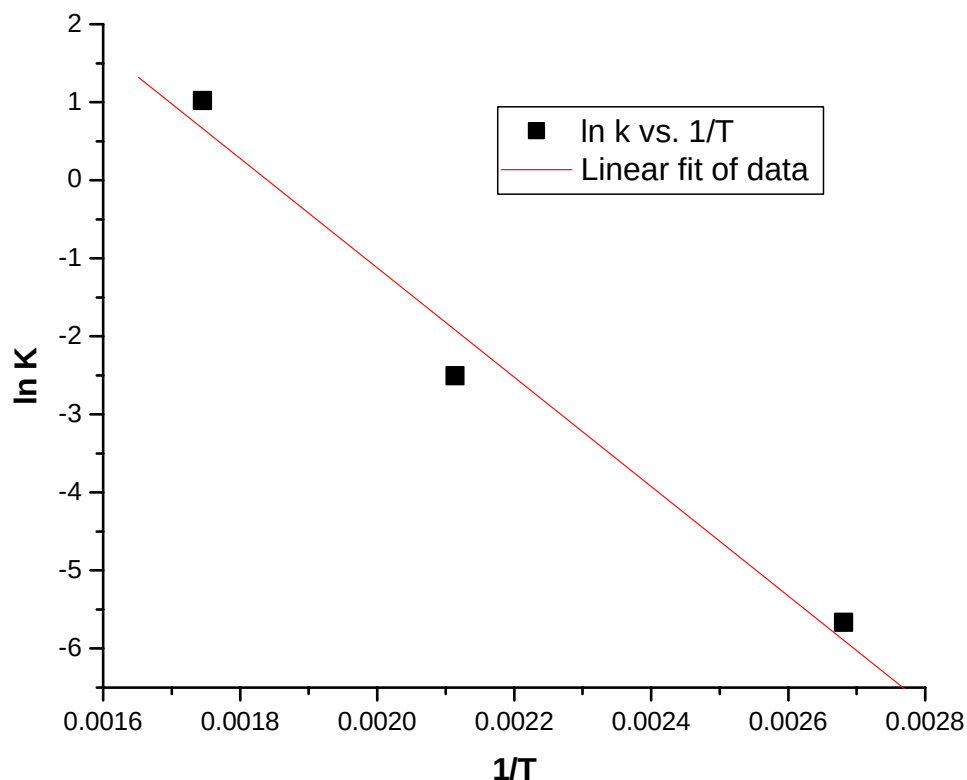


Figure 30 - Plot of the rate constant vs. temperature to calculate activation energy of the alloying reaction.

From the plot the activation energy for the reaction is 32 KJ/mol.

Mechanism: metal atom transfer

The process of digestive ripening has been utilized in our laboratories for years to modify and refine metal and semiconductor colloids. While the results of this process are unquestionably remarkable, the mechanism is not understood. The Ostwald process predicts only growth of nanoparticles as the high energy small particles combine to reduce surface

energy. Digestive ripening on the other hand involves growth of the smallest particles in solution only up to a threshold size. Meanwhile all larger particles in solution erode and reduce in size to this same equilibrium value. This process is tremendously useful not only in being able to reduce the size of most of the larger particles in solution, but by creating a very narrow size distribution of the particles, the significance of which is discussed in the introduction. The digestive ripening process has also proved to be extremely versatile as particle composition can be readily altered, and the size of the particles changed with use of different ligands.

Investigation of this process may help to give us some control over this reaction and allow us to take further advantage of digestive ripening. The modification of the size of the particles through this process illustrated in chapter 2 with silver nanoparticles is very interesting by itself and suggested the transfer of metal atoms between particles, for how else could the smaller particles grow and the larger particles erode except through atom transfer. Even more surprising, however, was the behavior of the particles when two different elements were added together. This alloying proved that metal atom transfer occurs between the particles in solution and that deep, complete homogeneity of the particles is achieved.

The concentration experiment shows that at higher concentrations the reaction proceeds faster, hence increased collisions between particles accelerate this process. This suggests metal transfer taking place during collisions. However, an excess of dodecane thiol was seen to be necessary for this alloy reaction to proceed. Particles that were washed of excess ligands and placed in otherwise the same reaction conditions merely agglomerated and fell out of solution. It can be suggested that the dodecane thiol itself grabs metal atoms off the surface of particles and transfers them to another particle.

There is another process taking place along with the metal atom transfer, and that is atom diffusion to the center of the nanoparticles. This is evident by the formation of homogeneous alloys of the metals and NOT core-shell particles. If atom transfer was the only process at this temperature, a coating of the opposite element would form on the particles but not a pure mixture. It is evident that a homogeneous alloy forms by the single, symmetrical UV-Vis peak and the homogeneity of the atoms seen in the high resolution TEM images. At 200 °C silver and gold in bulk placed next to one another will remain completely atomically separate. At the size of our particles, however, the atoms have to travel 3 nm to reach the interior of the particle to form a homogeneous alloy, and amazingly accomplish this feat. Similar experiments in the solid state, where Schaak's group has interacted copper and gold nanoparticles in the solid state, where the products were found to alloy at a temperature of 200-400 ° C¹³. Evidence of metal atom transfer between particles was found by Schaff and Whetten at this temperature¹⁴. This is another example of the wonderful change in properties of a material with the change in its size at the nanoscale.

Regarding the mechanism, I think complete alloying can be accomplished by: 1) surface atom exchange, 2) movement of atoms within each particle so that mixing occurs, 3) this happens over and over again until equilibrium concentration in each particle is achieved.

Summary

We have shown that through digestive ripening, alloying can take place between nanoparticles of different metals. The final product is a homogeneous intermetallic compound. This is shown through the uniformity of the surface plasmon UV-Vis peak and TEM images.

The formation of an alloy is unequivocally confirmed with the EDX data displaying the presence of both metals in a single particle. XRD data was taken to determine different crystal structures of the products, however they were indistinguishable from one another. This process has allowed us to have an insight into the mechanism of digestive ripening and how the reaction proceeds. This information may prove invaluable in the future as digestive ripening can produce such large amounts of uniform nanoparticles quickly and easily.

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Chapter 4: Microbiological activity of SMAD Prepared Nano-Sized Silver Powders

Silver as an antimicrobial agent in the past and into the future

Silver has been known for centuries to have antimicrobial properties. Some of the first recorded uses of silver date back to ancient Greece where silver coins were used to as a disinfectant of water and to prevent spoilage¹. The role of silver for medicinal purposes expanded with time and today silver is still used in the health care system for a variety of uses. Silver metal coatings on instruments such as catheters have cut down on the occurrence of infections. Standard delivery methods for silver ions are routinely accomplished with silver sulfadiazine cream and dilute silver nitrate solutions. Silver sulfadiazine cream has been found to be beneficial to treat ocular keratomycosis³ as well as being a common topical treatment on burn wounds⁴. It has been used to treat gonococcal and chlamydial ophthalmia neonatorum by using drops in the eyes of newborn children⁵.

As the nanotechnology field is blossoming the design of multifunctional materials is growing as well. New delivery methods of silver have been devised to maximize the slow release of the antimicrobial metal into its environment. Polyelectrolyte multilayer films are an example of this. These films are supports layered with alternating positive and negative ions incorporated into polymer chains. The supports are biocompatible and can provide a sustained release of silver ions from the polymer membrane⁷. Sharma described a procedure for impregnating the surface of alumina with silver metal as a method to produce a filter material. On a small scale the alumina support was found to be an excellent disinfectant of drinking water,

which he hopes can be expanded into use in community water systems². Bioactive glasses are new materials that have the innate ability to bind to both hard and soft biological tissues. The incorporation of silver through silver oxide has added an antimicrobial property as silver is slowly released from the material. These supports are now much more resistant to infection and have increased the success rate of implantation in living organisms⁸.

It wasn't long before scientists tried to apply the advantages of nanomaterials to creating more effective materials of silver. One of the first nanoparticle products put forth by a private company that turned a profit was from Acticoat⁶. This corporation created some wound dressings that involved incorporation of silver nanoparticles in wound dressings. These bandages were found to be magnificent in preventing infection and were also shown to increase the rate of wound healing compared to standard treatments. Despite the large potential for nanoparticulate silver to be a more effective antimicrobial delivery system, few laboratories are doing research involving nano-preparations of silver. A very effective synthetic and delivery method was devised by Balogh where silver ions are reduced to 1-3 nm particles inside dendrimer macromolecules. The highly branched nature of the dendrimers stabilizes the particles and the use of poly-amidoamine (PAMAM) polymer chains as the branching units make them very compatible with living cells¹⁰. There have been indications that silver oxide may have an even greater toxic effect on microbes than silver metal⁶.

Our research laboratory has been committed to preparing more effective chemical products through reducing the size of the material and still producing large quantities of products. This has the immediate effect of increasing the surface area of the substance providing more access to active metal atoms normally buried in the bulk of the solid. Secondly this has the effect of creating a high number of corner and edge sites on the surface of the powder that have

increased instability because of dangling bonds. These atoms may show new and more powerful chemistry than that observed by macro-sized materials. In this chapter we apply this philosophy to silver in the hope of dramatically improving the bactericidal activity of the metal.

To this end we have attempted several preparations of silver nanoparticles each with unique characteristics. These include high surface area dry silver powders, to particles with water soluble capping agents that trap the particles of silver metal at an incredibly small size. Other preparations were designed to emphasize exposure of the reactive metal powders oxidizing agents in an attempt to change the surface chemistry of the particles and improve their activity.

The bactericidal activity of the silver nanoparticles was tested against commercial silver powders of nanosized and standard dimensions and a solution of silver nitrate providing purely Ag^+ ions. The powders were tested against *Escherichia coli* as well as *Staphylococcus aureus* to judge the activity against both gram negative and gram positive bacteria. Viral testing was attempted as well to see if making the products nano-size has an effect on improving silver's normally low activity against viral pathogens.

Synthesis of nano-active silver particles

Several silver products were made to take advantage of different chemical interfaces. All syntheses were prepared from the Solvated Metal Atom Dispersion (SMAD) method as described in detail in chapter 2. This is a chemical vapor deposition reaction involving co-evaporation of large quantities of metal atoms and an initially stabilizing solvent onto a liquid nitrogen cooled surface. As the mixture of metal atoms and solvent warms, aggregation of the metal is slowed and eventually terminated by interaction with the solvent molecules. After

evaporation several adjustments were made to the procedure originally tailored for gold synthesis from Stoeva *et al*¹¹. Some of the information in finding the optimal conditions in tailoring the SMAD reaction to silver was determined from results from this chapter. The first silver nanoparticles produced here were protected solely by organic solvents. The studies of metal atom stability in various solvents provided essential information giving a strong foundation for future preparations of silver nanoparticles.

SMAD Silver powder

To maximize the surface availability of silver, the initial nanoparticle products were produced without the presence of capping materials included in the SMAD reactor. The particles were to be isolated by evaporation of the solvent after synthesis leaving a high surface area metal powder as the final form of the product. A second solvent was not needed for this system, since digestive ripening of the particles could not be accomplished without ligating species to refine the particles. Heating the nanoparticles in the organic solvents alone (no ligating species) resulted in Ostwald ripening and enlargement of the particle size. These factors keep this synthesis very simple. The size of the final particles was completely dependent on the ability of the initial solvent to passivate the metal atoms both during evaporation and after the warm-up process where the metal atoms interact with one another. The solvents tested were pentane, toluene, THF, isopropanol, acetone, butanone, and water.

Ligand capped silver particles

In an effort to further decrease the size of the silver particles and prevent the sintering and agglomeration of the previously described powders, water soluble ligands were introduced into the reaction chamber before evaporation. The synthesis was very similar to that used in chapter

2 with organic ligands and solvents. Here, acetone was used as a co-evaporation solvent with 60 ml of water placed on the bottom of the reactor intended to dissolve the ligating agent and be a solvent for the final particles. A large excess of 30:1 ligand to metal atoms was used in the reactor. After reaction acetone was removed from the colloid by evaporation under vacuum leaving a product of silver particles ligated in a water soluble ligand, excess ligand, and water.

Three different ligands were used for the experiments: 3-mercapto-1,2-propanediol, Sodium 3-mercapto-1-propanesulfonate, and Sodium 5-mercapto-1-tetrazoleacetate. These ligands were selected from a group of ligands previously used with gold aqueous colloids by Stoeva¹². Their selection and reactions with gold are expanded upon in chapter 5. They are listed as having very low toxicity in their respective MSDS and proved to be the most stable systems with the similar gold metal. Some attempts at digestive ripening were conducted on each silver colloid, however the particles were not stable to this treatment. This is likely the result of lower binding affinity of the ligands, or reaction with water at elevated temperatures.

Air-SMAD

To attempt to oxidize some of the silver metal particles, three separate procedures were applied. The first two involve a change in the reaction procedures during the initial formation of the metal particles, and the third is a post synthetic treatment. Once the metal atoms are evaporated from the bulk phase in the SMAD reactor they exist as highly reactive species frozen in a bed of solvent (acetone) which is cooled by liquid nitrogen. The extremely low temperature, passivation by solvent molecules, and high vacuum protect the particles from reaction. In an effort to oxidize the silver atoms the reactor is opened to the atmosphere at this stage. This has a two-fold effect on the reaction. Primarily this allows the atoms to interact with oxygen in the air and potentially react with one another. It also has the detrimental effect of warming the product

much faster than in a standard experiment. This is expected to cause a more rapid and severe agglomeration of the metal atoms and result in larger particles.

KOH-SMAD

In a second effort to modify the silver particles as they first form, potassium hydroxide was introduced in the reactor system. The KOH was dissolved in 60ml of isopropanol and placed on the bottom of the reactor. When the end of a standard evaporation was over the solvated metal atoms and clusters melted from the frozen solvent matrix and came into contact with the isopropanol/KOH solution.

Two preparations were attempted from this procedure. Isopropanol was used as the co-evaporation solvent in one reaction. This was done to improve the contact of the metal atoms with the basic solution in the reactor. Unfortunately isopropanol proved to be a very poor solvent for the stabilization of the metal atoms and quickly led to agglomeration before reaching the hydroxide. Acetone was used in a second reaction to circumvent this problem, and had sufficient miscibility with the basic solution.

SMAD-Heat treatment

Another method of oxidizing the surface of the particles was to heat the powder in the presence of air. This was done in a muffle furnace at temperatures of 60 °C, 100 °C, 150 °C, and 200 °C, and 250 °C. Each sample was heated for typically 3 hours.

Experimental: Millipore water was used in all reactions unless otherwise stated. Silver metal was purchased from Strem chemicals. Organic reagents and solvents were purchased from Aldrich as well as the silver powders used in biological testing. All preparations were viewed in

a Phillips 101 Transmission Electron Microscope (TEM) to analyze the particle size. The dry powders were further analyzed in a Bruker powder X-ray spectrophotometer using Cu K α radiation, surface area measurements were obtained on a Nova BET nitrogen absorber, and a Scanning Electron Microscope (SEM). All commercially purchased powders were subject to the same sampling conditions as our products.

Characterization of silver powders

Particle Size and Morphology

TEM Images

SMAD-Powder

Nanoparticulate silver was first observed by looking at TEM pictures of the products. When the SMAD prepared silver nanoparticles are first synthesized they begin as metal atoms embedded in a frozen organic solvent matrix. As this is warmed from liquid nitrogen to room temperature, the organic solvent melts and allows collisions and interactions between the metal atoms. Once the slurry reaches room temperature the product is relatively stable, however it has agglomerated significantly to produce 3-100nm crystals of silver metal as can be seen from figure 31. In order to isolate metal nanoparticles the solvent must be removed from the sample. This further induces aggregation as the solvent that was partially protecting the reactive metal particles from one another is removed. The resulting powder is somewhat porous, but has distinct surface features in the nanosized regime. However as can be seen from the images in

figure 32 the resulting powder is composed of micrometer sized chunks of silver. The use of different organic solvents leads to a slight change in particle size in the order of Butanone < Acetone < Toluene < THF < Pentane < isopropanol. Upon sonication when dispersed in water the SMAD powders break apart significantly and regain much of their lost surface area as can be seen in figure 33.

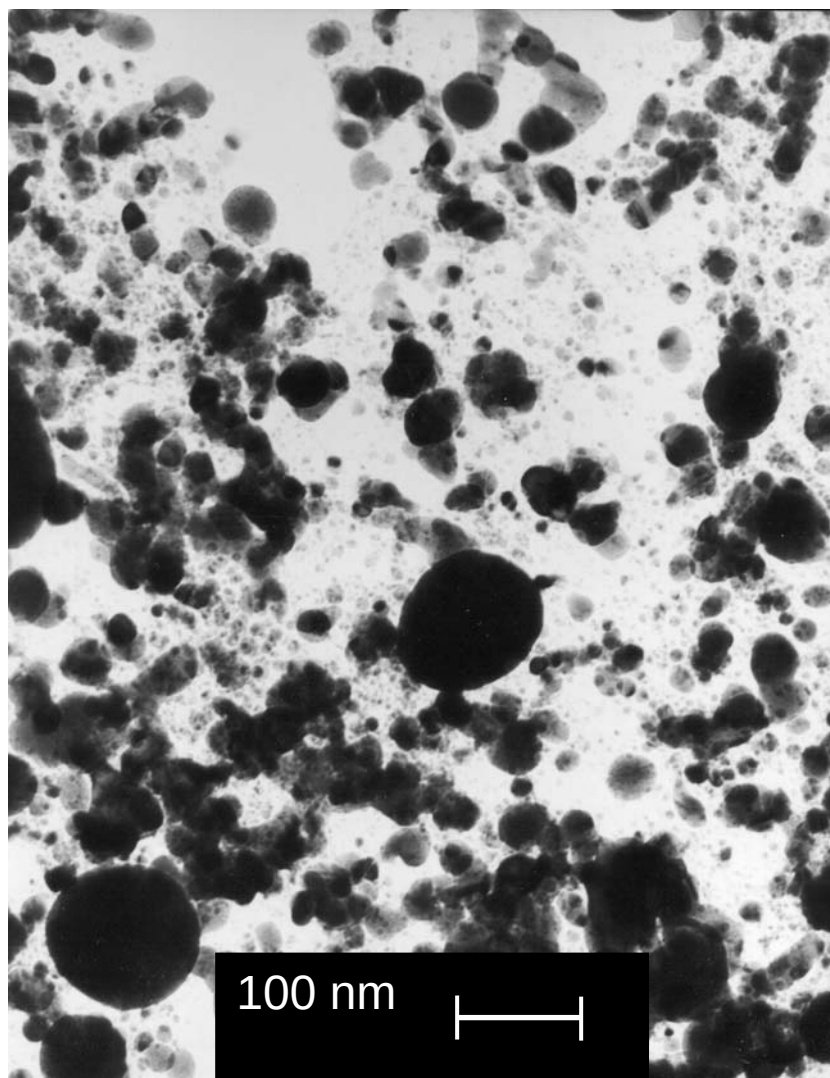


Figure 31 - TEM image of SMAD prepared silver metal in acetone solvent.

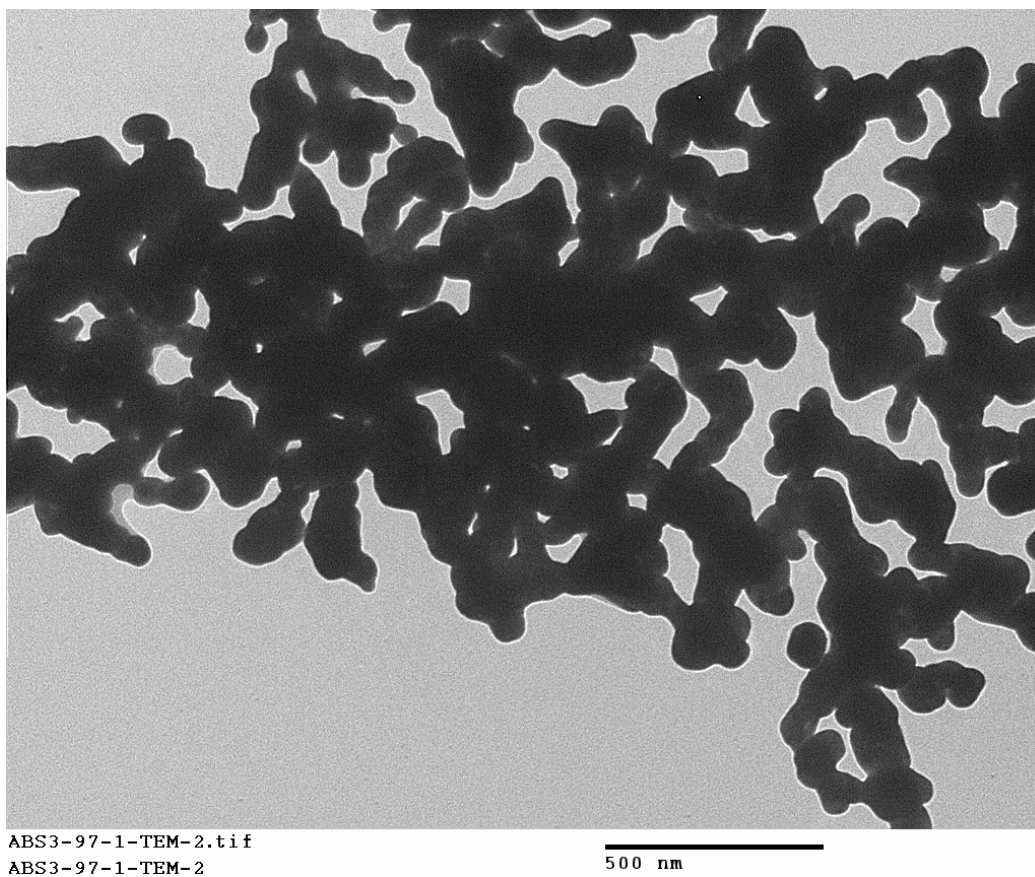


Figure 32 - TEM image of SMAD prepared silver powder.

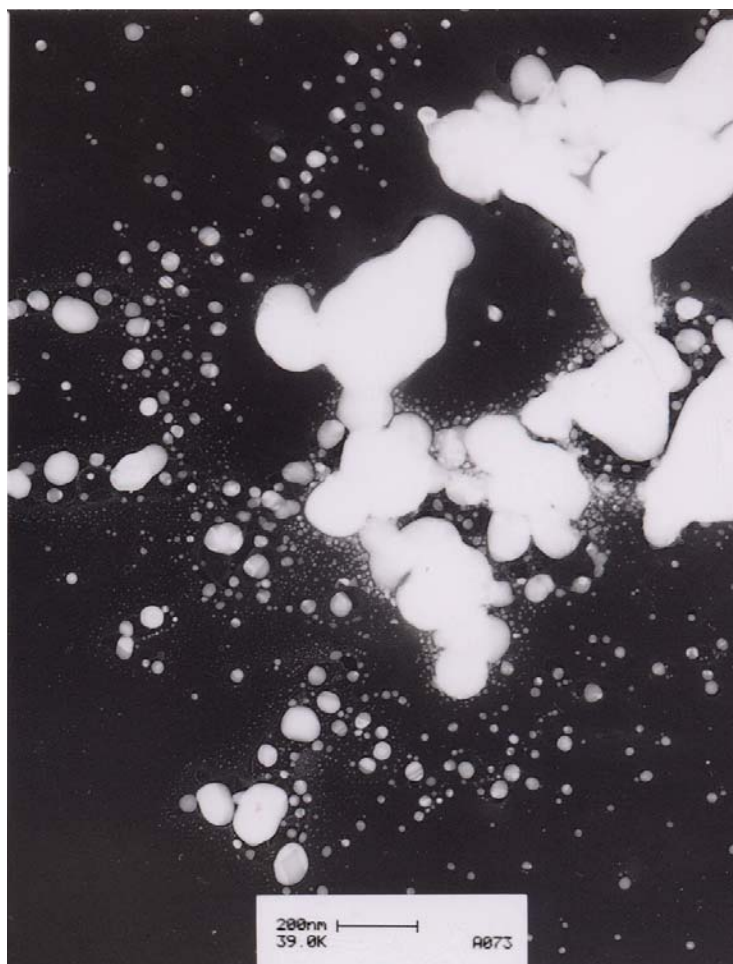
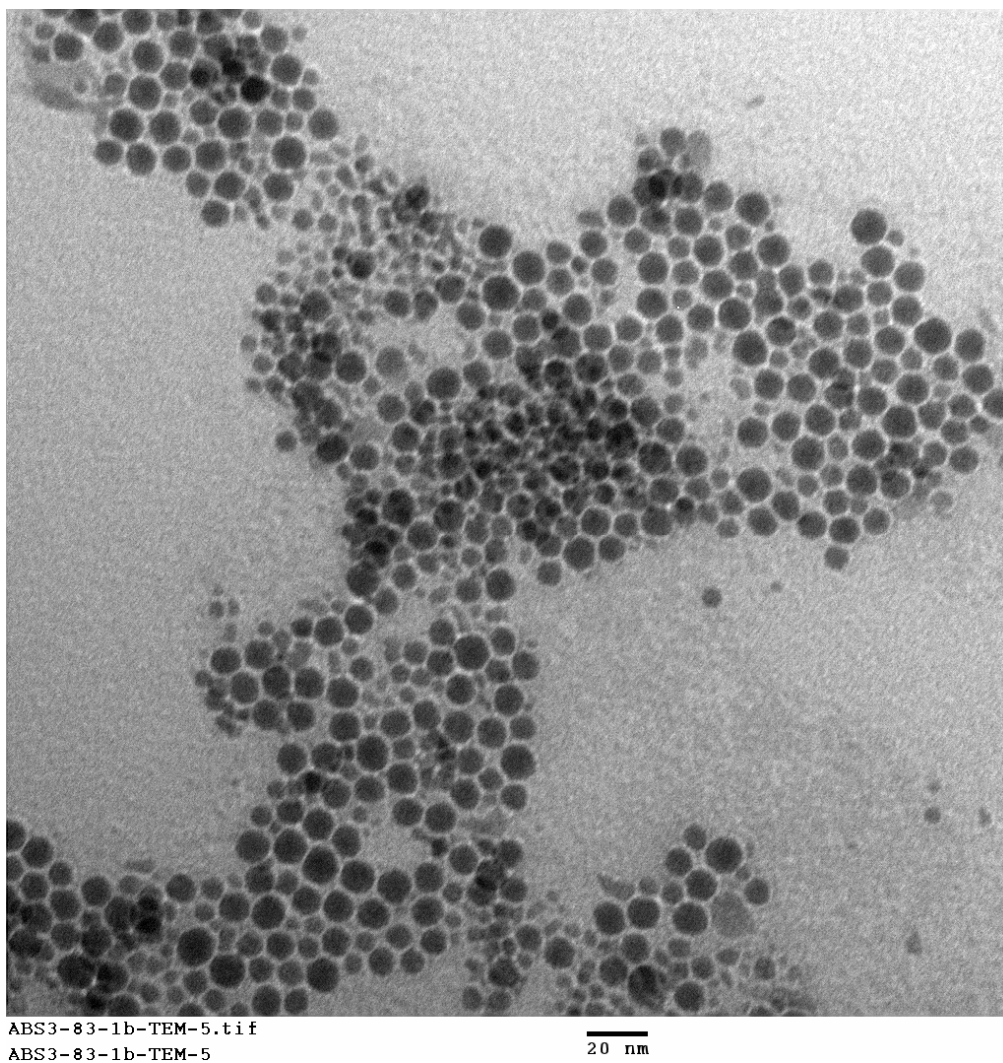


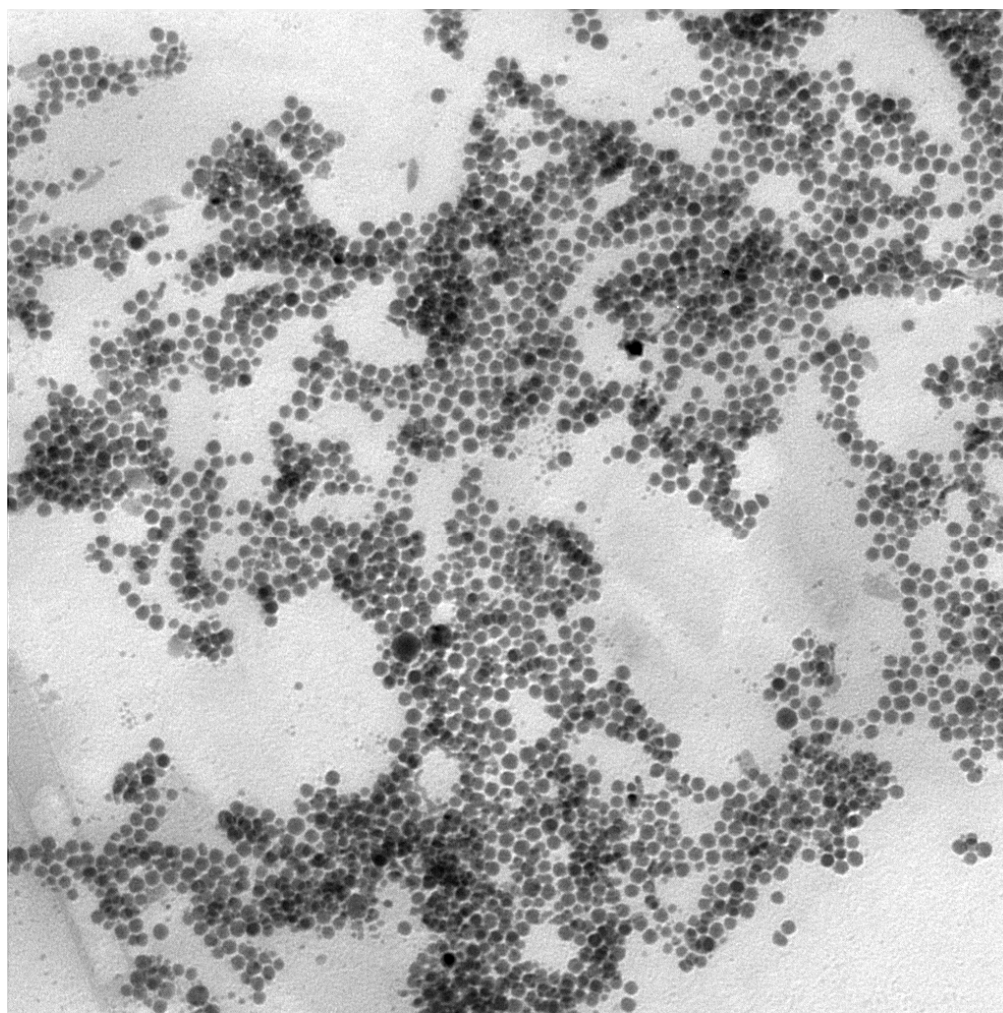
Figure 33 - SMAD powder after sonication.

Ligand-SMAD Colloids

In an attempt to try to prevent aggregation water soluble ligands were placed in the reactor so that when melting of the organic solvent takes place they would come into contact with molecules that would bind to the surface of the silver and provide a buffer from further inter-particle collisions. Three separate ligands were used which had varying success at passivating the silver nanoparticles evidenced by the particle size. The ability of the ligand to protect the nanoparticles was in the order of 3-mercapto-1,2-propanediol > sodium propane

sulfonate > sodium 5-mercapto-tetraazoleacetate. The results are displayed in the figures 34, 35, 36.





ABS3-83-1b-TEM-3.tif
ABS3-83-1b-TEM-3

100 nm

Figure 34 - 3-mercapto-1,2-diol protected nanoparticles

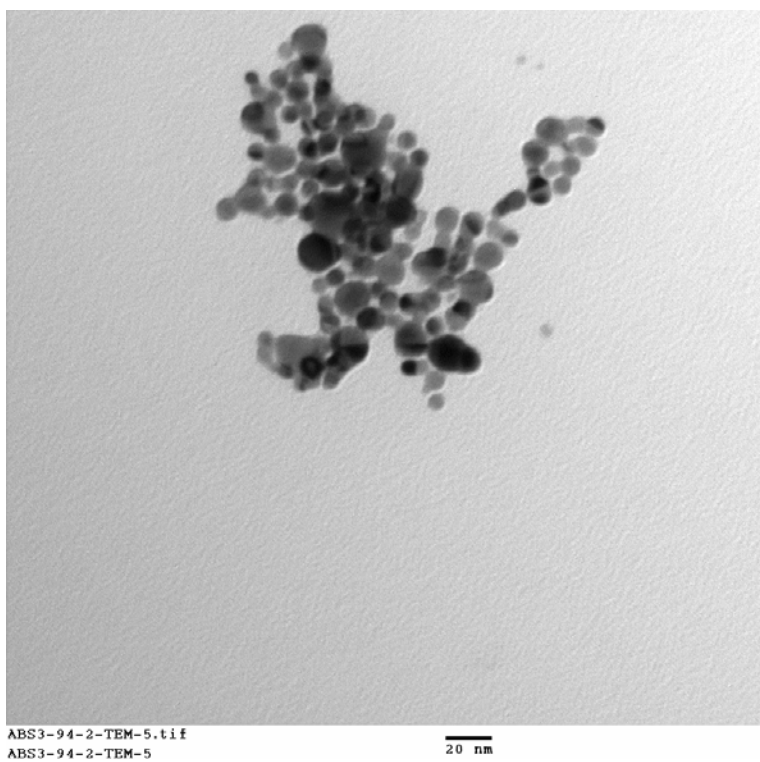
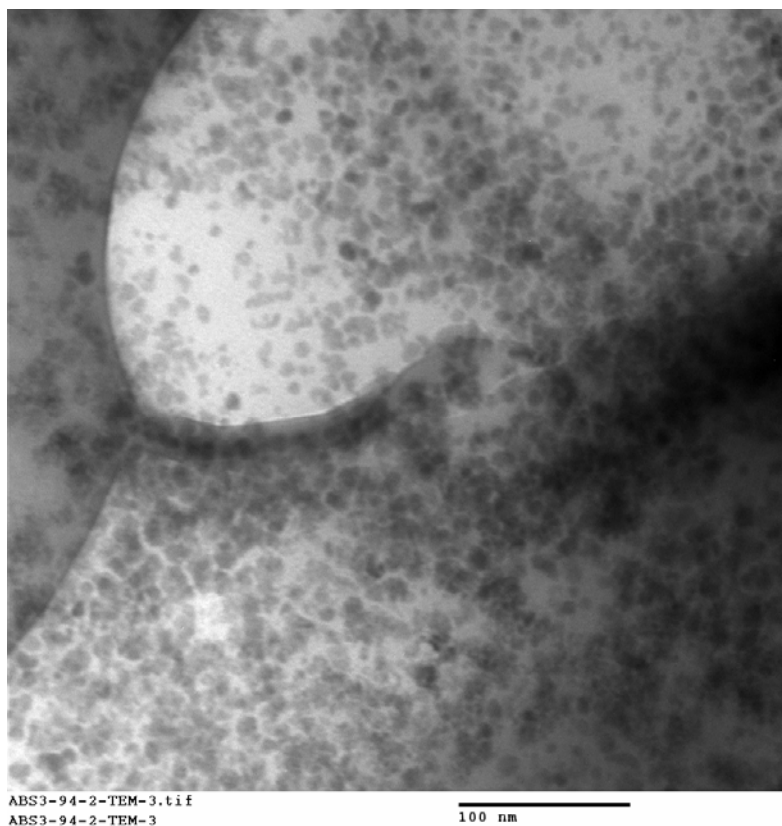


Figure 35 - Sodium 3-mercapto-propane sulfonate ligated silver particles

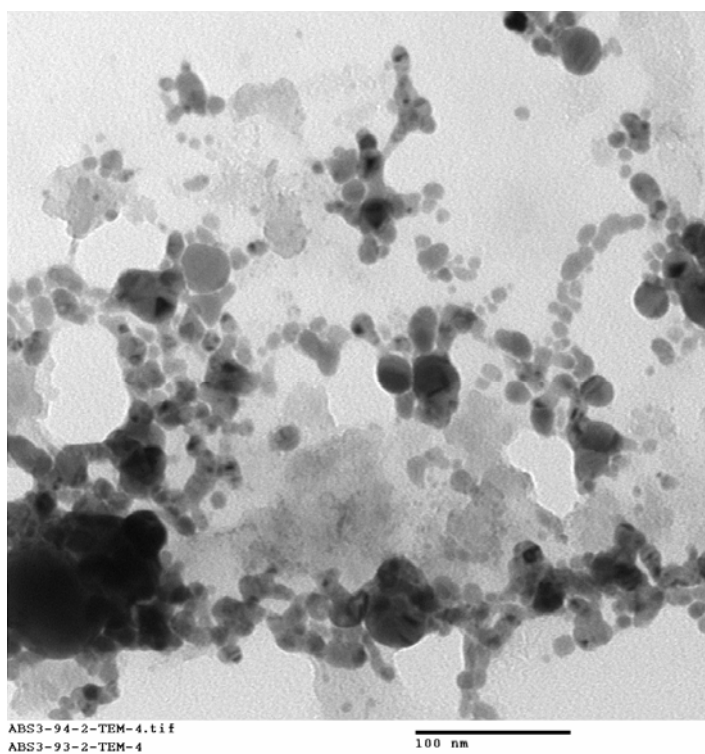
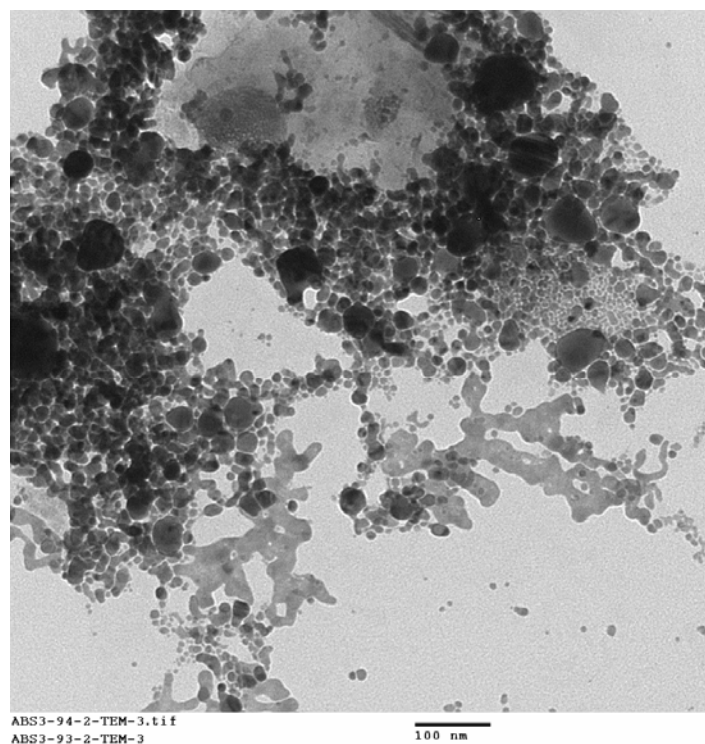
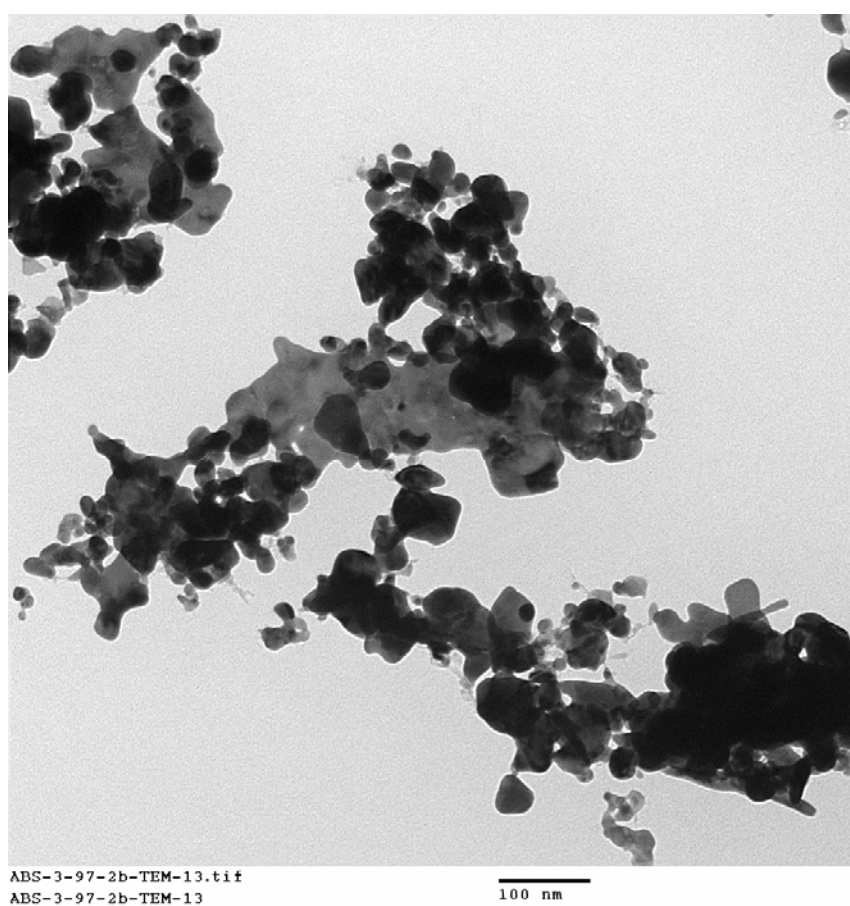


Figure 36 - Sodium 5-Mercapto tetrazoleacetate ligated silver nanoparticles

Air-SMAD powder

The metal atoms in this reaction are exposed to air at the stage when they are just beginning to come into contact with one another during the melting of the organic solvent. Surprisingly, exposure to oxygen did not increase the size of the final particles greatly as seen in figure 37. However there was some deviation from the mainly spherical particles.



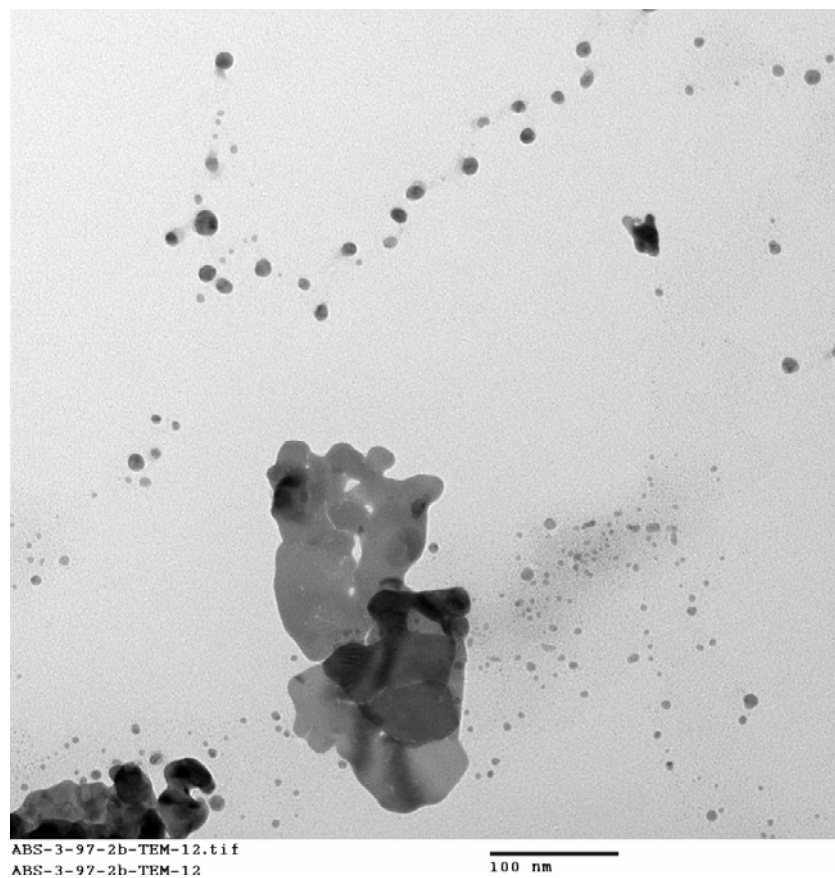


Figure 37 - TEM images of Air-SMAD powder

KOH-SMAD powder

The particle size from this preparation was increased markedly from other preparations even when acetone was used as the initial stabilizing agent in the synthesis. Isopropanol proved to be a very poor stabilizing agent and while it was needed to dissolve the KOH it had a negative effect on the size of the particles as can be seen in figure 38.

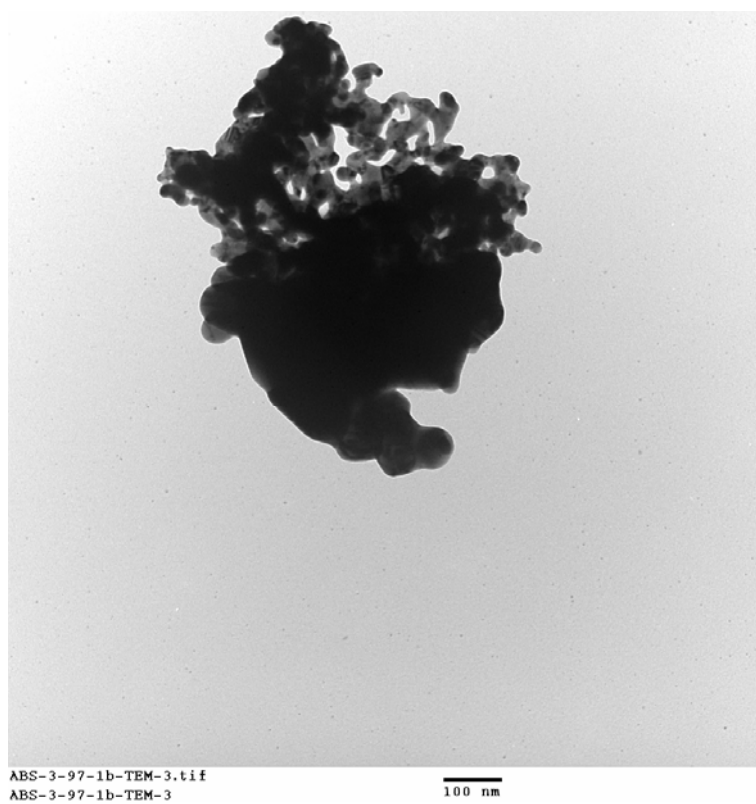
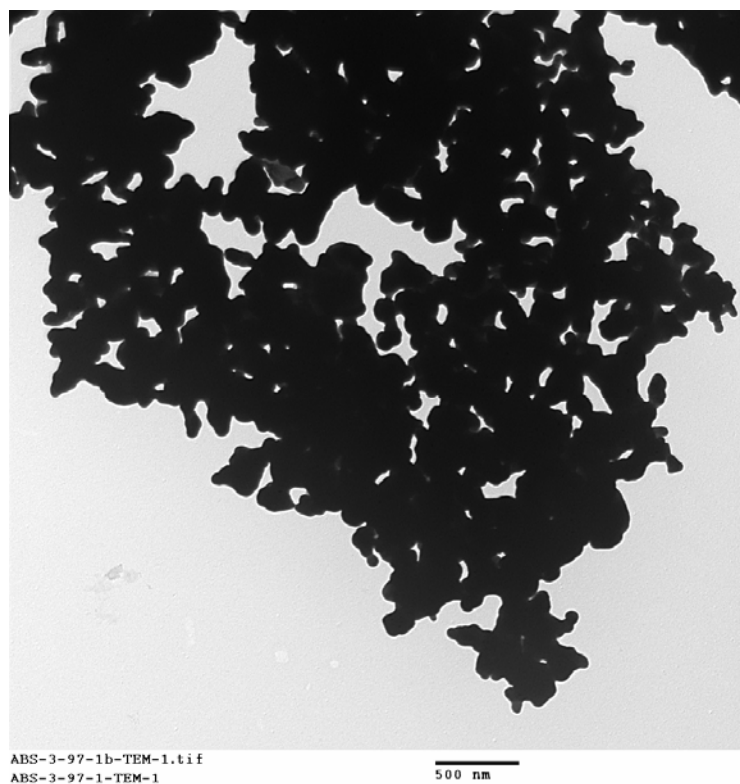
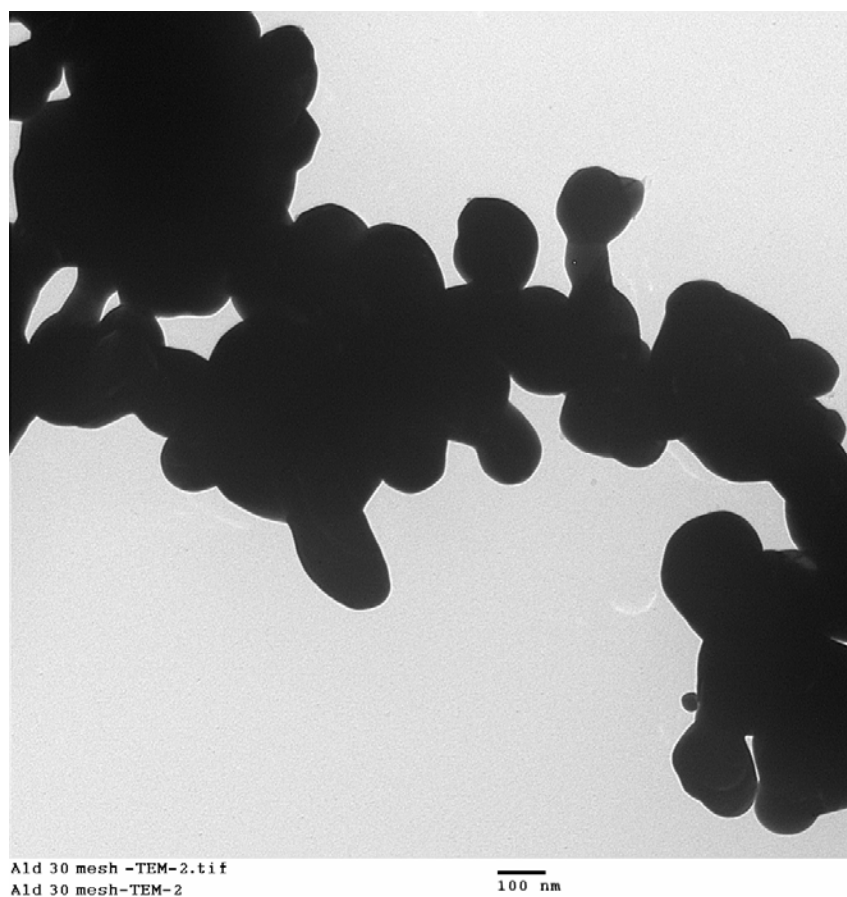


Figure 38 - TEM images of KOH SMAD powder.

Commercial Silver powders

Several silver powders were purchased from Aldrich to be used as standards of reactivity to our samples. TEM images of the commercial nano-sized powder show features that are not smaller than 100nm and overall the powders are $\sim 5\ \mu\text{m}$ in size seen in figure 39. The other silver powder was standard 60 mesh silver powder appears gargantuan compared to even this sample, seen in figure 40.



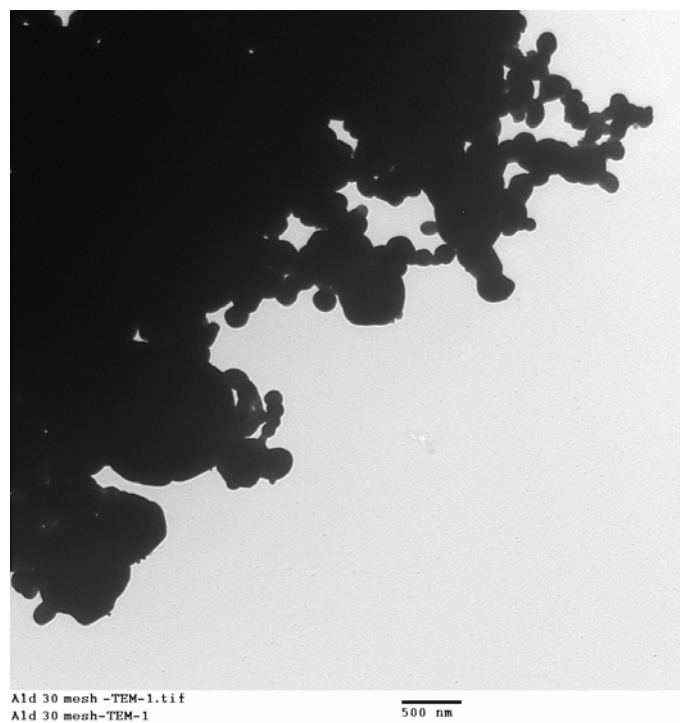


Figure 39 - TEM image of commercial nano-sized Silver Powder.

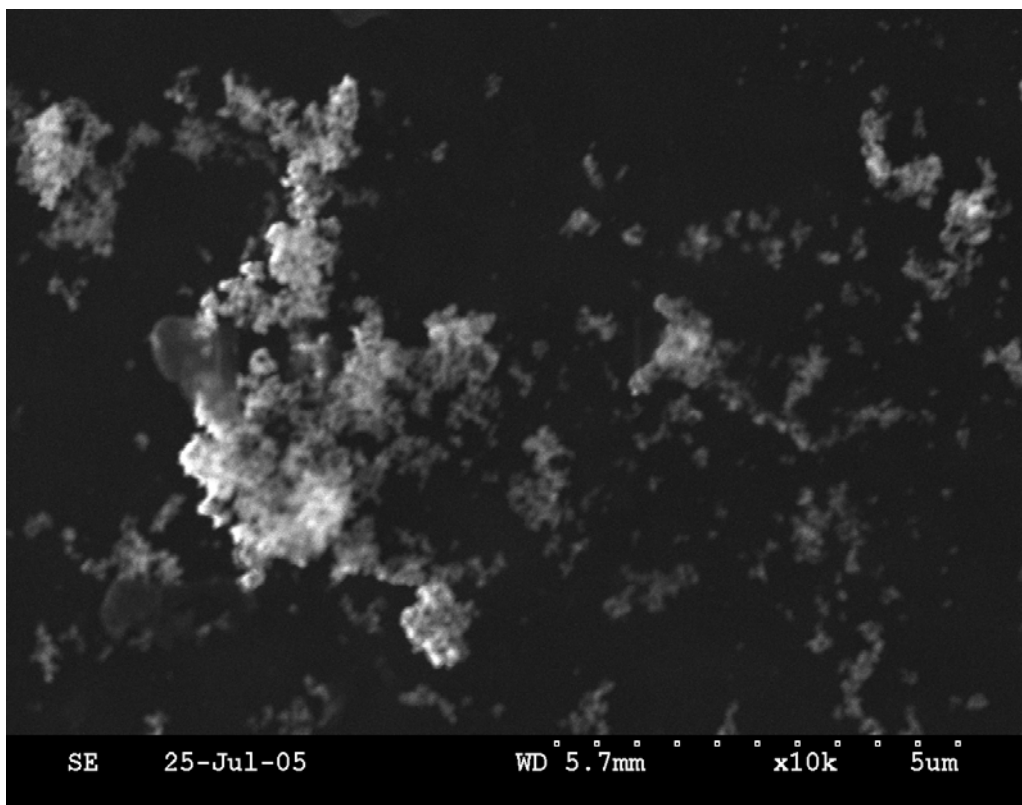


Figure 40 – Aldrich 60 mesh silver powder.

SEM

SMAD powder

The incredibly small surface features of the SMAD powders did not make quality imaging possible with the resolution of the microscope available. The perception of nano-scale morphology is alluded to, however cannot be clearly resolved as can be seen in Figure 41. Particle morphology is much more reliable from the TEM images for this sample.



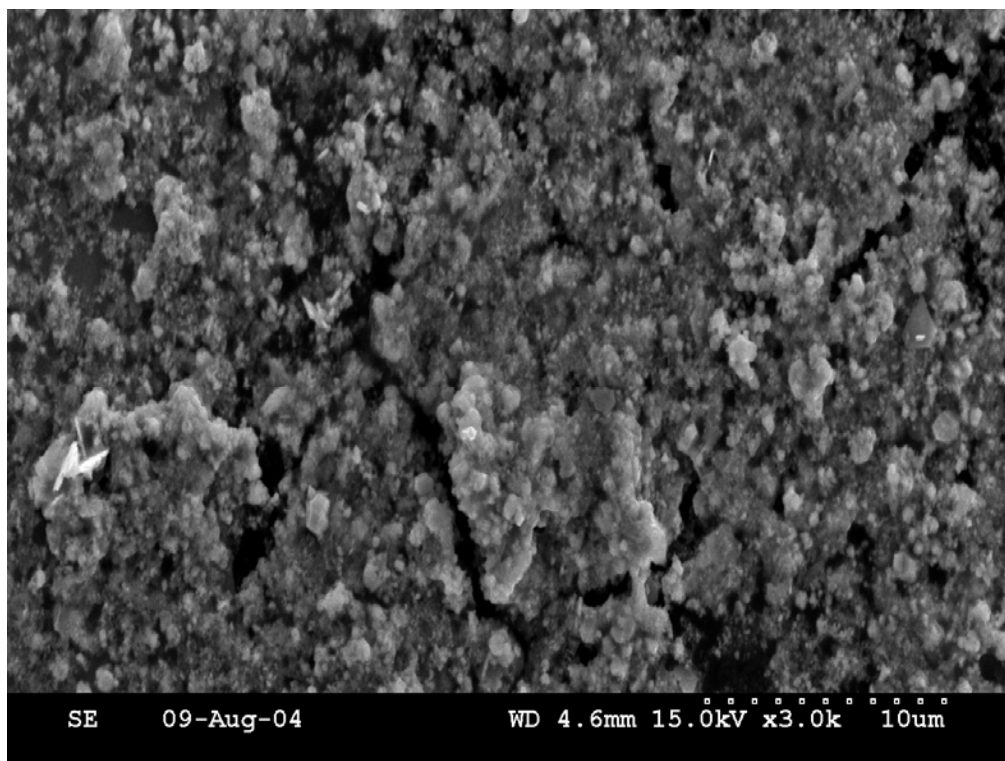


Figure 41 - High and Low magnification images of SMAD prepared silver powders.

Air-SMAD

The KOH-SMAD sample as well as the Air-SMAD sample did show a marked change in morphology from the standard SMAD synthesis. The AIR-SMAD sample displayed a propensity towards fibrous crystallites in addition to spherical agglomerates, seen in figure 42.

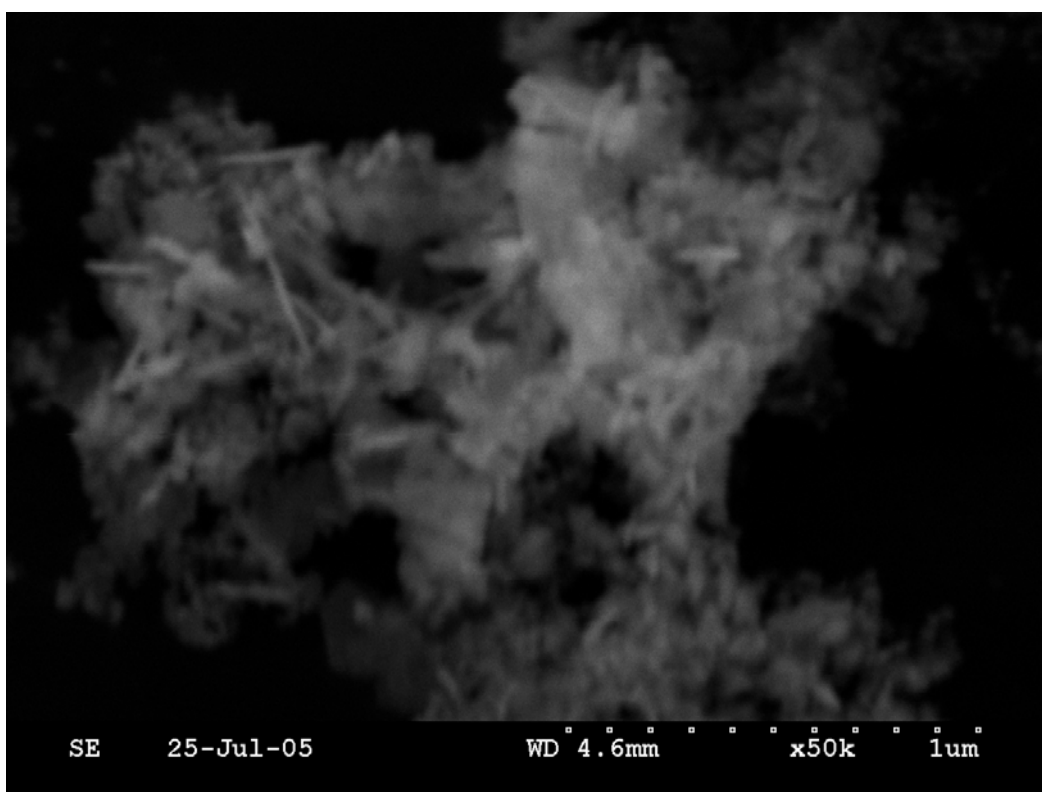
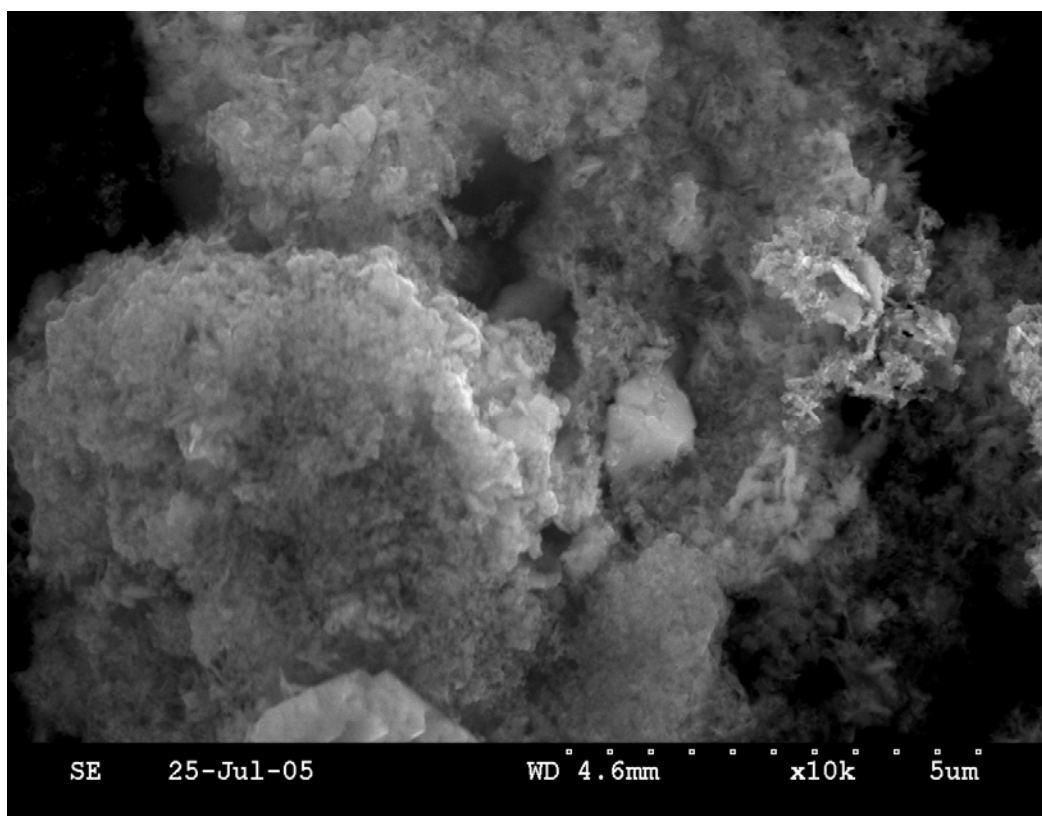


Figure 42 - SEM images of Air-SMAD powder displaying the fibrous nature of the powder.

KOH-SMAD

The KOH-SMAD sample had increased particle size as expected from being initially derived from a much poorer stabilizing solvent represented in figure 43.

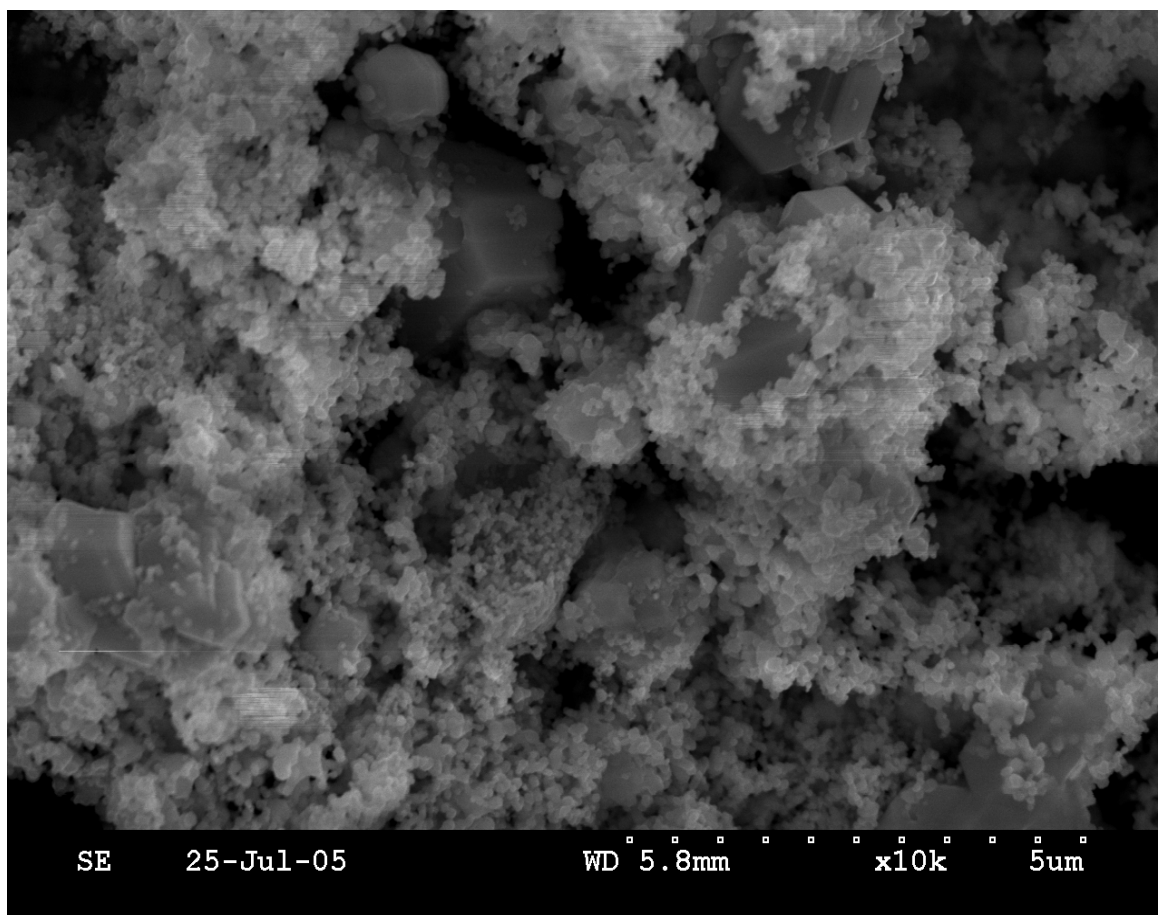


Figure 43 - SEM image of KOH-SMAD prepared nanoparticles.

Commercial silver powders

The SEM pictures for the commercial samples are very complementary to the TEM pictures. They clearly show the large particle size of over 1 μm and the lack of any interesting surface morphology and the likelihood of low chemical activity (see figures 44 and 45).

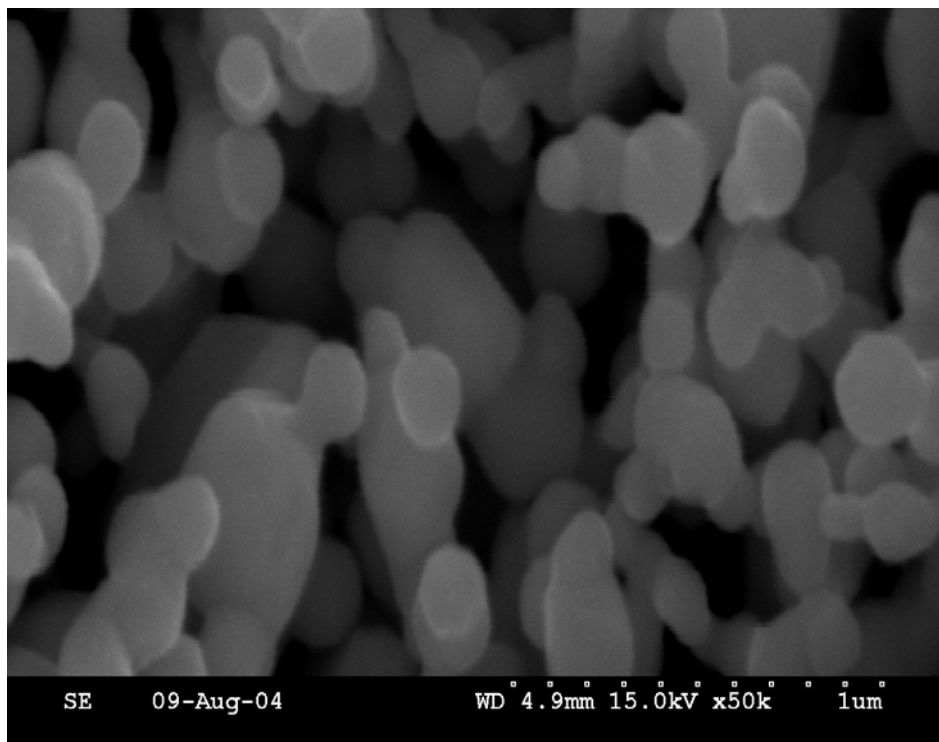
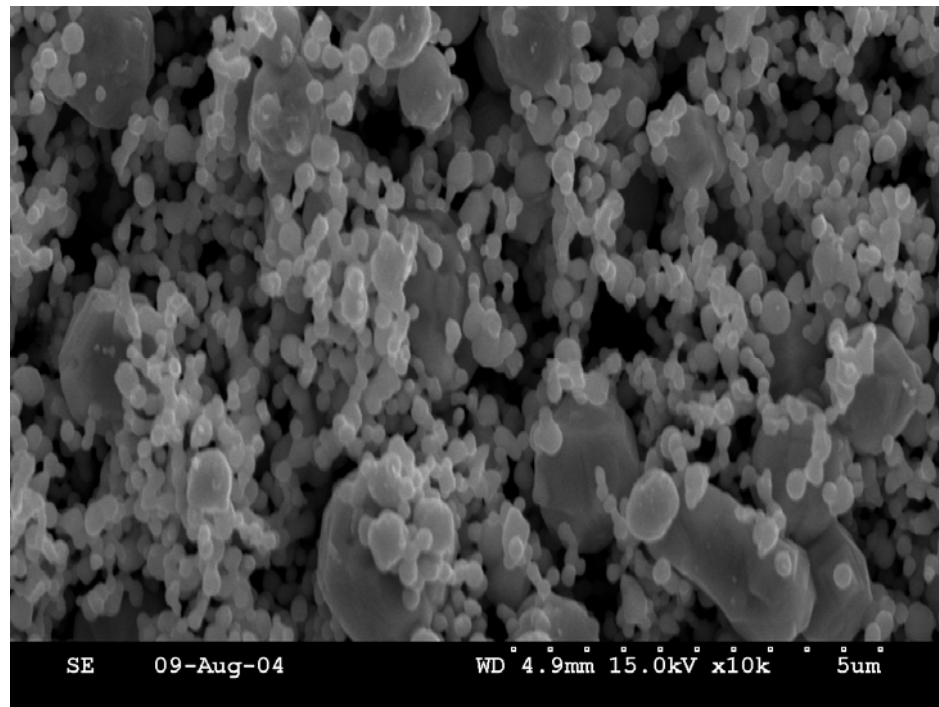


Figure 44 - Low magnification and high magnification SEM images of commercially purchased nano-sized silver powder.

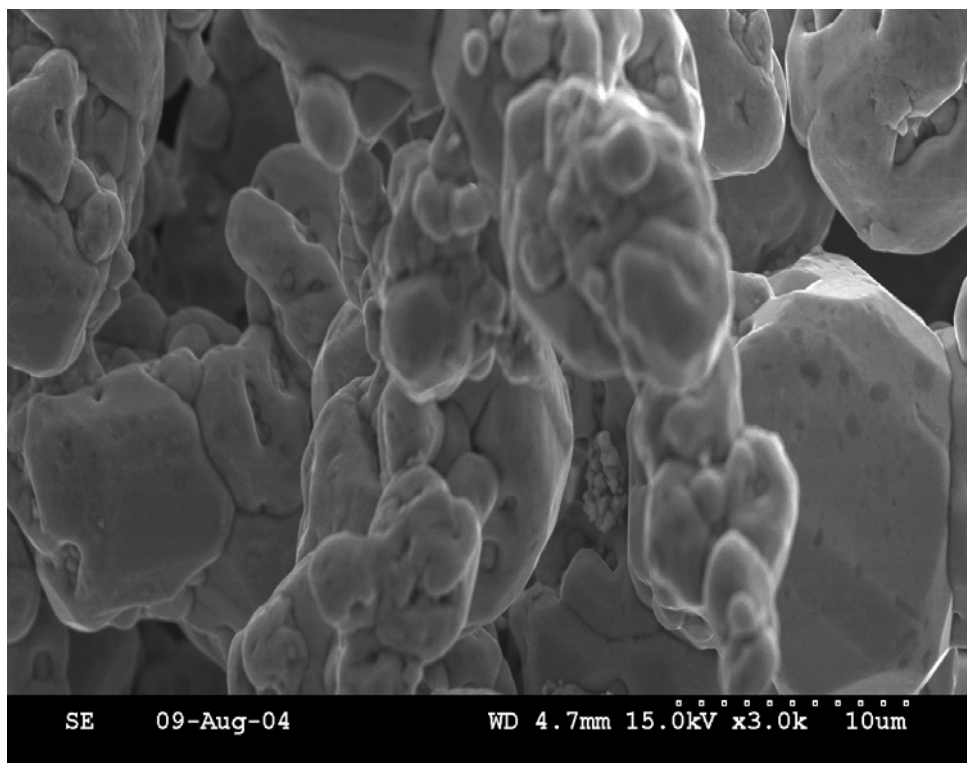


Figure 45 - SEM image of commercially purchased Silver powder 60 Mesh.

Ligand-SMAD

Considering the particle size of the water soluble ligand coated particles is an order of magnitude smaller than the SMAD prepared powder particles and these were past the limit of resolution for the instrument SEM images were not attempted for these preparations.

Particle surface area BET

As one measure of the activity of the silver powders, the available surface area was measured via nitrogen absorption/ desorption isotherm. A BET calculation of the data gave the following values for the pore size and surface area of the samples.

Table 3 - Surface area of SMAD prepared powders and commercial silver powder.

Stabilizing solvent	Surface Area
Pentane	5.1 m ² /g
Toluene	7.8 m ² /g
THF	6.0 m ² /g
Aldrich nano-sized	< 1 m ² /g

Commercially available nanoparticle samples did not have enough S.A. to register on the instrument. The SMAD prepared powders had the highest surface area as expected in lieu of the TEM data. The loss of surface area on going from the dispersed nanoparticles in the original organic solvent to a sintered dry powder is significant, however in order to remove all traces of organic residues and make them bio-compatible the process is necessary.

Delivery methods that can bypass this step by using ethanol as the organic solvent and leaving the particles suspended in this bio-friendly solvent would be advantageous. Alternatively the metal particle dispersion could be stored in the acetone solvent to be applied to polymer supports for physical separation as the organic solvent evaporates and leaves the metal precipitate with its high surface area. Attempts to exchange the solvent from organic to aqueous by first evaporating the metal into acetone, mixing with water and subsequent evaporation of the acetone also was observed to cause agglomeration of the particles and loss of surface area.

Composition of Powders

X-ray diffraction

To confirm creation of silver nanoparticles and test the purity of the sample, XRD was performed on all the samples. The major peaks for silver metal are as follows 38.1, 44.2, 64.4, and 77.5¹³. As can be seen from the figure 46 below all samples display strong peaks at each of these values. No other peaks can be discerned from the background of the diffractogram.

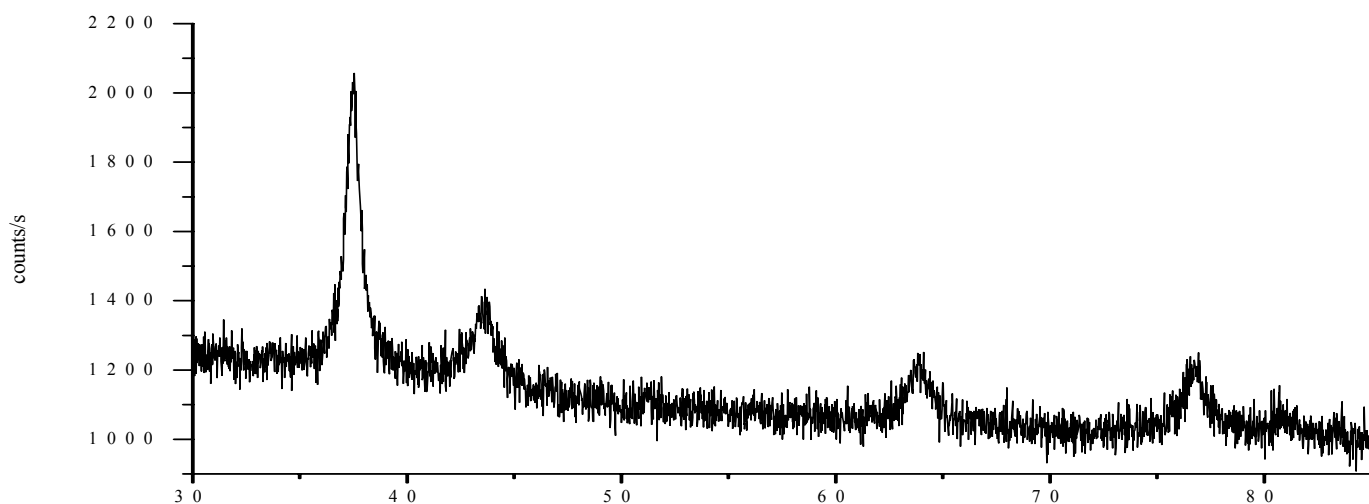


Figure 46 - XRD diffractogram for SMAD prepared silver powder synthesized with acetone as the stabilizing solvent.

The Scherrer equation was used to calculate the average crystallite size of each sample. An example calculation is provided below.

$$T = \frac{0.9\lambda}{B \cos\theta}$$

λ = wavelength of radiation (nm)

B = full width at half max of major peak in radians.

θ = position of peak in radians.

T = crystallite size in nm

Table 4 - Crystallite sizes of SMAD silver powders from Sherrer equation

Solvent	Crystallite Size
Pentane	18 nm
Toluene	23 nm
THF	17 nm
Acetone	11 nm

An XRD of the AIR-SMAD powder displays peaks solely for silver metal. Any silver oxide from the samples is either amorphous or not present. A comparison of the XRD spectra, Ag₂O, and AgO are shown in figure 47.

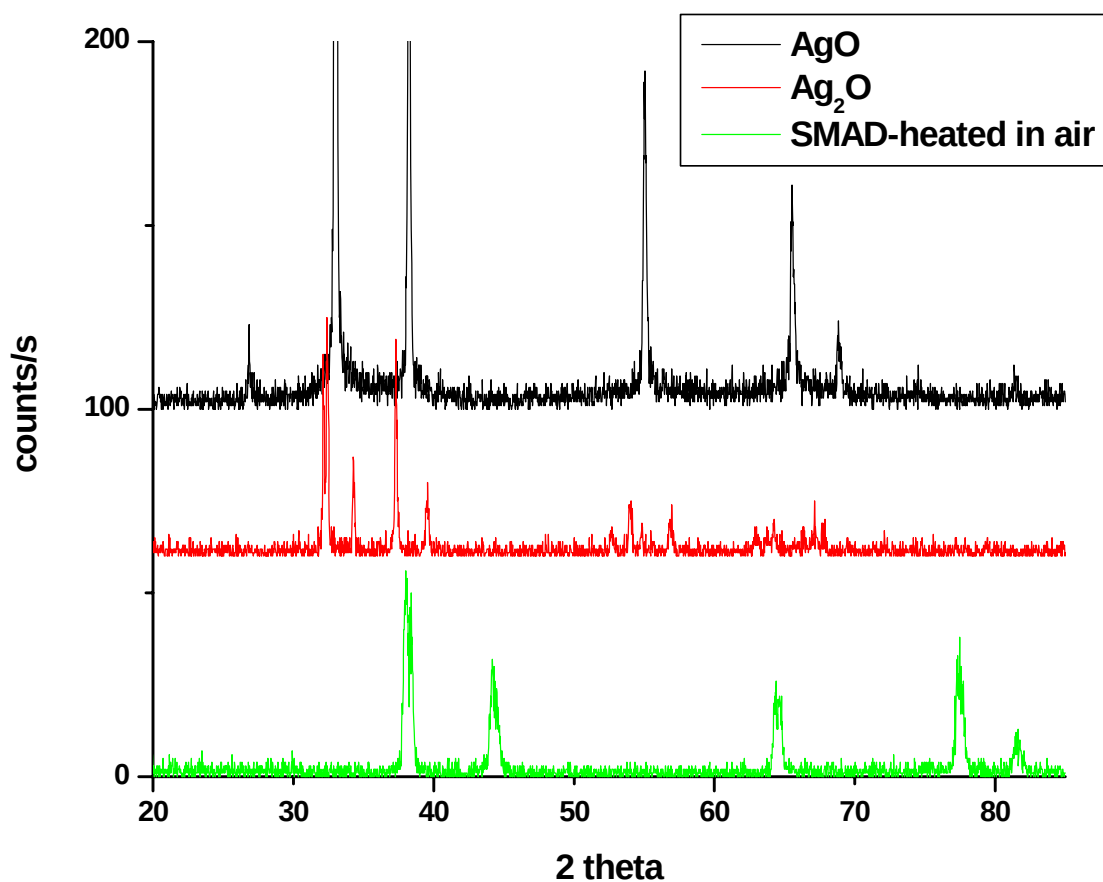


Figure 47 - XRD spectra of SMAD-Heat treated in air sample (green) vs. silver (I) oxide, (red), and silver (II) oxide (black).

EDX

To test for the presence of oxygen incorporated into the silver nanocrystals, EDX spectroscopy was applied to the samples. The SMAD prepared silver sample displayed no elemental oxygen peak; however a small percentage of sulfur was observed in the sample. This is believed to be tarnishing from the atmosphere reacting to the highly active surface of the powder. The lack of this peak in the other samples suggests lower binding and chemical

reactivity of the surface of the powders. Unfortunately even the SMAD samples that were heated in the presence of oxygen did not display an oxygen peak in the spectrum. Despite the efforts to oxidize the surface of the powders in the Air-SMAD, KOH-SMAD, and SMAD-Heat treated; no traces of oxygen were found for these samples. This suggests oxygen concentrations of less than 1% if they exist.

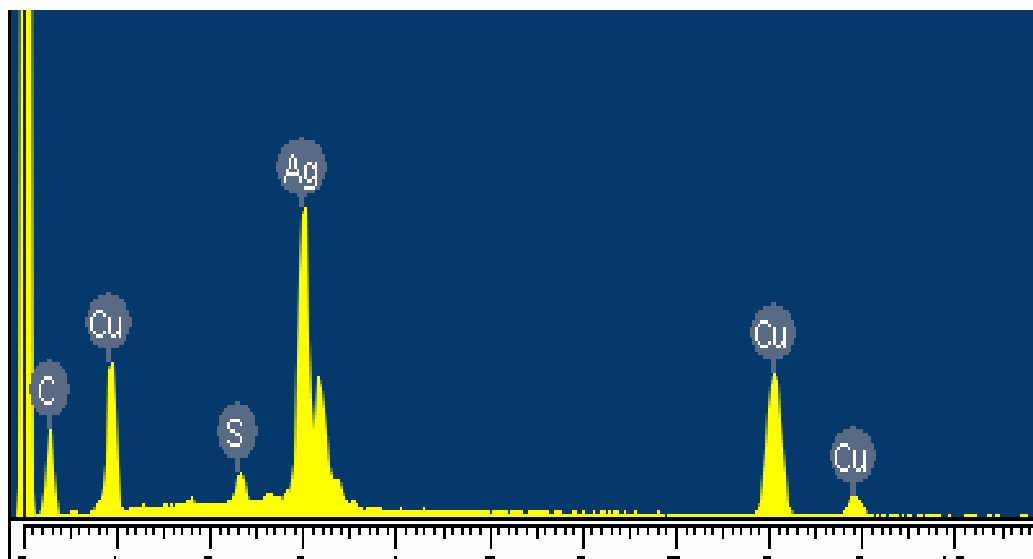


Figure 48 - EDX spectra of SMAD silver powder. The carbon and copper peaks are present because the sample support is a carbon coated copper TEM grid.

XPS

In a final attempt to detect the presence of oxygen in the powder preparations XPS spectroscopy was applied. Silver foil, Ag_2O , and AgO were purchased from Aldrich and used as standards in the instrument. Then the Air-SMAD, SMAD, and SMAD-heated samples were compared against these standards to determine the amount of silver oxide present in the powders. The peak shape and position of the standard samples are consistent with the literature and show

that there is a significant difference in the valence band peak in all three samples^{14,15,16}. The raw data from the SMAD samples must be analyzed to determine the contribution of each oxidation state to the sample peak. This procedure is hoped to be accomplished soon.

Microbiology Testing

Despite silver's extensive use throughout history as an antimicrobial agent, the discovery of penicillin caused a dramatic shift in the way bacterial infections were treated. The rise of resistant strains of bacteria, and the need for a more general disinfectant that can be active against all types of single celled pathogens is desired. To facilitate this need several pathogens were tested against the silver preparations in the hope of finding a novel and effective agent. A class strain of *E. coli* represented gram negative bacteria while *Staphylococcus a.* was used as a gram positive bacterium. Both bacterial strains are fairly robust and resist disinfection. The fundamental difference between gram negative and gram positive bacteria is the nature of the cell wall. Gram positive bacteria have an additional thick peptidoglycan layer that shows purple when treated with crystal violet during the Gram stain. Gram negative bacteria have an additional outer membrane but a much thinner peptidoglycan layer between its inner, and outer membranes. The drastic difference in the nature of the cell boundary in contact with the environment often dictates the basic response of the cell to environmental forces such as antimicrobial agents. In addition to bacteria MS2 a virus that is a surrogate of a human enterovirus was tested with the silver samples to gauge the effectiveness of nanoparticulate silver to viruses¹⁷.

Dry silver powder testing

Dry powders were found to be initially difficult to disperse in an aqueous environment. This is likely due to pockets of air in the pores of the powders, and an inability to break the surface tension of the water. Low power sonication of the sample for 5 minutes along with vigorous shaking of the sample sufficiently disperses the sample in water for testing. Typically 150mg of silver powder was dispersed in 10 ml of Millipore water. Aliquots of this mixture were either tested directly or diluted to appropriate concentrations.

Bacteria was grown by standard methods overnight in Luria broth (LB) in a shaking water bath set to 37 °C¹⁰. The bacteria solution was diluted to 10⁵ CFU (colony forming units)/ml to meet with EPA standards for bacterial reduction in drinking water¹⁸. Typically 1-2 mL of bacteria solution was pipetted into a 10 ml centrifuge tube with 1 ml of a silver dispersion, and 2ml of Millipore H₂O. After 10 minutes of contact time the tubes were centrifuged at 2500 rpm for 1 minute. This was found to be a treatment that could precipitate the silver powder, but leave the bacteria suspended in solution. A 100 µl aliquot was removed from the supernatant of the sample, diluted and plated in triplicate. The bacteria plates were of Tryptic Soy Agar and incubated overnight at 37 °C. Surviving colonies were counted and recorded.

To remove Ag⁺ from AgNO₃ samples an excess of NaCl was added to the sample and resulted in a white precipitate. This salt concentration was added to bacteria alone and was not found to have a detrimental effect on the cells. The same procedure was also used to precipitate the Ligand-SMAD samples, since the increase in ionic concentration of the solution caused the colloid to destabilize and flocculate which was then easily separated by centrifugation.

The results of these tests are displayed below. For each sample the concentration of silver in mg needed to completely kill the bacterial cells is typically an order of 10 greater than

that displayed if there are surviving colonies. All samples were tested against 10^5 CFU's with the number of survivors listed.

Table 5 - Bacteria survival vs. silver sample

Sample	Grams of silver sample (g)	E. Coli (10^5)	Staph A. (10^5)
No sample (control)	-	100,000	100,000
Powders			
Aldrich 60 mesh	0.015	79	53
Aldrich 60 mesh	0.0015		72
Aldrich nano sized powder	0.015	0	5,41,49
Aldrich nano sized powder	0.0015		84
AgNO ₃	0.0015	0	0
AgNO ₃	0.00015		0
AgNO ₃	0.000015		0
SMAD	0.015	0	0
SMAD	0.0015		1
SMAD	0.00015		53
Acetone, Oxygen	0.015		
Acetone, Oxygen	0.00075	1	2
Acetone, Oxygen	0.00015	1	0
Acetone, KOH	0.015		1000
Isopropanol, KOH	0.015		0
Isopropanol, KOH	0.0075	0	3
Isopropanol, KOH	0.0015	7	153
Ligand capped particles			
3-mercapto-1,2,propanediol	0.015		5000
3-mercapto-propanesulfonate	0.015	175	11
5-mercapto-tetrazolacetate	0.015	84	5000
Pure Ligand (no silver)	0.1 ml	10^5	
Ligand plus SMAD powder	0.015	10,000	1000
Ligand plus AgNO ₃	0.0015	500	100

* all values are an average of CFU's taken in triplicate.

It is possible that the bacteria were merely sticking to the heavy silver powder and being precipitated in the centrifugation showing as a false positive. To test this, the silver precipitate was taken from a sample and smeared wholly on an agar plate and incubated. Still no growth of any bacteria was seen showing that the cells were in fact no longer able to reproduce, if not killed.

To test for activity with time another set of experiments were carried out, that included results from the Air-SMAD powder and silver oxide samples. Two ml of 10^7 bacteria were introduced into an Erlenmeyer flask that contained 97ml of autoclaved Millipore water and stirred constantly. To this mixture 1 ml of silver sample was injected. 1 ml aliquots of the dispersion were typically removed at 0, 10, 30, and 60 minutes of contact time. Once the sample was removed from solution it was placed in a 1 ml Eppendorf vial, and centrifuged at 2500 rpm for 10 seconds. This was sufficient to bring the silver nanoparticles to the bottom of the flask while leaving the bacteria suspended in solution. From this tube 100 μ l of the supernatant was removed, then diluted to suspected concentrations of 3, 30, 300 CFU's and plated in triplicate.

Table 6 - Effect of silver product with time of contact

Sample	Quantity (ppb)	Surviving bacteria		
		0 minutes	5 minutes	30 minutes
SMAD	1500	50,000,000	0	0
	150	5000	1840	0
	15	5000	3600	670
Aldrich Nanosized powder	1500	12,400,000	1,000,000	32,000
Aldrich 60 mesh powder	1500	11,000,000	3,000,000	28,000
Silver pellet	1500	303,000	279,000	94,000
Air-SMAD	150	450,000	0	0
	15	5,000,000	4000	0
	1.5	5,000	40	0
KOH-SMAD	150	270,000	200,000	14,000
Ag ₂ O	150	46,000	0	0
Ag ₂ O	1.5	10,000	0	0
AgO	150	46,000	0	0
AgO	1.5	19,000	200	200
SMAD-Heat treated	1.5	18,900	11,300	6,000
3-mercapto-propane sulfonate	1500	256,000	233,000	133,000
5-mercapto-1-tetraazolacetate	1500	256,000	105,000	0
3-mercapto-1,2,-propanediol	1500	620,000	145,000	28,000
AgNO ₃	0.15	22,000	15,000	500

Disinfecting agent

Initially it was unknown what the active agent interacting with the bacteria was. The possible candidates are contact with the silver powder (presumably silver(0) atoms on the surface of the particle), Ag⁺ dissolved into solution from the silver powder, or Ag(0) atoms eroded into solution from the silver powders. To test for Ag⁺ activity the following experiment was

performed. 5 centrifuge tubes were filled with 2 ml of water. Two of these tubes had 1.0g of NaCl added. Two tubes, one with NaCl and one without, was added to the supernatant liquid from 1 ml of SMAD powder centrifuged at 2500rpm for 3 min. Two tubes, one with NaCl and one without, were also injected with 1ml of 15mg Ag^+ . These sample tubes were stirred vigorously in a vortex shaker, then to each 1 ml of 10^5 CFU/ml of bacteria was added. The samples were shaken again and allowed to be in contact for 10 minutes. The tubes were then centrifuged, and the supernatant was diluted and plated in triplicate. The results of which are displayed in the table below.

Table 7 surviving bacteria in the presence of silver samples, with and without NaCl.

Test tube contents with bacteria	Surviving bacteria
Water (control)	1000
SMAD supernatant	0
SMAD supernatant + NaCl	500
AgNO_3	0
AgNO_3 + NaCl	500

If Ag^+ is the only active agent in the SMAD powdered sample little to no activity will be found when interacted with NaCl. This was found to be the case, and a white precipitate was found in the SMAD supernatant sample as well as with the AgNO_3 . When either sample was introduced to a sample tube without NaCl the bacteria were killed 100%.

In light of this information the amount of Ag^+ dissolved into solution from the SMAD powders with time was desired. 1.5g of SMAD powder was sonicated and shaken into 40 ml of Millipore water in three separate batches. At times of 10 minutes, 30 minutes, 60 minutes, and

24 hours one batch was centrifuged and decanted. The conductivity of the supernatant of each batch was tested. The conductivity meter and cell was calibrated with a 0.01M KI solution. Standard concentrations of AgNO_3 were prepared at concentrations of 50, 25, 10, 5, 2.5, .5 ppm. The conductivity values for each batch were plotted vs. the standard fit of the known preparations shown in the figure below. The concentration of each sample is 10 minutes: 4.5 ppm, 60 minutes: 4.8 ppm, 24 hours, 8.0 ppm. It should be noted that the amount of silver used was 10 times as much as that interacted with the bacteria for a 150 mg sample. This suggests 0.4 ppm of silver ion is all that is needed to get into solution to kill the EPA level of bacteria.

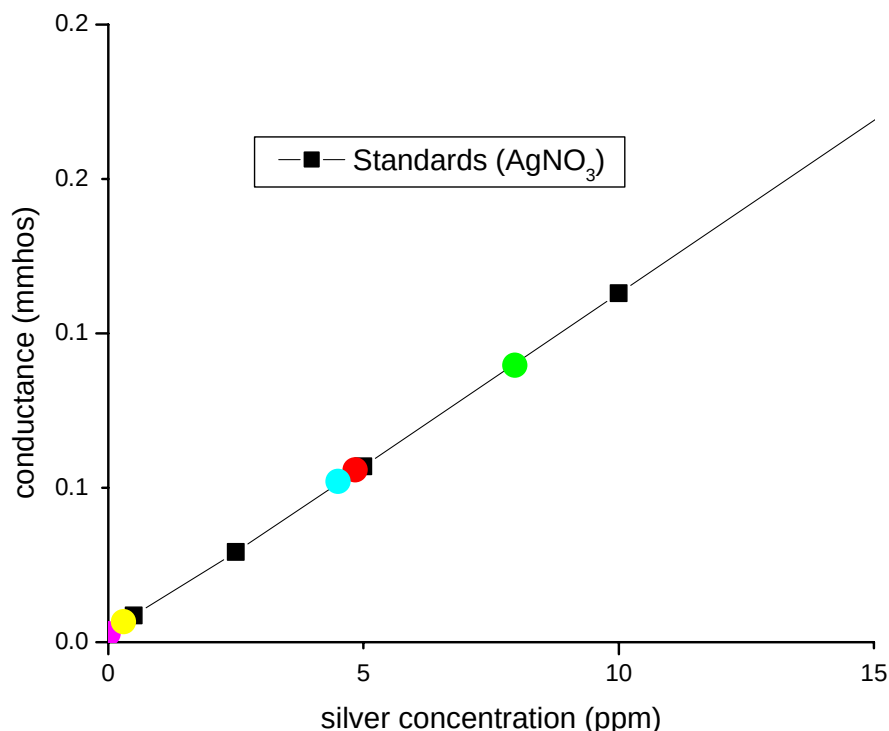
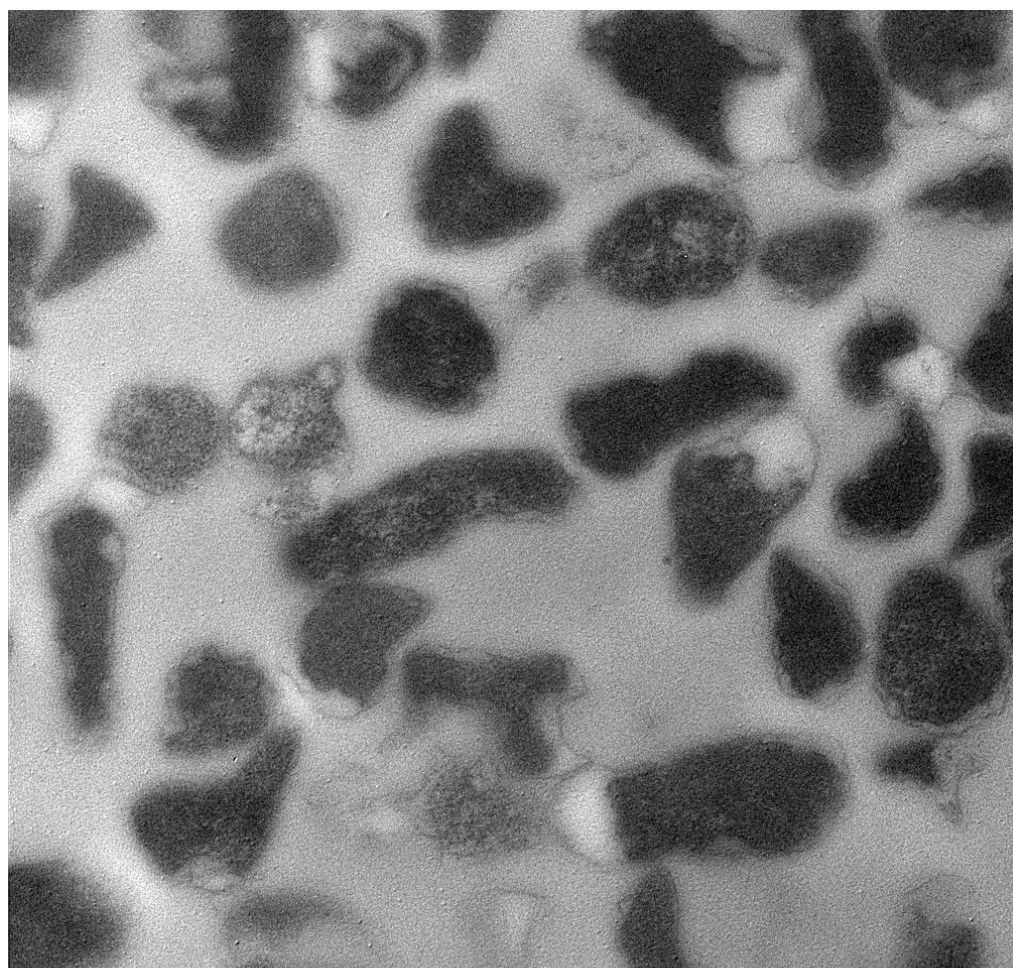


Figure 49 - Concentration of Ag^+ in solution from silver pellet 24 hours (yellow), SMAD powder 10 minutes of contact (blue), 60 minutes of contact (red), 24 hours of contact (green). Standards from silver nitrate and curve fit are in black.

Nanoparticle and bacteria interaction-TEM images

In a further attempt to understand the interaction of the silver nanopowders with bacteria, selected bacteria samples were stained and fixed after interaction with the silver products. To prepare the images *E. coli* cells were first captured in the exponential growth phase. This was found to be after 13 hours of incubation time under the laboratory conditions used. These bacteria were then interacted with an excess of silver material needed to kill the pathogens from previous results. These dead bacteria were separated as much as possible from the silver sample by centrifugation. The bacteria were then fixed according to standard procedures described here¹⁹. Bacteria was fixed with a 2% solution of glutaraldehyde for 16 hours, and washed 3 times by shaking for five minutes with a sodium cacodylate buffer. After every wash the sample was centrifuged at 12,000 rpm for 1 minute to separate the bacteria from solution. The sample was then stained with 1% osmium tetroxide for 1.5 hours. The sample was washed another three times and then dehydrated by washing with 50%, 60%, 70%, 80%, 90%, 95%, and 100% acetone for 5 minutes apiece. The sample was then washed with a 1:1 acetone to resin mixture, and finally set overnight in 100% resin. The sample tubes were heated in an oven to harden the resin and cut with a microtome diamond knife and viewed in the electron microscope.

Bacteria in contact with silver nitrate from our procedure reacted similarly to the literature^{19,20}. It appears the silver ions make it into the cell where they are reduced by the cell and attempted to be removed from the cell most likely by a reductase mechanism for cell self-defense²¹, as seen in figure 50. The SMAD powder has a very similar effect on the bacteria. It appears in this case however that the deposits of silver reduced in the bacteria are much smaller, and dispersed much more thoroughly throughout the cell as if the cell has a much harder time coping with this delivery, as shown in fig 53.



3h.tif
3 grid d3 h

500 nm

Figure 50 - TEM image of healthy bacteria.

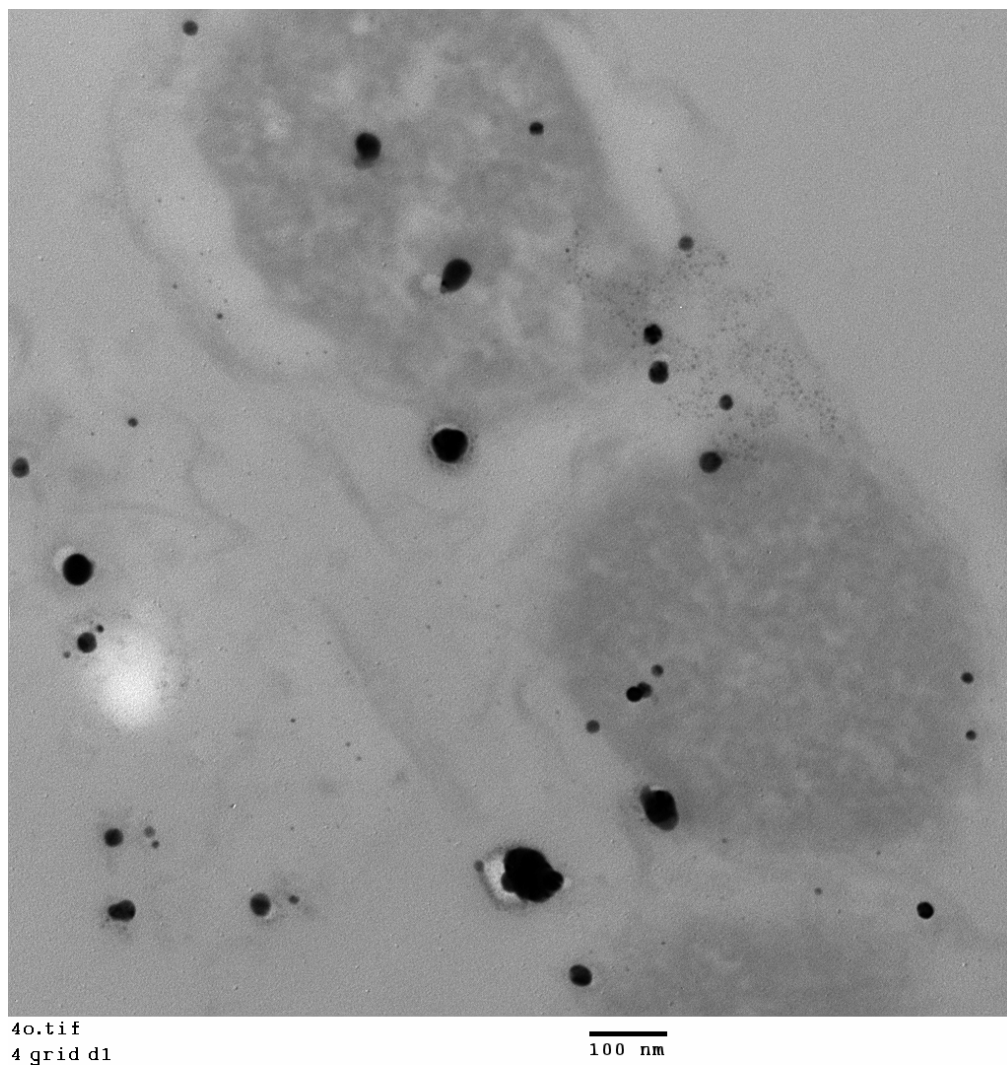


Figure 51 - TEM image of bacteria after interaction with AgNO_3 .

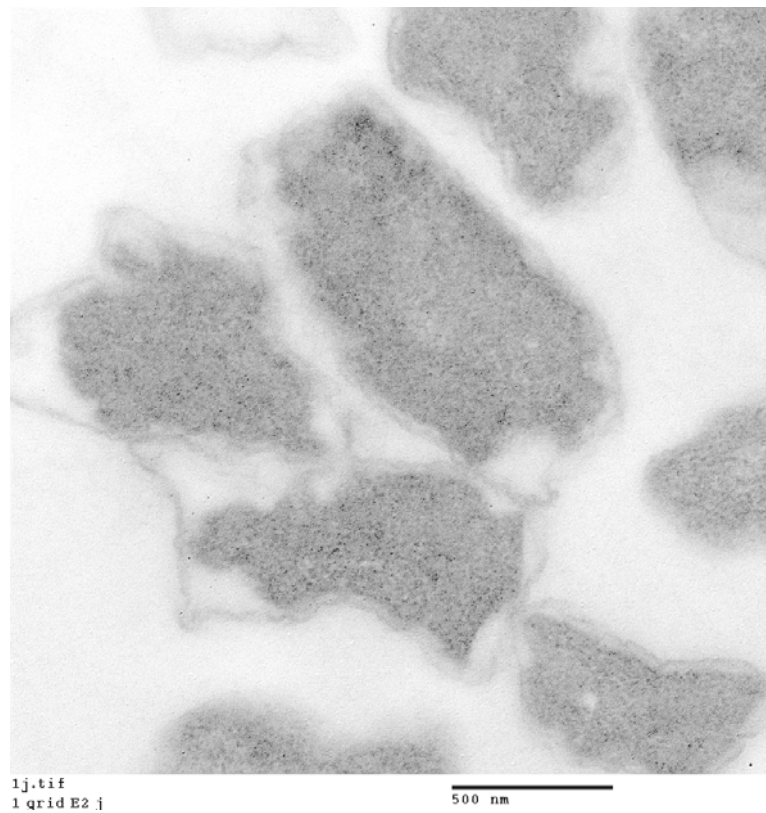
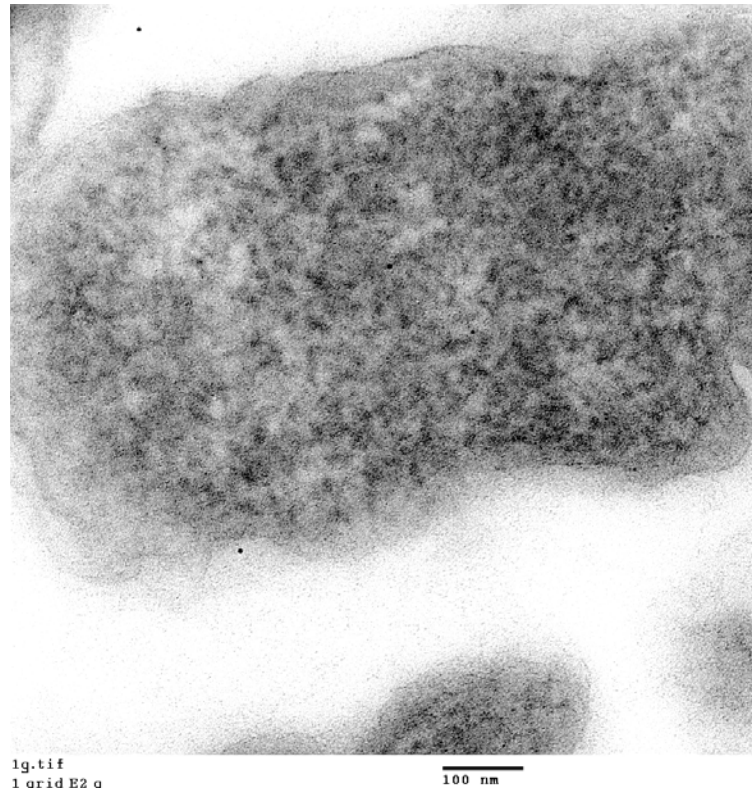
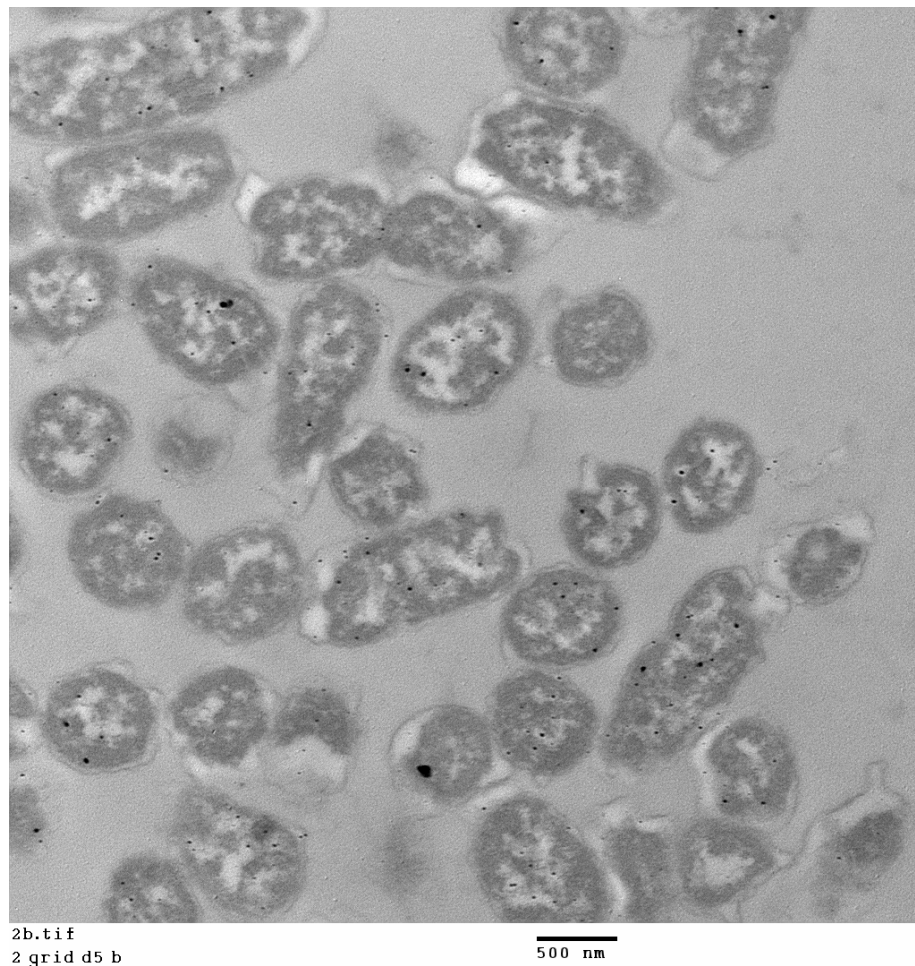


Figure 52 - *E. coli* interacted with SMAD powder.

The interaction of the Air-SMAD sample with bacteria was much more dramatic (figure 53). Large portions of the cellular contents seemed to have been completely eaten away. The cell appears to have little defense against this type of attack. It was hoped that images from either silver (I) or silver (II) oxide would be very similar to this activity but this was not found (figures 54 and 55). Silver (II) oxide appears to be able to infiltrate the cell much more effectively than Silver (I), however per gram they had very similar activity. There is some damage to the cell from the oxidized samples reminiscent of the Air-SMAD effect however the interaction is not identical.



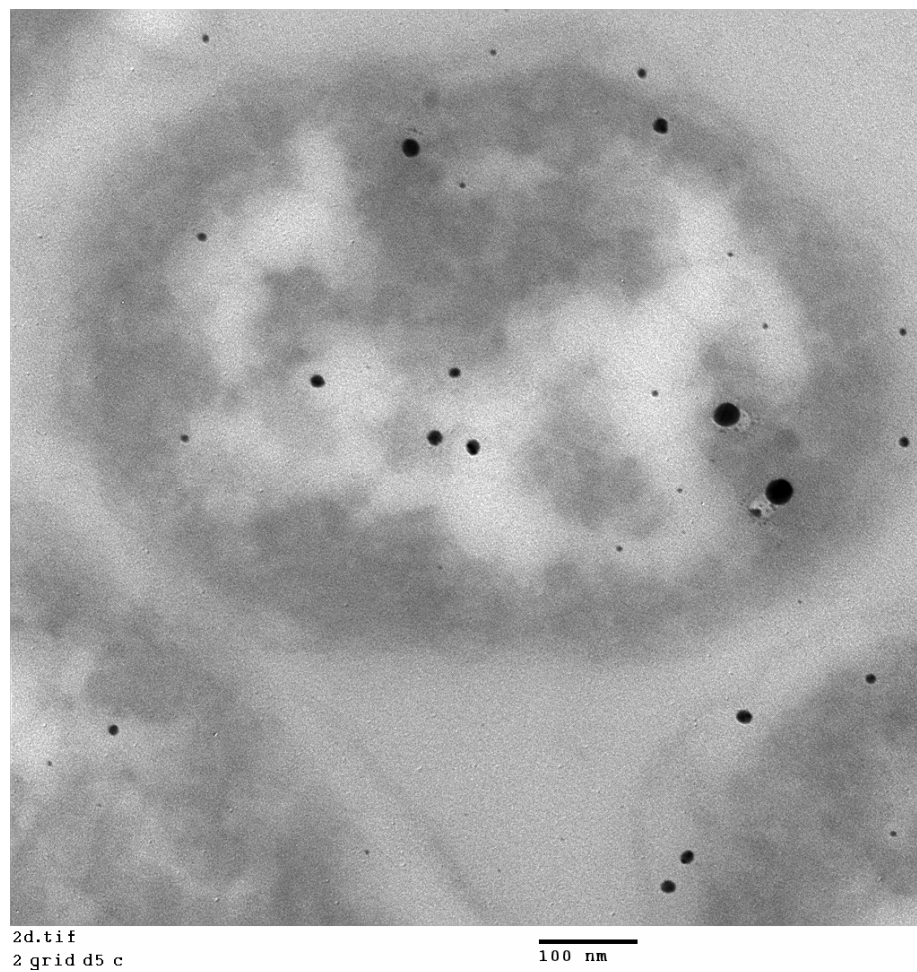


Figure 53 - TEM image of *E. coli* after contact with Air-SMAD powder.

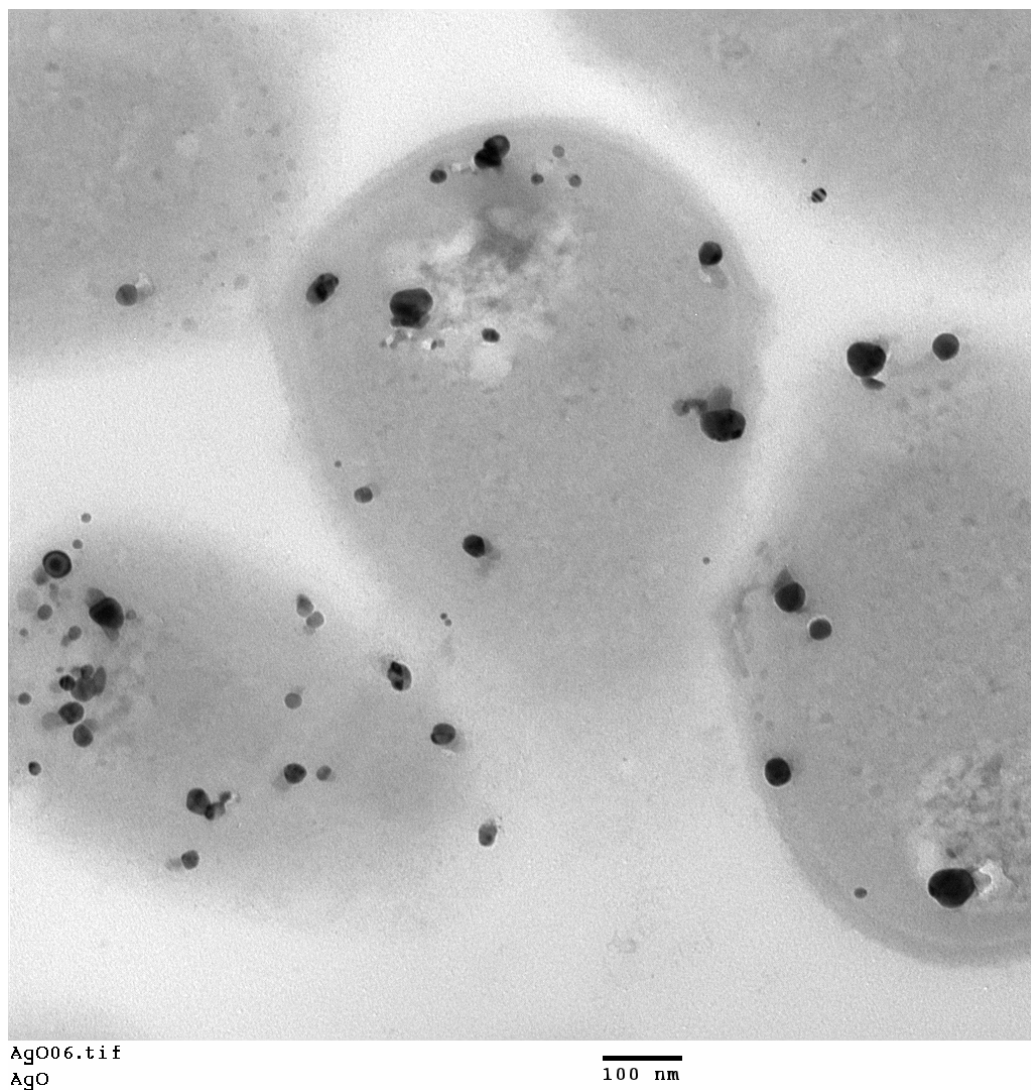


Figure 54 - TEM image of Ag₂O with bacteria.

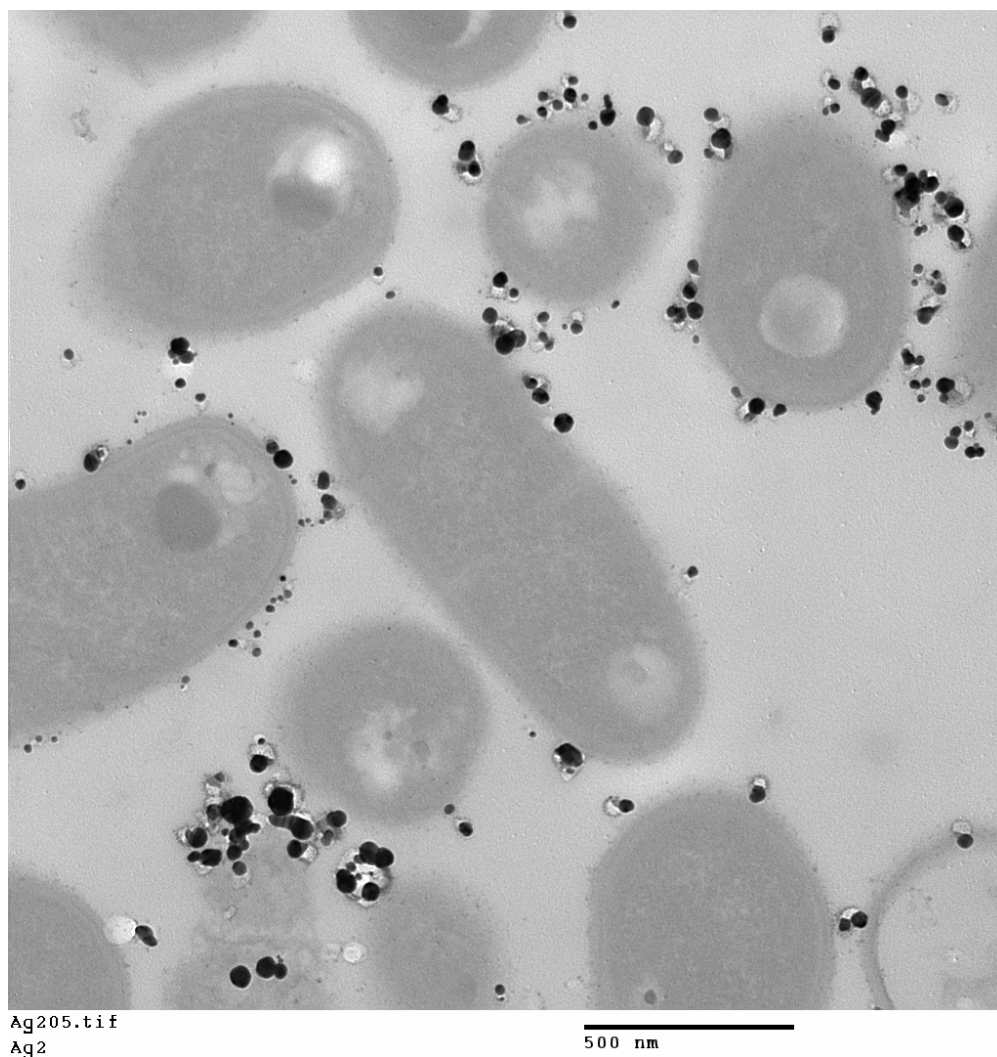


Figure 55 - TEM image of AgO with bacteria.

The interaction of 3-mercapto-1,2-propanediol capped silver particles with bacteria were also observed in the TEM in an attempt to understand their low reactivity. Ligand-SMAD sample was seen to be incorporated into some of the bacterial cells but their small size is retained, suggesting that they are still encapsulated in the initial ligand. SMAD heat treated samples were interacted with the same procedure. A few of the bacteria showed damage greater than that of untreated SMAD powder but definitely not to the extent of the AIR-SMAD powder.

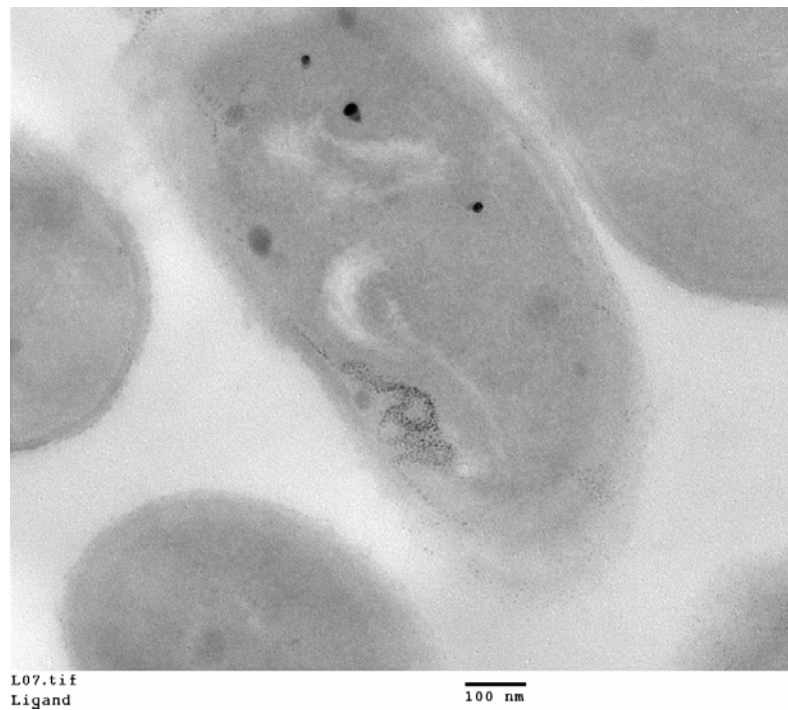
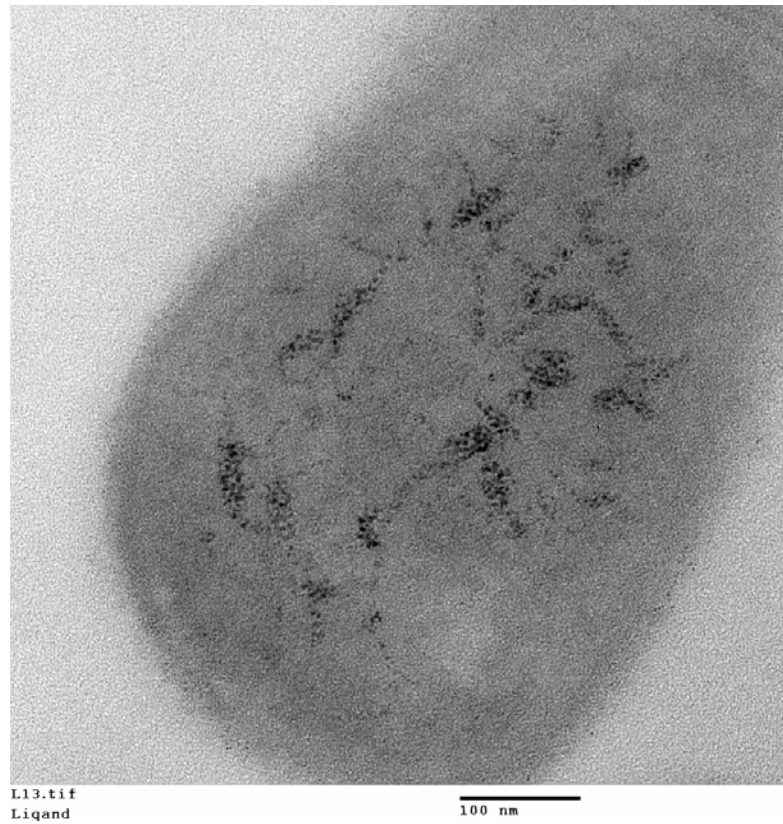


Figure 56 – Ligand-SMAD particle interaction with bacteria.

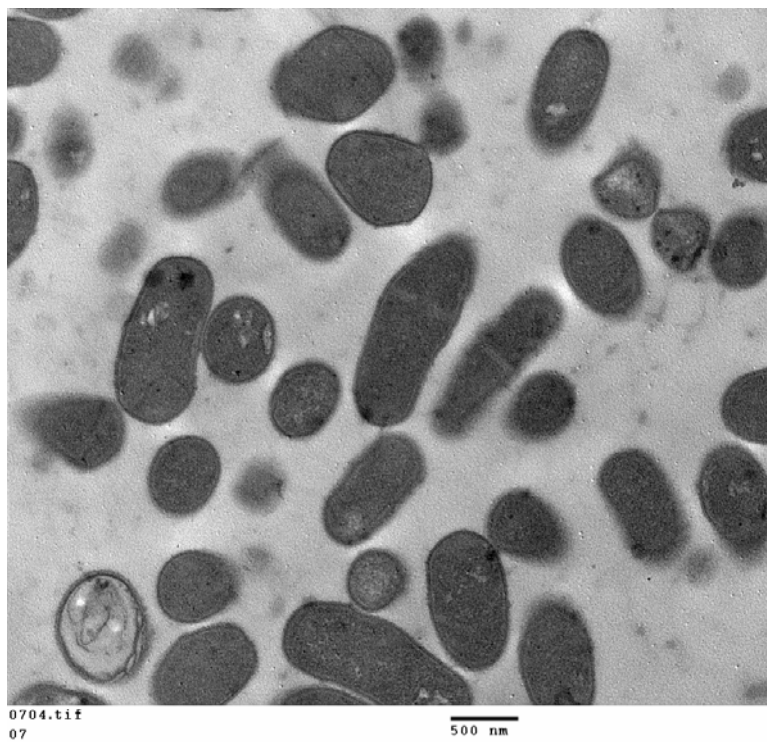


Figure 57 – SMAD-Heat treated powder interaction with bacteria.

Viral studies

The only positive results from anti-viral testing were from the Ligand-SMAD colloids. This was peculiar since this was the least effective of any of the materials tested previously with bacteria. When the ligand itself, without any presence of silver was tested against the virus very similar kill rates were shown. This suggests the surfactant-like ligands were able to break apart the viral cell and the silver had little effect. The Air-SMAD powder was not tested against the viral pathogens due to time limitation, but this would be a useful experiment.

Discussion of Silver Powder Activity

SMAD-powders

The SMAD powders out performed their commercial counterparts in the bacterial mortality by an more than an order of magnitude. This product has a higher surface area, and overall much smaller surface features. This translates into more active sites for the material. These features provide a quicker and more thorough means for the silver powder to erode silver ions into solution that interact and destroy the bacterial cells. The concentration of silver ions eroded into solution was measured by conductivity and were found to emit as much as 50 times as much silver as a solid silver pellet. The silver ions are then available to infiltrate the inside of the *E. coli* cells where they are reduced by the cell, but become incorporated into the proteins and DNA of the cell where they denature the proteins and prevent cellular function, or

reproduction in the case of DNA. This material performed up to its expected potential, however much of the surface area, and presumably activity is lost when the solvent used in synthesis was removed. This may be circumvented by using solvents that are more human-friendly and leaving the particles solvated until application.

Ligand-SMAD colloids

The ligand passivated nanoparticle synthesis was very successful. Silver nanoparticles of 6 nm were prepared and were highly dispersible in aqueous solution. The increase in surface area of the particles, and improved solubility were thought to improve the toxic effect of the nanoparticles. Unfortunately it appears that the available metal to interact with the bacteria was actually reduced as the ligands covered the particles too completely, and did not allow any loss of silver into solution. This led the silver colloids to perform under our expectations. This was confirmed by adding free ligand into an active silver suspension of SMAD powder, or AgNO_3 . This was found to deactivate, or complex with the free silver ion and reduce the antimicrobial effect as seen in table 6.

Air-SMAD powder

The Air-SMAD powders were created in an attempt to chemically modify the surface of the silver metal to an oxide. Upon doing this the particle size was sacrificed only very minimally but the effect on the bacteria, which is evidenced in figure 53, was dramatic. The huge holes in the *E. coli* after interaction, and the decrease in concentration of silver needed to destroy the bacteria cells were amazing. However the confirmation of the presence of oxygen on the surface

of the powders is still lacking. There is clearly a different chemical interaction with the bacteria that is more similar to the action of AgO on the bacteria. Also the increased activity is in line with the activity of the two standard silver oxide powders. XPS studies are being conducted to detect the presence of trace oxygen on the powder.

Heat-Treated powders

Like the Air-SMAD sample this powder was prepared with the intention of at least partially oxidizing the surface of the silver powders. Heating the already high surface area powders in the presence of oxygen was hoped to maintain the nanosized surface morphology yet change the chemical activity. The oxidation of bulk silver is known to occur at 200 °C. SMAD powder was heated in air at several temperatures, many lower than this amount due to an increased likelihood of reaction from the more active powders and a hope to prevent sintering. These powders displayed a slight increase in activity over the SMAD prepared powders, however more testing needs to be done with a variety of temperatures.

KOH-SMAD powder

This preparation was an attempt to modify the silver powders with liquid phase chemistry in the reactor when the metal atoms are still very reactive in the SMAD vessel immediately after melting. It was hoped that a much larger portion of the silver powder could be surface modified in this manner. To encourage immediate reaction isopropanol was used as the initial solvent in the SMAD synthesis, because of its ability to dissolve KOH so well. An initial amount of KOH was dissolved with the isopropanol on the bottom of the reaction to interact right after melt.

Isopropanol proved to be a much poorer stabilizing agent for the initial silver atoms and led to rapid agglomeration and a much poorer particle size. Even when this reaction was attempted with acetone as the stabilizing solvent the particle size was not much improved, and this product did not perform any different from standard SMAD prepared samples. Strong oxidizing agents such as hydrogen peroxide, may lead to a more improved product.

Conclusions

The above experiments were performed with the intent of finding a more effective antimicrobial agent. While the activity of the SMAD powders is lower than that of pure silver nitrate, it has the ability to kill bacteria very effectively and over long periods of time. The powder can be dispersed in water, but is not washed away by it and would likely perform very well on a polymer support in contact with aqueous solutions. Silver nitrate is more of a “one shot agent” that is easily washed away in water. The original powder was able to outperform the commercially available powders by a large margin. Further studies showed that an air-treated SMAD sample was able to have remarkable killing power against bacteria and is a promising product. While the surface chemistry of this sample is yet to be identified some studies are still taking place.

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Chapter 5: Versatility of Digestive Ripening

Introduction to misc. reactions

Nanoscience has not just grown in the past decade, but exploded. As one of the more popular buzzwords in academic circles, research involving new findings in nanoscience is always noteworthy. Several of these topics are discussed here, involving reactions that took place in our laboratory, but often were collaborations with fellow scientists who have investigated the reactions much more thoroughly. This chapter provides an introduction with current relevant work related to each topic as well as experiments conducted in our laboratory and their preliminary results. Among these topics include the growth of nanocrystal superlattices, the synthesis of nanoprisms, the synthesis of water soluble gold particles, and the creation of a unique nanoparticle catalyst system. These materials each have exciting possibilities that span applications from photonic crystals, bizarre optical responses, biosensors, and soluble metallic Pd catalysts supporting the highly oxidizing polyoxometallate macromolecules.

Controlled growth of Nanocrystal Superlattices

Self-assembly of nano-scale materials is paramount in the integration of these unique species into new technology¹. Lithographic methods have been developed to manually imprint or write nanoscale features on components; however this one by one approach is extremely time consuming and occurs at a length scale that is very hard to control, except with exceptionally

expensive equipment². In an attempt by scientists to mimic nature self-assembly procedures are being investigated, where the materials organize themselves through identification with one another and weak interactions³. This is mimicry from the fundamental organization of biological cells. Cellular membranes are mainly composed of molecules with polar head groups and apolar fatty acid chains. In solution they assemble to form a double membrane that gives cells their shape and their distinction from the environment. Another famous example of self-assembly found in nature is DNA recognition and replication.

If nanoscale materials can be coerced into a self-assembly mechanism, larger and potentially more complex structures will become possible to produce synthetically. Ordered arrays have been found to make fantastic sensors that are small in size and can therefore detect miniscule amounts of material⁴. The simplest model of ordering is the stacking of spherical particles. Polystyrene spheres have been used as a laboratory model for this system⁵. These polymer spheres can be created with a high degree of monodispersity and are a good approximation of hard spheres. Many ingenious materials have been constructed from these materials⁶. A self-assembled superlattice can be used as a template where the space between the particles is infiltrated with a chemical precursor to silica or titania. The reverse-pattern is formed by solidifying the reagents between spheres, then removing the spheres selectively with acid. This inverse structure can again be used as a template to create the ordered arrangement of a material with a higher dielectric constant than the original polymer. If the structure is made with perfect enough crystallinity and made of a high dielectric material they create a region where light of a select range of wavelengths is forbidden, or a photonic crystal⁷.

Creating channels through this material allows the lossless transfer of light. This feature can be used to create entirely optical computers, and enhance the efficiency of organic LED's,

and high efficiency molecular lasers⁷. The length scale of the periodically repeating structure determines what wavelength will be forbidden by the material. Meter sized periodicity can be created manually in the laboratory with wooden sticks, how this leads to very large components, and can only block light of micrometer wavelengths⁸. Nano-scale periodic materials, are small enough to be incorporated into electronic devices, and are able to block visible light wavelengths and smaller.

Naked metal nanoparticles are very reactive especially towards one another, and will not stay monodisperse in a solution. As seen from chapter 2 there is a common solution to this problem by covering the surface of the metal particles with an organic ligand that prevents aggregation⁹. These products can also be formed with a very narrow size distribution and could be good candidates for self-assembly. The question arises however of whether the ligands will arrange on the surface of the metal in a perfectly spherical orientation, or will they be unorganized and not produce an ordered crystal. Wang has shown that dodecane thiol protected gold nanoparticles are in fact highly faceted crystals, and not perfect spheres¹⁰. On each facet of the crystal the dodecane thiol molecules order normal to the surface and into solution. Nanocrystals precipitated from solution will take advantage of this arrangement as dodecane thiol ligands will interdigitate as the van der Waals forces between the apolar chains induces attraction for one another. This causes the facets of the crystal to order across from one another and is translated across the superlattice.

Attempts to manipulate this behavior have been moderately successful. The chain lengths of the capping ligand can be altered for increased or decreased spacing between the particles³. This also has a large effect on the solubility of the nanoparticles, which will favor solubility in longer-chained thiols and precipitation for shorter chain thiols. Changing the metal

from gold to silver has been found to create slightly better nanocrystals as the angle of the dodecanethiol ligand was found to be further off 90 deg than with gold. This seems to aid in precipitation of regularly ordered superlattices. The use of silver as a construction material is more difficult due to its increased reactivity.

Crystal growth has been a key to purification of chemicals for a long time. Solids tend to form as pure substances rejecting impurities from solution. Defects arise in crystal growth that will tend to cause a solid with various crystal planes, and orientational growth. Seeding is also important during crystallization such that a small number of seed crystals will often give rise to a small number of large uniform crystals whereas a large number of seed crystals typically give rise to large numbers of small crystals. Slow crystal growth has been found to reduce the number of crystal defects as mistakes have time to be erased by re-dissolution into solution. Particles that are placed incorrectly are more soluble in solution to those that are in a well established order. Given time this particle will return to solution and redeposit correctly. If the crystal growth is fast the defect will become incorporated in to the crystal forever and lead to defects.

As crystals become larger in solution the force of gravity begins to draw them down the bottom of the reaction vessel and will limit the surfaces of the crystal that are available for growth. To overcome this conditions of microgravity are often sought after for growing large crystals that are more difficult to handle in standard environments¹¹. The lack of gravity allows the crystals to remain in solution indefinitely and can continue to grow in a very controlled manner. Unfortunately microgravity is a difficult situation to reproduce. For short intervals airplanes can mimic this by a series of parabolic dives in the upper atmosphere. Travel to outerspace is necessary for longer exposure to microgravity.

The following experiments were conducted with an extraterrestrial reaction in mind. Previous superlattice growth in the laboratory involved the use of 4-Tertbutyl-toluene is a perfect solvent for this experiment since the liquid will only evaporate very slowly, allowing the crystals to stay in solution for a very long time without being disturbed. For this reaction however the unique conditions must be taken into consideration. The crystallization must be simple enough to initiate by untrained astronauts and proceed without their interference. Also the lives of the astronauts must be given high consideration so use of highly toxic substances should be avoided at all costs as well as using a completely contained apparatus.

To facilitate these needs the apparatus below was designed and constructed with the aid of the glass blowing specialist at our institution Jim Hodgson. The apparatus consists of two chambers, one in which the nanoparticles would be kept at the point of saturation in solution. The other would contain a highly absorbing material such as MgO. This powder was intended to slowly reduce the volume of solvent when a passage between the chambers was opened. Thus the solution of nanoparticles would reach super-saturation and begin to precipitate. The loss of solvent was hoped to be constant and very slow but to be accomplished in the time frame of the space mission. To this effect the key controllable parameter in this experiment is the absorption rate of the solvents in the apparatus. Two variables were tested, changing the nature of the solvent, with regard to boiling point and the use of slightly elevated temperatures were monitored to increase evaporation rate if needed.



Figure 58 - Apparatus used to control the evaporation of solvent from a nanoparticle colloid to induce slow precipitation of the colloid.

The temperature was controlled by placing the apparatus in a water bath with a feedback mechanism to monitor the water temperature. The solvent loss was indicated periodically on the outside of the apparatus and carefully measured by syringe after the experiment was completed. Water was used as a solvent in case the only particles allowed by the shuttle crew and NASA were 100% non toxic. In this case water soluble nanoparticles would need to be used, the synthesis of which is described below for gold and in the previous chapter for silver. The result of the solvent evaporation study is listed in the table below.

Table 8 - Solvent absorption in apparatus per day with solvent type.

	Amount of solvent loss in ml/day			
	25 deg C	35 deg C	45 deg C	b.p.
water	nil	0.05	0.09	100°C
toluene	0.08	0.16	0.26	111°C
heptane		0.36	>0.33	98°C
hexane		0.5	>0.5	69°C

Our laboratory is a perfect fit for this experiment. Not only do the particles produced synthetically here have a very narrow size distribution, but they self-assemble to form large and beautiful regular superlattices under normal gravity. Also our synthetic procedure is one of very few that can produce high gram amounts of nanoparticles of this quality.

Our previous superlattices were grown from high boiling solvents. This aids in two ways. Synthetically this provides a high temperature reaction for digestive ripening. Equally as important it allows for slow evaporation of the solvent. All superlattices shown have been grown on TEM grids by deposition of a small amount, typically 1-3 μl of solvated nanoparticles on a carbon coated copper grid. Sample preparation has been conducted with several solvents including toluene (b.p. 110 deg C) by Stoeva *et al* and found many nice structures could be formed from the slow evaporation of this solvent¹². Superlattices formed by Smetana *et al* were made using 4-tertbutyl toluene that allowed for extensive crystal formation, yet needed to be placed under vacuum to remove most heavy solvents¹³. The presence of excess organic ligands

and solvent hinders clarity of samples in the microscope to some extent, but allows for more time for superlattice formation. Some examples of self-assembled structures are shown below.

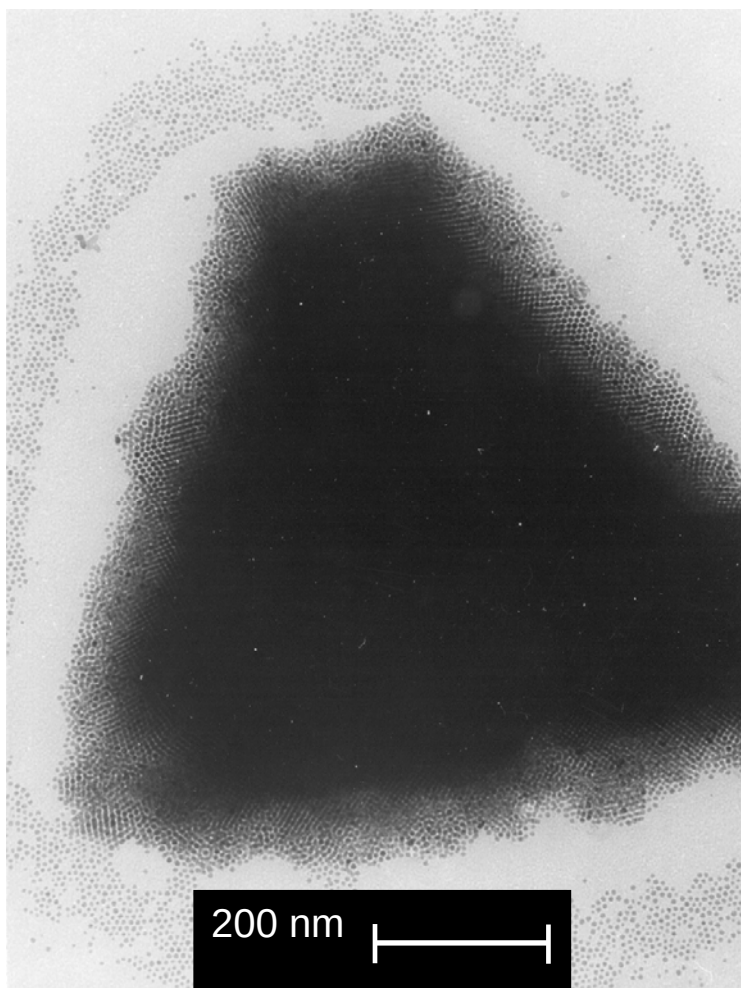


Figure 59 - Superlattice of silver nanoparticles ligated with dodecane thiol.

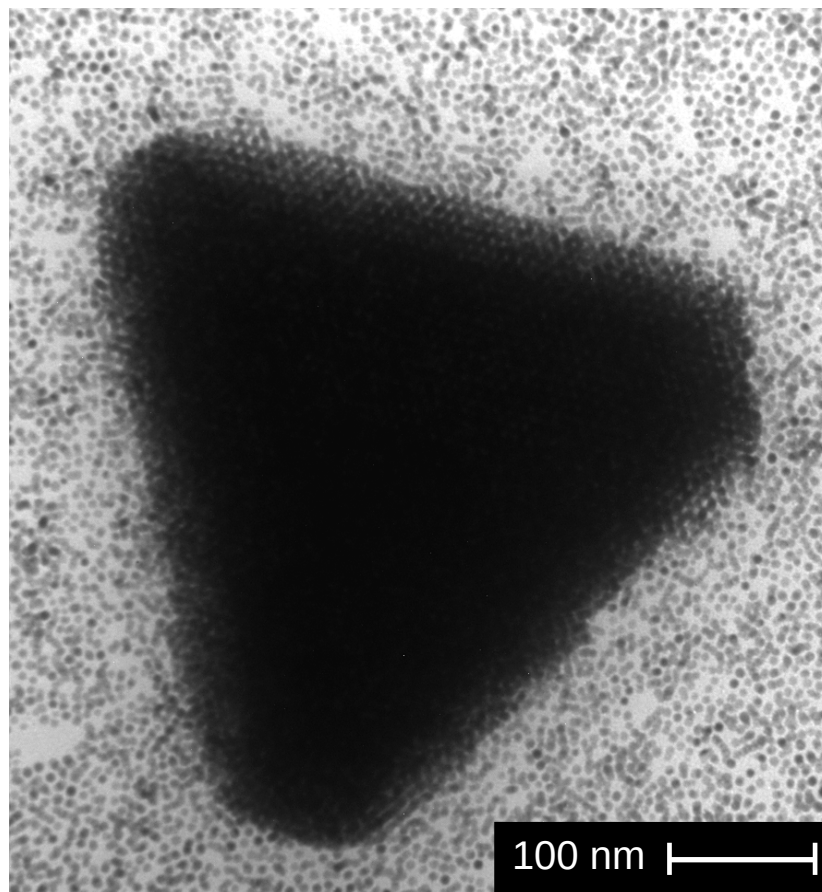


Figure 60 - Superlattice of gold nanoparticles ligated with dodecane thiol.

Unfortunately funding was cut short before any further experiments were conducted. Future work would involve trying to find the saturation concentration of the silver colloids and actual nanocrystal growth conducted in the apparatus in figure 58. In theory a large solid crystal would have been produced in the reaction chamber, which could be observed by high resolution SEM imaging to determine the degree of defects in the crystal, and the size of what may have been the world record self-assembled nanocrystal superlattice.

Prismatic Nanocrystals

In the world of metal nanomaterials the first products realized were spherical particles⁹. As new synthetic strategies were found and different ligand materials were produced spherical particles became the norm and lost their excitement. As synthetic conditions were altered nanocrystals were soon found to grow in different geometries from nano cubes to wires, rods, tubes, and triangles¹⁴⁻¹⁷. One of the first notable examples of this is the photo-induced growth of silver seed particles into triangular prisms by Mirkin¹⁸. Changing the shape of the nanocrystal has drastic effects on the optical absorption of the particles. New quadrupolar absorptions become important yet can still be calculated with advanced theoretical calculations¹⁹. It can be seen that the particles now absorb in more than one area of the spectrum as the mean free path of the electrons will be different in the x, vs. the y, or z direction unlike the spherical case. The formation of these materials is usually dependent on using more than one ligating agent absorbing to the surface of the particles²⁰⁻²¹. It is theorized that different ligand molecules will bind preferentially to different surfaces of the growing crystal. For example this could allow for rapid growth in the x, or y, dimension and minimal growth in the z if a stronger ligating agent binds to this plane.

The nanoprisms in our laboratory, like the first reaction of xenon gas with fluorine, were an unplanned discovery. The conditions of the experiment were as follows. A raw product from the SMAD reactor of 4-tert-butyltoluene, trioctyl phosphine (TOP), and silver nanoparticles was the precursor. In an attempt to produce spherical particles digestive ripening was performed on the particles in a Schlenk tube set up as detailed in chapter 2. The chemical mixture was brought to reflux under a constant flow of argon. However the rubber septum used in the

experiment was quite old, and presumably some oxygen entered the reaction vessel. It is proposed that some of the TOP in the vessel was oxidized to TOPO which provided a second ligand in the solution for reaction. The ligands could have preferentially bound to different faces of the growing nanocrystal and result in the various structures shown in figure 61. IR spectroscopy was run on the reaction products however no P=O stretching peak was observed among the other solvent stretches. This is likely due to the signal being masked due to the small concentration of the ligand.

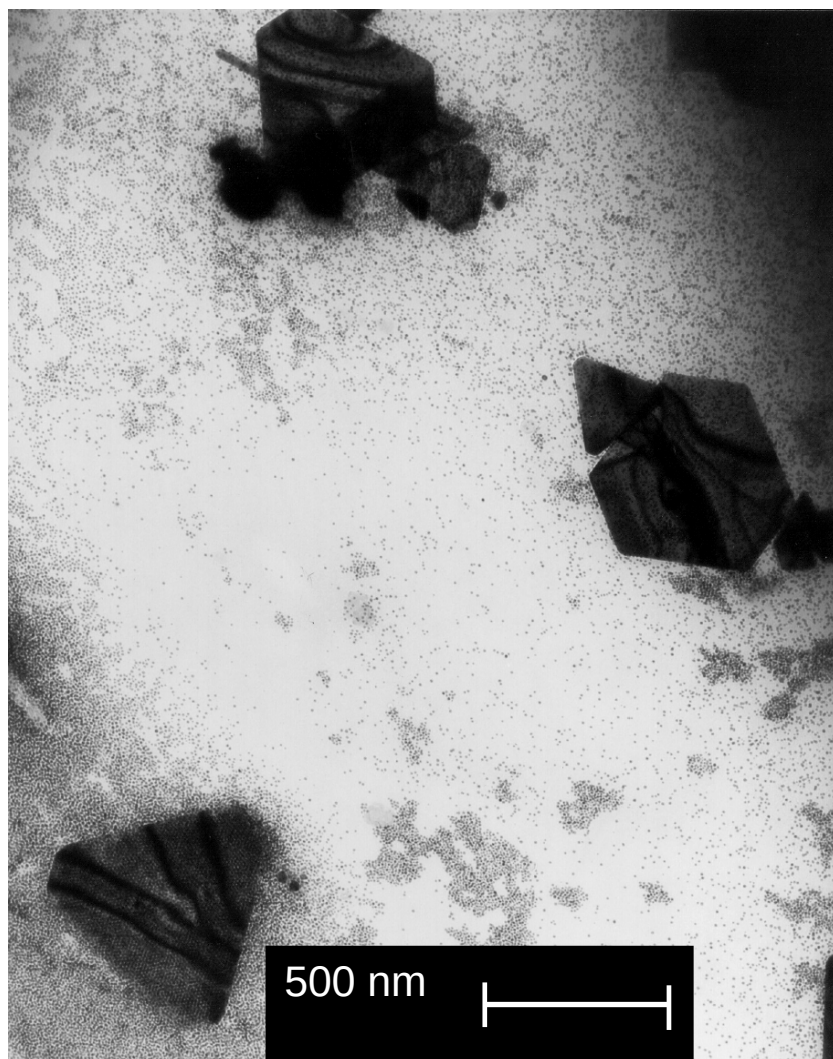


Figure 61 - Mixture of small spherical nanoparticles and larger triangular nanoprisms.

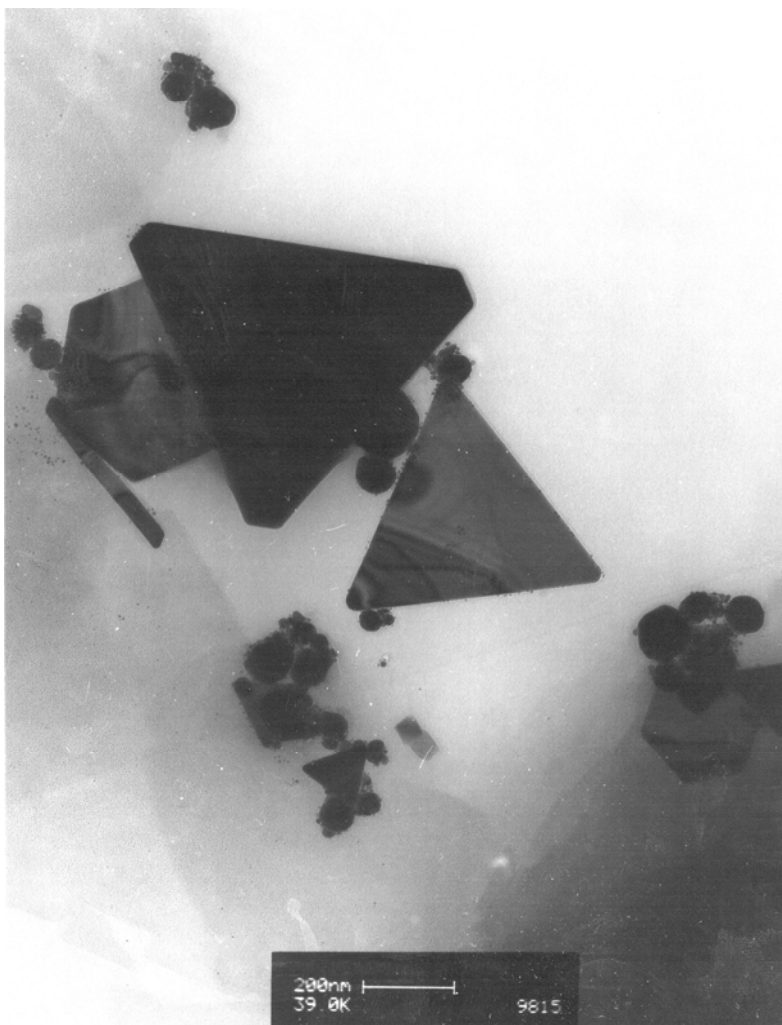


Figure 62 - Silver nanoparticle rod, hexagonal prism, and triangular prisms.

While it was not investigated fully the significance of the reaction is that digestive ripening is taking place in solution to a new effect. The spherical nanoparticle precursors are being broken up and caused to grow into geometrical crystals as new atoms are delivered to the prismatic seeds. This experiment shows that the experimental that most defines the quality of metal nanoscale products at Kansas State University is versatile to synthesize even the most strange products and can compete with many complicated synthetic strategies, while remaining simple to conduct.

Aqueous Gold Colloids

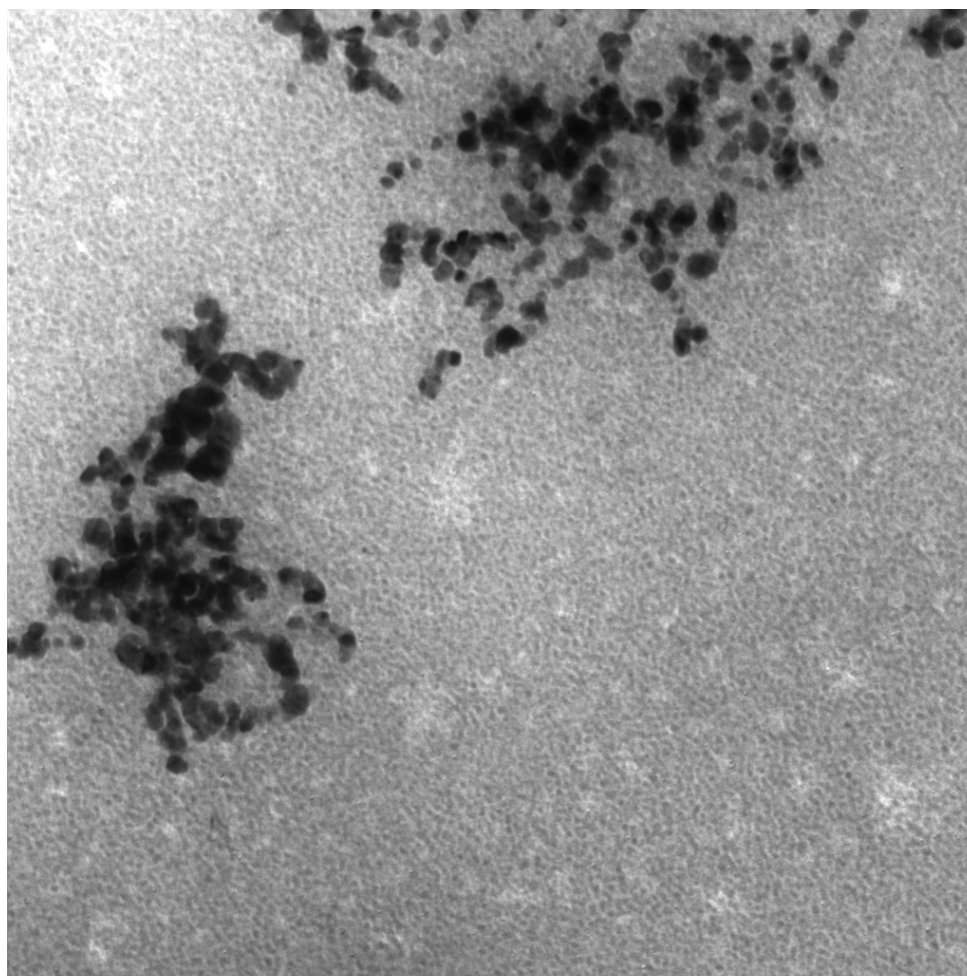
Metal nanoparticle applications are being fashioned in two distinct channels.

Nanoparticles for electronic and optical applications do not have any problems when synthesized from organic solvents. The other emerging technology is biological applications of nanoparticles whether as sensors, drug delivery devices, or therapeutic agents²². Since biological systems find most organic solvents extremely toxic, water soluble nanoparticles must be created for practical applications. The change from organic to aqueous systems will require the ligands surrounding the particles themselves to change as they are ultimately responsible for the solubility of the particles.

Choice of ligand becomes much more complicated than in the previous discussions in this dissertation. Not only are aqueous colloids much harder to synthesize, data on ligand quality is much more rare. The paramount issue in this experiment is the toxicity of the ligands used to encapsulate the nanoparticle. It is completely useless to find an accurate and painless sensor for glucose monitoring if the sensor itself causes death of the patient. With this in mind all ligands used in this study were chosen first for their low toxicity. All materials are listed as an irritant at worst in their material safety data sheet (MSDS) this being the same toxicity as NaCl it was felt they would be sufficient. The second challenge was to ensure the solubility of the nanoparticles in water which would require either a charged or highly polar functionality. Lastly the ligands must have a high binding affinity for the nanoparticle themselves and would likely need a free mercapto group. The brunt of this work was completed by Savka Stoeva and recorded in her own thesis²³. The work done here was to reproduce her most stable products and to conduct

zeta potential analysis of the product colloids to determine their stability in biological applications.

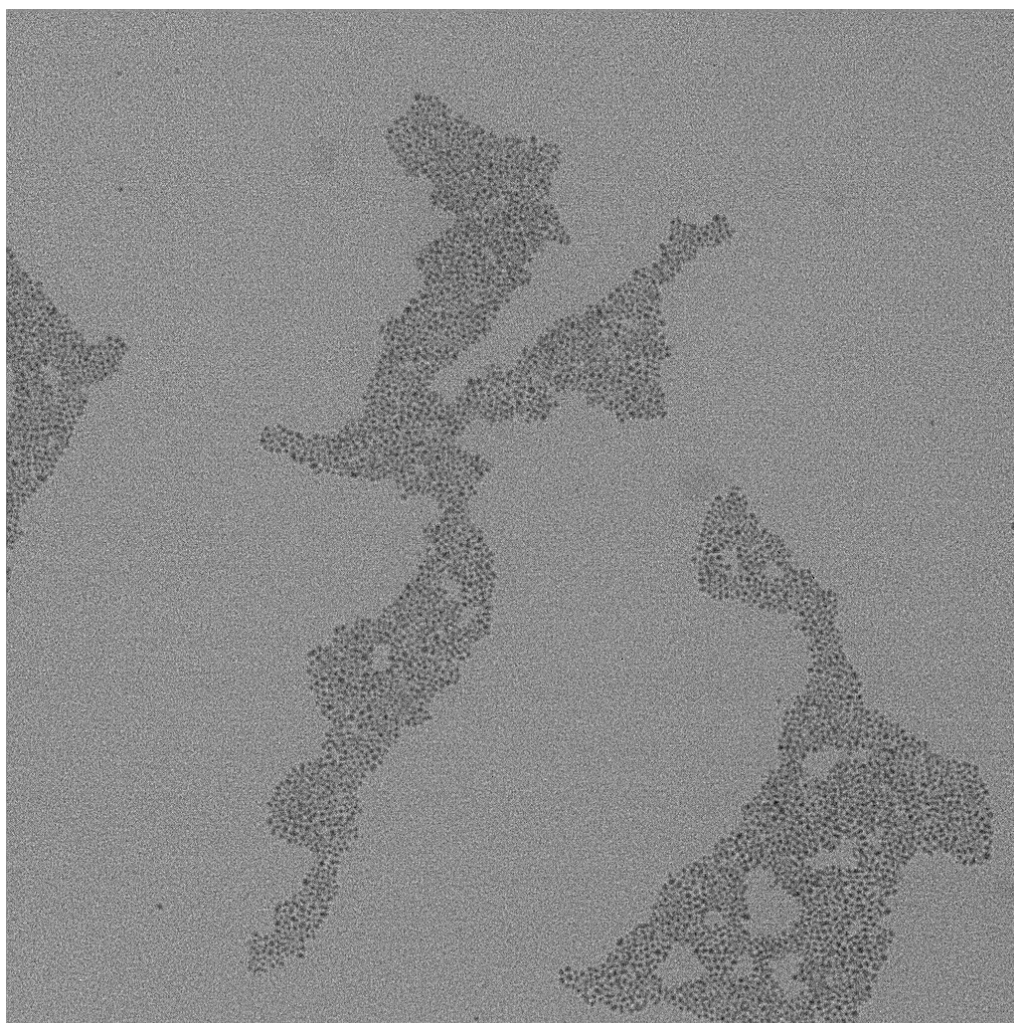
Several alterations to the standard SMAD reaction were required to produce the particles. The first was a decided change in synthetic philosophy as the previous enemy to monodisperse particles, water, was introduced as a reagent in the reactor. Millipore water was used for all reactions, detailed in this section, and was bubbled with argon before use to remove dissolved oxygen. An increased molar ratio of ligand to metal atoms was used from the previous 20:1 ratio to a 30:1 ratio. Three ligands were used: 3-mercapto-1,2-propanediol, Sodium 3-mercapto-1-propanesulfonate, and Sodium 5-mercapto-1-tetrazoleacetate. The evaporating solvent was still acetone. No digestive ripening was performed on the particles as this quickly caused aggregation and enlargement of particle size.



ABS3-65-3-TEM-1.tif
ABS3-65-3-TEM-1

100 nm

Figure 63 - TEM of 3-mercapto-1,2-propanediol.



ABS3-76-1b-TEM-1.tif

ABS3-76-1b-TEM-1

100 nm

Figure 64 - TEM of Sodium 5-mercapto-1-tetrazoleacetate.

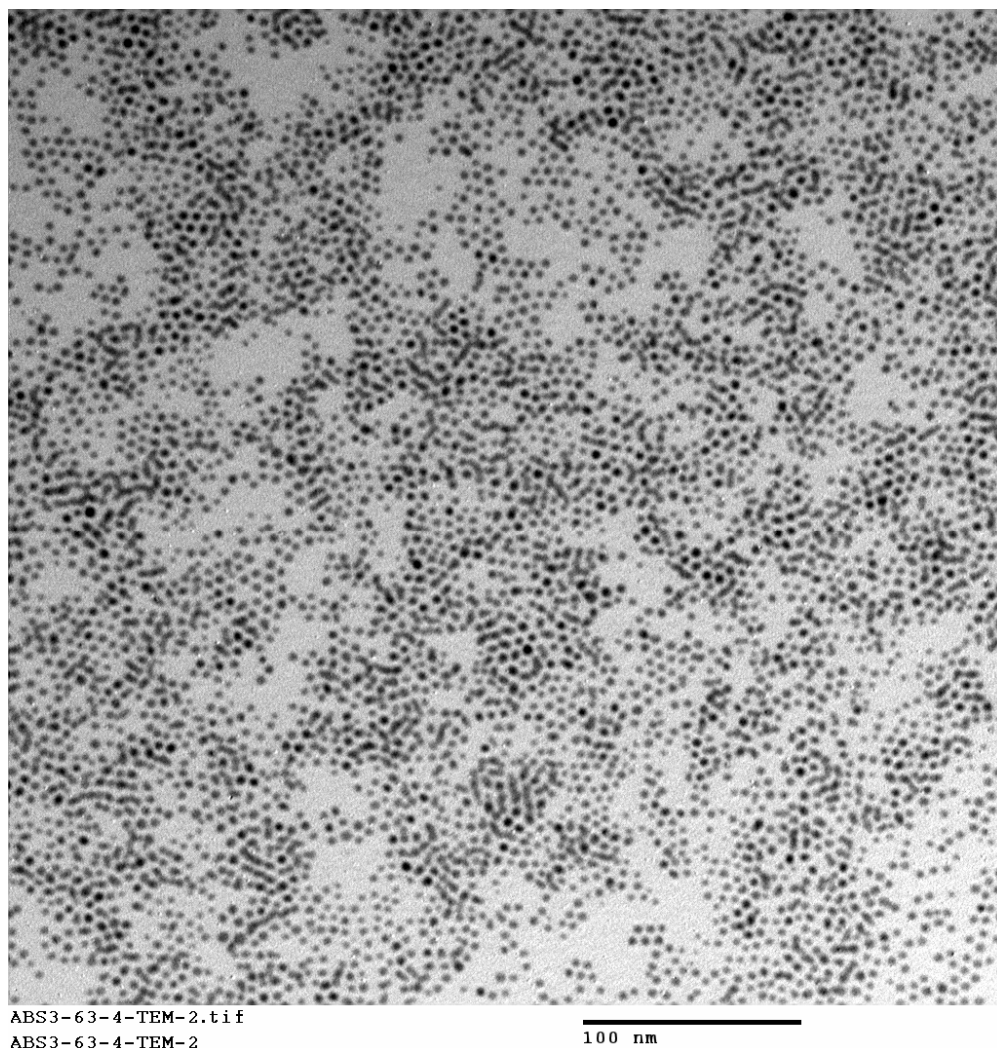


Figure 65 - TEM of Sodium 3-mercapto-1-propanesulfonate.

The stability of the particles was tested in by two methods. UV-Vis spectra were obtained for each product and at concentrations of 0.0 – 0.5M NaCl. The salt was added to increase the overall charge in solution thereby decreasing the electric double layer surrounding the particles making them soluble in water and eventually causing them to precipitate from solution. The surface plasmon resonance peak from the gold particles, as well as the background scattering of the sample was monitored. As the particles came out of solution the SPR peak

would broaden and shift. Also increased scattering from the larger agglomerates in solution was monitored by an increase in the baseline extinction.

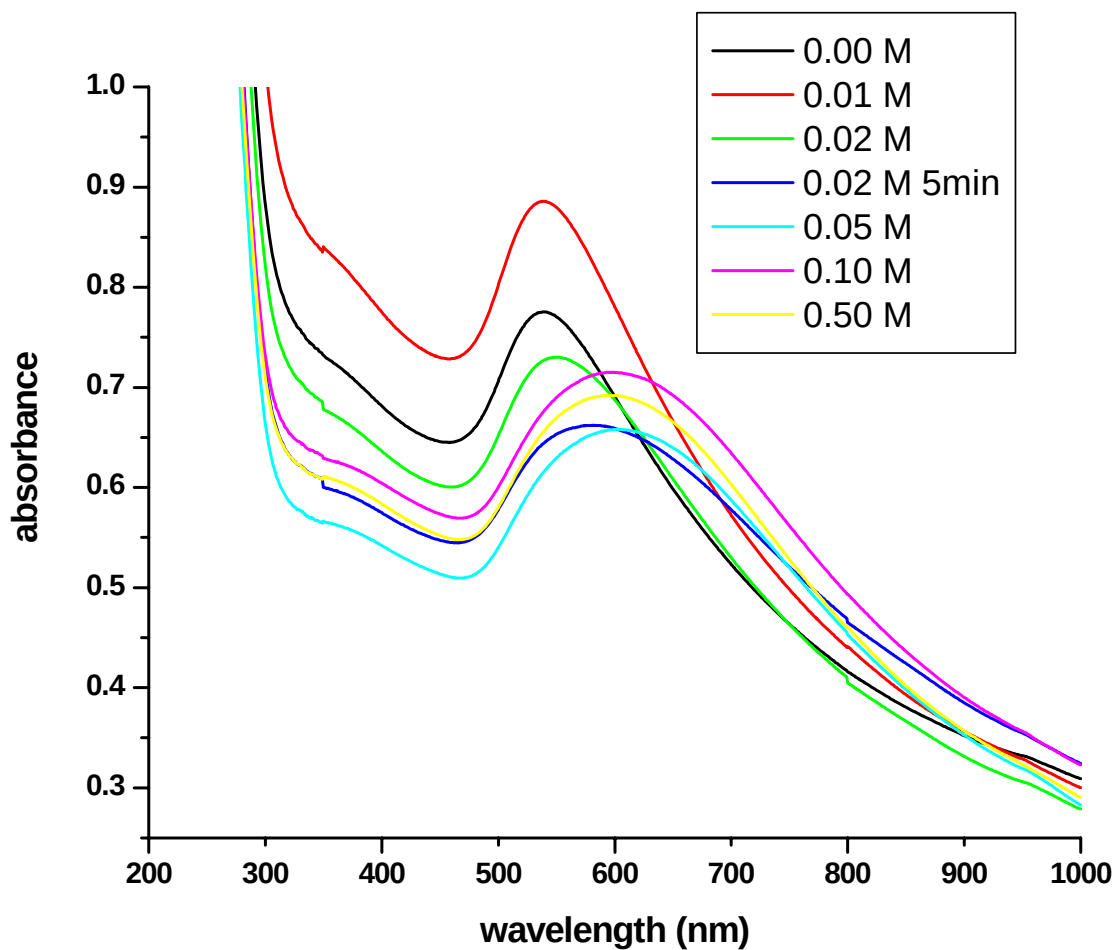


Figure 66 - UV-Vis spectra of 3-mercaptopropyl-coated gold particles at increasing values of NaCl salt concentrations.

Zeta potential measurements were also taken at several concentrations of NaCl. It was observed that when the colloid appeared stable at low concentrations of salt the zeta potential

peak was constant. After the solution reached a critical value for dissolved ions the solution would aggregate and cause a large peak shift and broadening of the signal. The parameters used in the experiment were as follows: cell Temperature 25 °C, Frequency range 500 Hz, Frequency shift 250 Hz, Voltage 5V, on time 3s, off time 1s, time for analysis 240s. The data was collected at a value of .16 mm cell height, and an average of the 17.6° and 26.3° laser angles which gave more reliable results than the 8.9°, and 35.2° values.

Table 9 - Zeta potential values for each colloid at two salt concentrations, one that is a stable colloid, and the second one that causes aggregation of the particles from solution.

Nanoparticle Ligand	Zeta Potential	NaCl Conc.
3-Mercapto-1,2-propanediol		
Before Aggregation	-37.1 mV	0.005M
After Aggregation	-11.5 mV	1.000M
5-Mercapto-tetrazoleacetic acid sodium salt		
Before Aggregation	-18 mV	0.001M
After Aggregation	-44.3 mV	1.000M
3-Mercapto-propane sulfonic acid sodium salt		
Before Aggregation	-22.8 mV	0.005M
After Aggregation	-29.9 mV	1.000M

It was feared that the SMAD synthetic method may not be ideal for the synthesis of water-soluble metal particles for the reactive metal atoms are quickly exposed to water and may react violently as they are occasionally pyrophoric. The particles produced were not quite as monodisperse as the original colloids produced by Stoeva however the quality of the colloids was still fairly high and were able to lend themselves to further testing. The particles proved to be resilient to high salt concentrations that were made to mimic biological systems.

Polyoxometallate protected Pd nanoparticle catalysts

In another set of experiments performed as a complement to another research group the SMAD method was used to prepare POM protected nanoparticles of Pd. This research was originally performed by Dr. Lawrence D'Souza under the direction of Prof. Ryan Richards. This laboratory produced high quality nanoparticle catalysts by wet chemical reduction of PdCl_2 by NaBH_4 . These particles were tested and found to be extremely effective catalysts for the hydrogenation of alkene bonds, reporting over 139,000 total turnovers. Unfortunately when submitted for review the nature of the stability of the particles was called into question, whether it was in part due to excess ions from the reduction step or solely the POM molecules. To circumvent this issue and test the catalysts without any presence of counter ions the SMAD method was employed.

As with other materials the ideal conditions to use Pd in the SMAD reactor had to be investigated. Acetone, THF, Acetonitrile, were all used as initial stabilizing agents in co-evaporation with Pd. While the Pd particles had good dispersability in THF and acetone, Acetonitrile proved to be a poor stabilizing agent for palladium metal. The smallest particles were obtained using THF as the stabilizing solvent, a representation of the particles is given in figure 67 below.

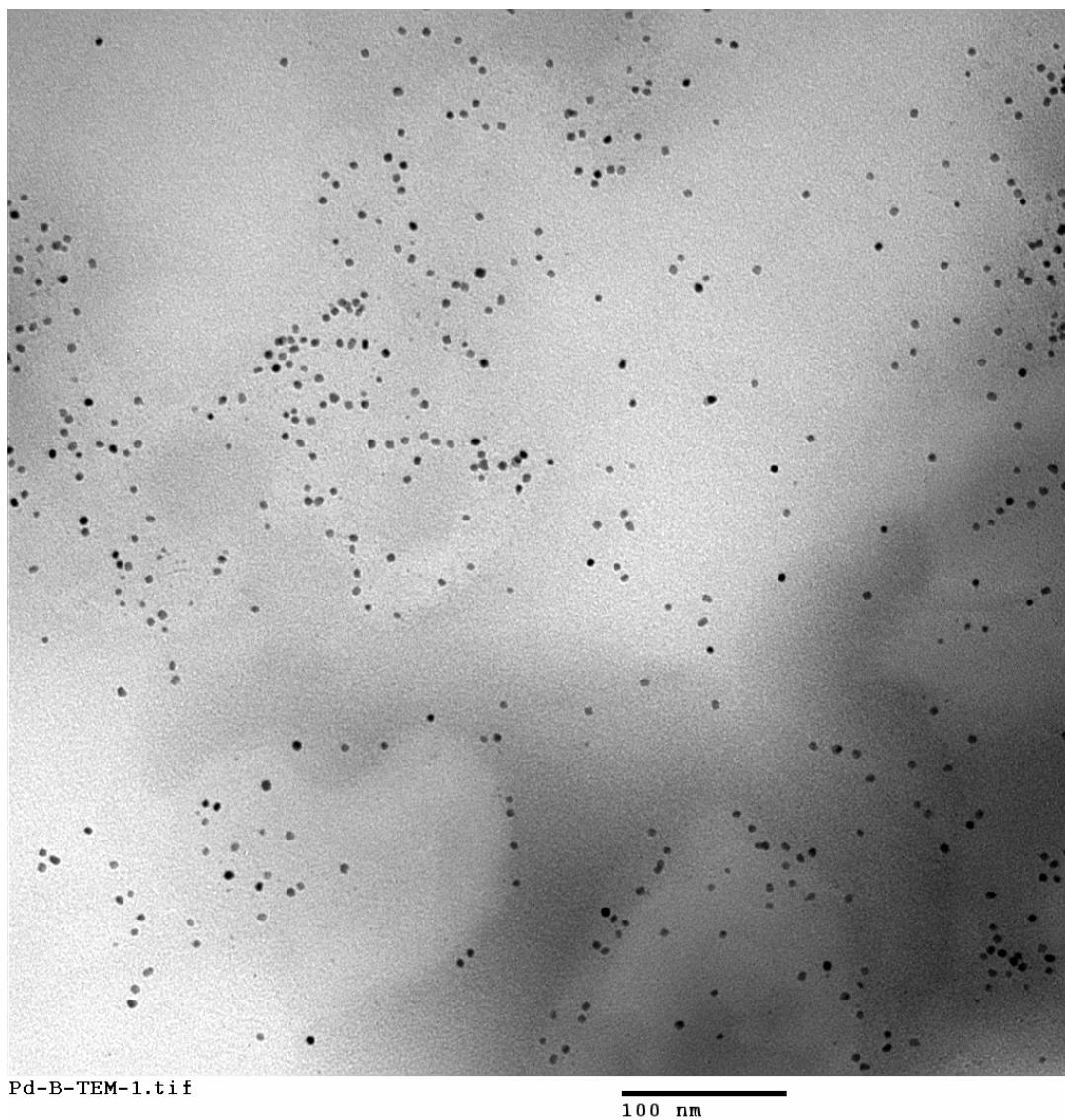


Figure 67 - TEM of Pd nanoparticles solvated in THF with no protecting ligands.

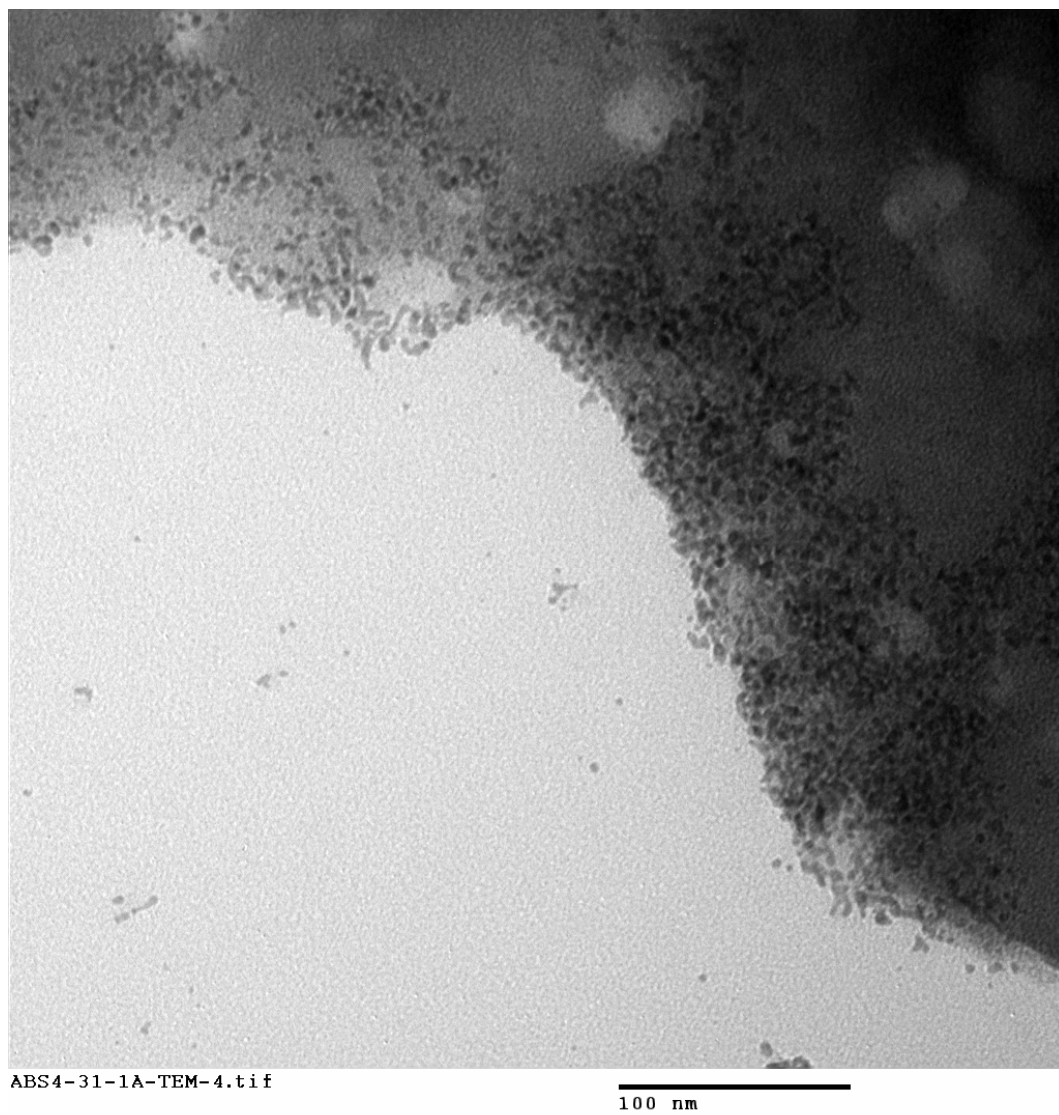


Figure 68 - TEM of Pd particles capped with $[\text{P}_2\text{W}_{18}\text{O}_{62}]^{6-}$ POM.

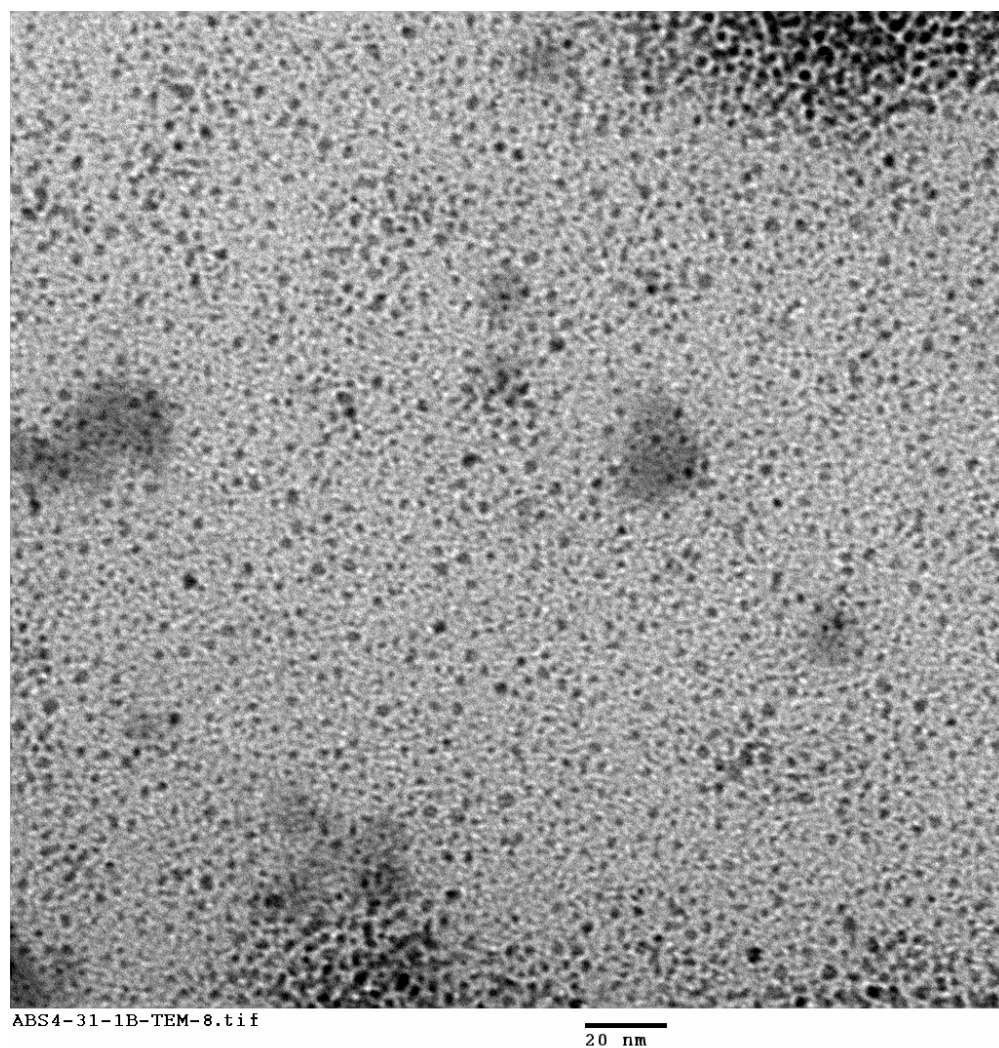


Figure 69 - TEM of Pd nanoparticles ligated with $[\text{P}_2\text{W}_{15}\text{O}_{59}]^{12-}$.

The above samples were sealed in sample vials under argon and sent to Germany for further testing and catalytic trials to compare to the wet chemical reduction products. The trials are currently underway and it is hoped that similar reactivity is found for both syntheses to

validate the hypothesis that the excess ions in solution have little to no effect on the chemistry of the reactions.

Conclusions

This concludes a brief description of several interesting short projects related to the main topic, that of nanoparticles and their chemical properties and manipulation. It is interesting to note that much of the new and exciting chemistry being done with metal nanoparticles can be made possible in one laboratory through variation of one technique. These projects do highlight the possibilities that have inspired so much creativity on the part of nanomaterial scientists.

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CONCLUSION

The results of each chapter individually were very promising. While there is always room for improvement in reaction yields and understanding, quality products were produced at every stage of synthesis that lent themselves to at least some measure of characterization and testing. That being said, this research would improve immensely with more thorough characterization of the products. The sensitivity of nanoscale products, and their difficulty to fully explore is an obstacle the entire world is facing with these materials. Much exciting work is being done, and with every new publication of the many nano-science journals now in press, some command of the materials is being gained. The vast maturation of synthesis in this area in the past decade is astounding, this is truly hot chemistry at the moment.

A more complete understanding of the mechanism of digestive ripening has been a goal of this group for several years. As with all chemical processes further understanding will lead to greater control of the products and open many more opportunities for the research to take off. As a graduate student project I found myself exceptionally fortunate to have been involved in the major fields of technology, materials science, nanoparticle synthesis, bio-technology. Due to the depth of the respective fields and limitation of time the project only scratched the surface of the growing amount of fascinating work being done, but it was a privilege none-the-less.