FOCUS ON TOMORROW

RESEARCH FUNDED BY WORKSAFEBC

# Determining the Stability of Nanoparticles in Solution and Implications for Using these Materials

April 2012

Principal Investigator/Applicant
Byron Gates

RS2009-DG04

WORK SAFE BC

WORKING TO MAKE A DIFFERENCE

Additional copies of this publication may be obtained by contacting:

Research Services
6951 Westminster Highway
Richmond, B.C. V7C 1C6
Phone (604) 244-6300 / Fax (604) 244-6299
Email: resquery@worksafebc.com

WORK SAFE BC
WORKING TO MAKE A DIFFERENCE

WORK SAFE. FOR LIFE.
WORKERS' COMPENSATION BOARD OF NOVA SCOTIA

# Determining the Stability of Nanoparticles in Solution and Implications for Using these Materials

*Idah C. Pekcevik,[†] Maryam S. Mahmoudi,[†] Michael T.Y. Paul,[†]*

*and Byron D. Gates*[*†]*

[†] Department of Chemistry and 4D LABS, Simon Fraser University, 8888 University Drive Burnaby, BC, V5A 1S6, Canada

* To whom correspondence should be addressed. Email: bgates@sfu.ca Tel. (+1) 778-782-8066. Fax. (+1) 778-782-3765.

## SUMMARY OF PRIMARY RESEARCH FINDINGS

1. We have reviewed the existing protocol for laboratory and chemical signage indicating the presence and use of nanoparticles. We found the existing labeling of containers and workspaces using nanoscale particles to be inadequate. The suggestion is made for adopting a new system that is informative and simple to use by experts and non-experts alike.

2. We have reviewed the possible resources that can be used to analyze the stability of nanoscale materials with the goal of identifying those techniques that are simple and inexpensive, such that these techniques could be widely implemented in most work places. Our goal was to create a comprehensive approach to study stability of nanoparticles, and specifically particles suspended in various solutions.

3. We developed a protocol for assessing the stability of colloidal dispersions of nanoparticles, and used gold nanoparticles as a model system. The protocol uses the techniques identified in line #2 above.

4. We assessed the long-term stability of colloidal nanoparticles, demonstrating a correlation of this stability to the quality of the surface chemistry of these particles to resist oxidative damage and other changes. Month-long experiments were carried out on gold nanoparticles as a model for other particles. This particle type was chosen for its widespread use in the literature, and the relative inertness of gold to many other materials. Our results show that these particles can have short-term stability over a few days, but become destabilized after a week or more. Results were compiled from four independent experiments to determine the reproducibility of the results associated with these studies.

5. We have also suggested, through the use of preliminary results, a method for optimizing the surface chemistry of nanoparticles in order to make particles that have longer-term stability.

## IMPLICATIONS FOR POLICY AND PREVENTION

1. Workers using nanoparticles in the work place should implement signage to indicate the presence of a potential hazard. The risks of working with aerosols of nanoparticles are great. Those risks associated with solutions of colloidal nanoparticles, such as those used in these studies, are not negligible. For example, spills containing nanoparticles can volatilize or otherwise come into contact with the worker. Prevention of exposure and containment are essential, but communicating this potential hazard is equally important. A standardized policy should be implemented for nanoparticle signage.

2. Simple and inexpensive techniques are available for those using colloidal dispersions of nanoparticles to determine the stability of these materials to a variety of environmental conditions. These techniques include optical absorption, dynamic light scattering, and zeta potential measurements. They provide the workers with the skills necessary to monitor and assess the stability of their materials, and to assess the potential risks of using a material. Work place policies should require workers to implement a standardized process to assess nanoparticle stability. Policies and methods for determining particle stability currently vary between work places, and are often not as rigorous as methods presented here.

3. The knowledge of potential instabilities in colloidal nanoparticles could have large implications in the interpretation of results on nanoparticle toxicity. Many nanoparticles are being injected into cell cultures or animals to predict the toxicity of these materials. If, however, the stability of these materials and the quality of their surface chemistry to resist change in these environments has not been established, it could be a challenge to interpret and/or reproduce the results of these toxicity studies. The fact that a material is changing over time does not imply toxicity, but rather a change into a form or composition that has not been accounted for in the interpretation of the results. Rigorous analysis is necessary to correlate the results to potential changes to the particles over the course of a study.

4. The protocols for and results from monitoring the stability and quality of a nanoparticle that are suggested by these studies could alter the way we handle these nanoscale materials, as well as the methods used to prepare these materials. These protocols are developed and demonstrated using gold

nanoparticles as a model system. Further work is needed to understand the implications of these results in prior studies on gold, the stability of other nanoparticles, and the need for further changes in policy.

## EXECUTIVE SUMMARY

Nanoscale particles are of increasing concern to our employees and to our communities. The technological impact of these materials is anticipated to be large, but the environmental and health impact could also be just as large. Concerns have arisen over recent years in the use of these materials in technological advancements. These concerns are not misguided. Further research is necessary to determine the validity of claims that these materials are going to revolutionize the industrial sector while not also compromising the health of generations to come. The focus on developing nanoscale particles has brought attention to the fact that many of the studies performed on nanoscale particles are lacking in terms of the necessary protocols and details that are essential for safely using and handling these materials. As nanoscale particles are further developed and implemented in advanced materials and technologies, it is imperative that we also develop our understanding of how to best handle, store, and otherwise use these materials. In addition, we must understand the long-term stability of these materials. We seek to assist in addressing these needs through this study.

There are a number of concerns regarding the use of nanoscale particles. These include the biotoxicity and ecotoxicity, persistence and accumulation (in the environment or biological systems), transport in biological systems or the environment, and bioavailability. Research into each of these areas is very important, and much of this research has been gaining momentum in laboratories around the world. Equally of concern is the importance of informing those exposed to or potentially exposed to nanoscale particles about their potential hazards. Avoiding contact with these materials is the best preventative measure, but in the consideration of the possibility of exposure it is imperative to educate and inform workers on the appropriate use, storage, and handling of these particles and materials containing them.

The goal of our research is to establish simple procedures that can be used to prepare, handle and label nanoscale particles that are used world-wide. The initial aim is to understand the stability of these materials and to develop new protocol for safely using nanoscale particles. One of the primary outcomes of this research is the development of a set of protocol for monitoring the stability of nanoscale particles and assessing the long-term effects of aging on the stability of these materials. Results are presented in our work for a particular type of nanoparticle, but these studies could be easily extended to particles of other compositions, dimensions and shapes. Another outcome from this research is the development of a new method of labeling for bottles or laboratories that contain nanoscale particles, which can be also extended to products that incorporate these materials. We have determined that current practice for labeling bottles and other storage vessels, as well as rooms in which nanoparticles are used should be revised for the safety of all workers. We provide a new method of signage that could be used to indicate the presence of nanoscale particles.

We evaluated a number of techniques for their applicability in measuring the properties of nanoparticles, as well as monitoring any changes in these properties. The primary property of interest for our studies is the stability of the nanoscale particles in solution. This property is a function of the composition, size, shape, and solubility of the nanoparticle. A particle that is initially determined to be stable might become unstable after a longer period of time in storage, transport, or use. The preferred set of techniques would be able to monitor a change in stability of nanoparticles over the course of a long period of time, such as days or months. Given the intended length of the proposed experiments to assess the long-term stability of nanoparticles, the techniques should also be both relatively inexpensive and easy to implement. Techniques were also evaluated on their ability to provide information on stability of nanoparticles in aqueous environments, relevant to use or contact with biological systems. Another

important criterion in the selection of the appropriate techniques was the ability to integrate the results of all the measurements, creating a thorough depiction of stability for the nanoparticles.

From the wide number of analytical techniques that were evaluated, a subset was chosen that met the criteria listed above. These techniques included optical absorption, dynamic light scattering, and zeta potential measurements, as well as occasional use of electron microscopy analysis for further verification of the results. These chosen techniques were used to establish a protocol for assessing the potential instability of nanoscale particles. We synthesized gold nanoparticles with well-defined shape, size, composition, and surface chemistry. Each of these features could contribute to variations in the long-term stability of the nanoscale materials. The surfaces of these nanoparticles were decorated with organic molecules that stabilized the particles in aqueous solutions. Stability of these particles was determined as a function of solution composition and temperature. Month long studies were carried out to assess the long-term stability of the nanoparticles, but we recognize that longer-term studies may also be necessary in the future. We determined that the stability of these particles correlates with the quality of the surface coatings that stabilize the nanoparticles.

Our results suggest that the methods used to prepare nanoparticles and, importantly, their surface coatings are critical to determining the long-term stability of these materials. It is this surface coating that interacts with the environment surrounding the nanoparticle. Every nanoparticle has a surface coating. Examples of a surface coating include an oxide, an organic polymer, or self-assembled monolayers of organic molecules. The composition and uniformity of the coating, and its interaction with the core of the nanoparticle determines the robustness of this layer to oxidative or other methods of degradation. Degradation of the capping layer will expose the underlying particle to chemical and physical attack from the environment. These instabilities can result in particle aggregation or the release of material from the nanoparticle. To avoid degradation of the nanoparticles it is important that the stabilizing layer remain intact throughout their use. We demonstrate that creating a stable capping layer, and thus a stable nanoparticle, requires a high quality capping layer. The results suggest that the capping layer should be covalently linked to the nanoparticles and contain minimal irregularities that could expose the underlying material to the surrounding environment.

We believe that it is important to standardize the protocol for preparing nanoparticles and their capping layers. The method of preparing the capping layer determines the presence or absence of weak points within this coating that are subject to long-term chemical degradation. Creating a standard method of preparing nanoparticles and their surface coatings will be essential for those studies focused on determining the toxicity, transport, accumulation, persistence, and reactivity of nanoparticles. Further work is necessary to understand the implications of the quality of a capping or stabilizing layer with the outcome of these types of tests. More research is also required that looks into the longer-term stability of other nanoparticles that are being synthesized, and correlating these tests with the strategies to stabilize these materials.

Communicating the use of nanoparticles, as well as the potential hazards associated with these particles is essential for a safe work environment. Workers, visitors, and first responders in an emergency need to be prepared for the potential hazards they might encounter in a work environment. One of the best methods of communicating chemical safety information to workers is demonstrated through the United States National Fire Protection Agency (NFPA) hazard diamonds. These diamonds provide an overview of the information on health hazards, flammability, reactivity and other specific hazards that a worker might encounter when entering a laboratory or opening a bottle. This type of signage is essential and should be extended to the use of nanoparticles. There are a number of methods that have been proposed for use as signage to indicate the presence and hazards associated with the use of nanoparticles. After

reviewing the proposed hazard warning labels for the use of nanoparticles, we suggest a revised approach that builds from the existing conventions of warning labels and the NFPA standards.

A warning system should be adopted for all shipping containers, waste containers, and bottles holding nanoparticles, as well as all work environments in which these materials are manufactured, processed, or otherwise used. Our suggestion for the new signage is shown in Figure A, which is derived from the NFPA standard for labeling chemical hazards.

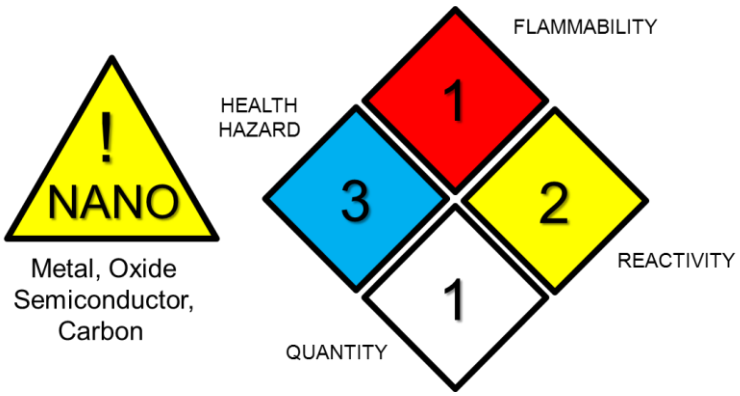


**Figure A.** Proposed label for indicating the presence of and hazards associated with nanoparticles.

The proposed label in Figure A indicates to all workers the presence of potentially hazardous nanoparticles. We recommend that this label be visible on all products and bottles containing nanoparticles. In addition, all work environments containing nanoscale particles should be implemented to indicate to visitors and workers alike the hazards within that environment. This sign should be posted in addition to the NFPA hazard diamonds. To avoid confusion, the style presented in Figure A includes an additional warning for specific nanoparticles (materials listed under warning sign) in contrast to the NFPA hazard diamonds that are general to all chemicals. The warning of nanoparticles at the start of the label in Figure A also indicates the specificity of the following hazards associated with nanoparticles. The values on the hazard diamonds are specified as a number between 0 and 5. A higher the number on the diamond indicates a greater the hazard. We believe that the quantity of material present should be indicated in its own diamond. We have replaced the diamond typically used for specific hazards—other than health, flammability or reactivity—with quantity of material present. The health hazards could include the biotoxicity, as well as persistence and accumulation in biological organisms. The flammability and reactivity are parameters that could be more easily tested for these materials. Each of these hazard diamonds will be a function of the composition, size, and capping layers on the nanoparticles. More research is required in order to implement the proposed hazard warning, which includes standardizing the values assigned to and the types of tests required for each category. The initial stages of this labeling could start with the 'nano' warning label on each bottle containing nanoparticles with as much information regarding the hazards outlined on an associated Material Safety Data Sheet. The proposed labels could, however, serve as the ultimate goal of keeping all workers and visitors in a work environment informed of the potential hazards.

The initial deliverable of this research includes the recommendation to adopt new protocol for handling and preparing nanoscale particles. This recommendation is supported by analytical data that validates the recommended procedures for monitoring and assessing nanoparticle stability. Longer-term success will be determined over time as more data is necessary for assessing the stability to aging (e.g., shelf-life) of nanoscale particles, the ability of other researchers to adopt these techniques, and finally the ability to predict the stability of other nanoparticles. This work represents one more step towards developing an extensive understanding of the health and safety aspects of nanoscale particles in the work place. Further work is necessary to determine the longer-term stability of nanoparticles and to extend these results to particles of other compositions (e.g., oxides, semiconductors, carbon). In addition, further work is required to create appropriate labels that indicate the hazards associated with nanoparticles. These include nanoparticles being studied for their catalytic, mechanical, antimicrobial,

and image enhancing properties. The implications of this work extend beyond our local community, to other provinces and to businesses and research laboratories around the world.

**Keywords:** nanoparticle stability, gold colloids, defects in capping groups, self-assembled monolayers, temperature stability, biological stability, work place hazards, nanoparticle hazard labels

## RESEARCH PROBLEM AND CONTEXT

The stability of nanoscale particles is an important concern. Instability implies unwanted changes in the properties of the particles. These changes could be as simple as aggregation of the nanoparticles to form a larger mass, which no longer retains the properties or dimensions of the original particles. The ability of these nanoparticles to resist change relies on how they interact with their surroundings. The solubility and specific functionality of the particles is often determined by the single molecule thick coatings on the nanoparticles' surfaces (e.g., biologically active molecules).[1-6] Changes to the surface properties of the particles can be easily controlled by tuning the composition of these molecular coatings.[7-10] The ability of the nanoparticles to retain these specific properties does, however, depend on the resistance of the molecular coating to degradation, such as oxidative damage or other types of displacement of these molecules from the surfaces of the particles.

Nanoparticles are widely pursued for their unique properties relative to their bulk counter parts.[11-13] For example, the relatively high surface area to volume ratio of these particles is desirable for their increased catalytic activity.[14] This increased activity can be partially attributed to the increased density of the edge and corner sites on the faceted surfaces of the crystalline nanoparticles.[15-18] The catalytically active sites are potential weak points in any molecular coatings, and must be capped with surfactants to stabilize the particles against unwanted interactions with their surrounding environment. These less desirable interactions include aggregation of the particles when dispersed in solution. The assembled surfactant molecules protect the particles from aggregation by creating a dense molecular packing that covers the crystalline facets. Defects in these self-assembled molecular coatings can expose the highly active surfaces of the particles.[19-20]

Molecules within self-assembled monolayers (SAMs) are stabilized through their interactions with the surfaces of nanoparticles and with the other molecules in the monolayers. Individual molecules in the monolayers adhere to the nanoparticle surfaces through a head group, and the same molecules

interact with neighboring molecules through van der Waals forces to assemble into the single molecule thick coatings.[19, 21-22] Molecular-based defects within these monolayers can weaken the ability of the coating to resist chemical or physical attacks. These molecular defects include the incomplete packing of molecules in the monolayers or the competitive adhesion of other surfactants onto the surfaces.[23-26] Molecules adsorbed within or onto the monolayers can diffuse away from the surfaces, exposing unprotected surfaces of the nanoparticles.[27-29] Oxidative damage can also lead to the loss of covalently linked molecules from the nanoparticle surfaces.[30-31] Damage to the molecular coating on the surfaces of the particles by any of these processes could lead to precipitation from solution or other unwanted changes to these nanoparticles.

Gold nanoparticles are widely studied as a platform for many different applications. Gold is also more resistant to oxidation than many other metals.[11, 19] Under specific conditions, colloids of gold can be prepared that are stable over relatively long periods of time.[11] Gold nanoparticles are easily decorated with monolayers that are terminated with a wide range of chemistries, such as peptides and strands of DNA.[32-33] Surface plasmon resonance of the gold particles is useful for monitoring binding and/or release of molecules from receptive ligands bound to the surfaces of these particles.[34] These gold nanoparticles can also be used as imaging agents in biological systems.[35-37] In addition, photothermal properties of these particles can activate chemical processes (e.g., molecular release) associated with molecules bound to their surfaces,[38-41] or lead to the destruction of cancer cells.[42-45] Many of these properties are, however, irreversibly altered or otherwise lost altogether if the particle changes its shape, size, or composition. Self-assembled monolayers can, for the most part, protect the nanoparticles from these unwanted changes. It is, therefore, essential to understand the stability and uniformity of these molecular-scale coatings.

The integrity of SAMs covering many types of nanoparticles can be indirectly assessed by monitoring changes in the properties of the particles. Some properties, such as the size or solubility of the particles, need to be maintained over long periods of time. For example, when used for imaging or photothermal

therapy within biological systems the gold particles must remain stable for at least a few days.[46-52] Nanoparticles can be introduced into systems of interest by intravenous injection or direct injection into tissues (e.g., tumerous growths).[47,53] Some nanoparticles might remain within the biological system for extended periods of time, while many other particles are filtered from circulation.[48-52] The particles must remain stable until they are entirely cleared from the system. To assess the stability of each batch of synthesized gold nanoparticles, whether produced for use in biological systems or other applications, it is important to identify a simple set of informative procedures that can be executed in laboratories world-wide. These procedures would need to monitor the stability of the nanoparticles over multiple days or months, and the techniques should be as simple, quick and inexpensive to implement as possible. Tests for evaluating the robustness of the SAMs (or other stabilizing coatings) should also closely match the conditions of the intended applications for the nanoparticles. The chosen analytical procedures should include the ability to change the relevant variables, such as establishing conditions that are physiologically relevant. This integration of test conditions with appropriate analytical techniques can assist in determining the integrity of nanoparticles' coatings and correlating this assessment with the methods used to prepare these protective coatings.

A set of complementary analyses are used in our study to monitor the stability of gold nanoparticles and, indirectly, the integrity of their surface coatings. The techniques used in this study monitor the changes in the chemical and physical properties of gold nanoparticles decorated with SAMs. Each of the individual techniques in this study is commonly used for nanoparticle characterization. We use these techniques in a parallel analysis to provide a comprehensive understanding of changes to particle stability. One of the outcomes of this analysis is a set of procedures that can be used to assess the quality of coatings on many different types of nanoparticles. These studies are essential for providing a range of useful information about the stability of nanoparticles and their surface coatings without requiring expensive and vast quantities of analyses. Through this type of study we demonstrate that the integrity of SAMs on gold nanoparticles depends, for the most part, on the methods used to prepare these single

molecule thick coatings. The information learned from this type of analysis can be widely used to assess the stability of nanoparticles and the quality of their surface coatings. The implementation of such procedures can guide the further improvement of these coatings from the design of the capping molecules to the methodologies used to assemble them onto the nanoparticles.

## METHODOLOGY

*Synthesis of Gold Nanoparticles.* Gold nanoparticles were synthesized and studied for their relative stability at different temperatures. Solutions of sodium citrate capped gold nanoparticles were prepared with a few modifications to a published procedure.[54] One of the most important steps of this process was thoroughly cleaning the glassware required for the reaction. Glass flasks and Teflon® coated stir bars used in this synthesis were first soaked for ~15 min with a 15 mL solution of aqua regia. The aqua regia solution was prepared from a 3:1 (v/v) solution of hydrochloric acid (36.5 – 38.0% in water; Anachemia Canada, Inc., Richmond, BC, Canada) and nitric acid (68 – 70% in water; Anachemia Canada, Inc., Richmond, BC, Canada). *CAUTION: Aqua regia solutions are extremely corrosive. This solution should be handled with extreme care.* The aqua regia soaked flasks and stir bars were rinsed with more than 500 mL of 18 MΩ·cm high purity water (water purified from a Barnstead Nanopure DIamond Life Science water filtration system). After the rinsing procedure, the flasks and stir bars were further cleaned with for ~15 min with a 15 mL of piranha solution. The piranha solution was prepared from a 7:2 (v/v) mixture of concentrated sulfuric acid (Anachemia Canada, Inc., Richmond, BC, Canada) and a 30 % by volume aqueous solution of hydrogen peroxide (VWR International, Mississauga, ON, Canada). *CAUTION: Piranha solution is a strong oxidizing agent and reacts violently with organic compounds. This solution should be handled with extreme care.* These flasks and stir bars were subsequently rinsed with more than 500 mL of 18 MΩ·cm water, and dried in an oven at 120°C.

The gold nanoparticles were synthesized from a solution of gold (III) chloride. These solutions were prepared by dissolving approximately 100.67 mg of $HAuCl_4 \cdot 3H_2O$ (99.9% pure; Sigma-Aldrich, St. Louis, MO, USA) in 50 mL 18 MΩ·cm water to prepare a solution of 5.11 mM gold salt. Stock solutions of the gold salt were prepared at least 24 h prior to each synthesis of gold nanoparticles. For the synthesis of the gold nanoparticles, a solution of 0.022 mM sodium citrate tribasic dihydrate (99.0% pure; Sigma-Aldrich, St. Louis, MO, USA) was prepared by dissolving 6.58 mg in 5 mL of 18 MΩ·cm water. The gold stock solution was further diluted prior to use in the synthesis of gold nanoparticles. A 1.45 mL aliquot of the gold salt solution was diluted to 50 mL with 18 MΩ·cm water. This diluted salt solution was transferred to a 250 mL round bottom glass flask. A water cooled condenser was attached to this flask and the solution brought to reflux using an electronic heating mantle. Meanwhile, the solution of sodium citrate was heated to 60°C and then quickly added to the boiling solution of tetrachloroauric (III) acid. The combined solutions were allowed to reflux for another 10 min. During this time, the solution changed from a colorless to a dark purple and finally to a red color.

The resulting red colored solutions contained dispersions of gold nanoparticles. These particles had a characteristic surface plasmon resonance absorbance peak centered at 521 nm.[55] Copper grids coated with carbon and Formvar (300 mesh; Electron Microscopy Sciences, Hatfield, PA, USA) were used to prepare the nanoparticles for analysis by transmission electron microscopy (TEM). Aliquots from the gold nanoparticle solutions of ~10 μL were drop cast onto the TEM grids and dried under vacuum in a desiccator. Diameter of these particles was measured using a Hitachi H-8000 STEM operating at 200 kV. From this TEM analysis the average diameter of gold nanoparticles used in these studies was 21±2 nm.

*Preparation of Monolayer Protected Gold Nanoparticles using Polysorbate 20 Surfactants.* The exchange of citrate molecules on the surfaces of gold nanoparticles for alkanethiols can destabilize the nanoparticles.[56, 57] This destabilization can result in a significant loss of nanoparticles from solution.

One method to stabilize the gold nanoparticles is to introduce Tween® 20 (polysorbate 20 surfactants) into the solution during the exchange process.[56. 58, 59] All glassware was cleaned and prepared by the procedures described above. The gold nanoparticles were modified with 16-mercaptohexadecanoic acid (MHDA) purchased from Sigma-Aldrich (St. Louis, MO, USA) at 90% purity, which was used upon receipt without further purification. To prepare the monolayer protected gold nanoparticles, 25 mL of a solution containing the citrate-capped gold nanoparticles was mixed with 25 mL of 0.32 M Tween® 20 (Sigma-Aldrich, St. Louis, MO, USA; 8.32 g dissolved in 25 mL phosphate buffered solution at pH 7.2). This mixture was allowed to sit, undisturbed in a round-bottom flask for 30 min. Then 1.5 mL of freshly prepared 1 mM MHDA (dissolved in ethanol) was added to the round-bottom flask containing the gold nanoparticles and polysorbate 20 surfactants. The reaction mixture was stirred at 22°C (maintained by an oil immersion bath) for 30 min, 4 h or 2 days. When the designated period of time for exchange of the capping groups was complete the MHDA capped gold nanoparticles were transferred to 1.5 mL Eppendorf tubes for purification. These solutions of alkanethiolate modified gold nanoparticles were purified of excess alkanethiols and polysorbate surfactants with 3 steps of centrifugation (13,200 rpm for 10 min), decantation of supernatants, and re-suspension in 10 mM phosphate buffer at pH 7.2 (sodium phosphate monobasic and sodium phosphate dibasic; Caledon Laboratories Ltd., Georgetown, ON, Canada). Some polysorbate surfactants remain adsorbed onto the surfaces of the gold following this purification process.

*Preparation of Monolayer Protected Gold Nanoparticles without using Polysorbate 20 Surfactants.* The citrate capped gold nanoparticles were also modified with MHDA without the addition of polysorbate 20 surfactants in order to correlate the nanoparticle stability to the presence of this surfactant during the formation of the alkanethiolate monolayers. To describe the procedures briefly, 1.5 mL of freshly prepared 1 mM MHDA (dissolved in ethanol) was added to a round-bottom flask containing a 10 mL solution of citrate-capped gold nanoparticles. The reaction mixture was stirred while being held at 22°C (using an oil immersion bath) for 30 min, 4 h or 2 days. When the exchange period was complete,

the MHDA capped gold nanoparticles were transferred to 1.5 mL Eppendorf tubes for purification. These solutions of alkanethiolate modified gold nanoparticles were purified of excess alkanethiols with 3 steps of centrifugation (13,200 rpm for 10 min), decantation of supernatants, and re-suspension in 10 mM phosphate buffer at pH 7.2.

*Testing the Stability of Monolayer Protected Gold Nanoparticles*. The stability of MHDA modified gold nanoparticles was tested at different temperatures over the period of ~1 month (or 672 h) by immersing these solutions of gold nanoparticles into water baths maintained at 22.0±0.3°C, 37.0±0.5°C, or 45.0±0.5°C. These temperatures were chosen to include average physiologically relevant conditions, as well as near ambient conditions and an elevated temperature for accelerated degradation. Solutions of nanoparticles were continuously stored at elevated temperatures to promote both oxidative damage to the SAMs and the diffusion of adsorbed species away from the surfaces of the particles. These solutions also contain species (e.g., phosphates) that compete with the SAMs to bind with the surfaces of the gold nanoparticles. Four duplicates of the gold nanoparticle solutions were evaluated under each of the experimental conditions. For consistency between the experiments, each synthesis of gold nanoparticles was divided into 3 equal portions. All of these portions were evaluated in parallel, but each portion was tested at a different temperature. All solutions were held at the set temperature without agitation and in the absence of light during the experiments. Aside from the influence of temperature, the pH and composition of the buffer solution can also influence the stability of the monolayer protected gold nanoparticles. For these studies, the MHDA capped and purified gold nanoparticles were suspended in a series of solutions containing different buffers. These buffers included phosphoric acid/sodium phosphate monobasic (pH 3.0 solutions), acetic acid/sodium acetate (pH 5.0 solutions), sodium phosphate monobasic/sodium phosphate dibasic (pH 7.2 solutions), tris borate EDTA (pH 8.3 solutions), sodium phosphate dibasic (pH 9.1 solutions), and sodium carbonate (pH 11.2 solutions). Solutions containing different buffers were prepared using gold nanoparticles that had either included or excluded polysorbate 20 surfactants during the assembly of the MHDA monolayers. This comparison of

polysorbate and non-polysorbate stabilized nanoparticles is important to assist in determining the role of this surfactant on the ultimate stability of the nanoparticles in different solutions.

*Monitoring the Properties of Monolayer Protected Gold Nanoparticles*. Various properties of the nanoparticles were monitored at regular intervals (0, 24, 48, 72, 168, 336, and 672 h) during month-long studies. The suspensions of monolayer capped gold nanoparticles were monitored by a combination of ultraviolet-visible (UV-Vis) absorption spectroscopy, zeta potential (ZP) measurements and dynamic light scattering based particle size analysis (PSA) measurements. The UV-Vis absorption spectra were recorded from 300 to 900 nm on a Cary 300-Bio UV-Visible spectrophotometer. Samples of the suspended gold particles were agitated by hand for at least 30 s and transferred by pipette into quartz cuvettes with a 1 cm optical path length. The UV-Vis spectra for solutions of gold nanoparticles held at different temperatures (22, 37, or 45°C) were recorded at regular intervals during study. At each time interval, we also acquired PSA measurements on the same solutions using a Malvern Zetasizer Nano-ZS. Solutions were contained within folded capillary cells (Malvern, Westborough, MA, USA). The solutions of gold nanoparticles were loaded into the capillary cells in a similar manner to that described for the UV-Vis spectroscopy. Zeta potential measurements for each of these solutions were also acquired using the same Malvern system and capillary cells that were used for the PSA measurements.

## RESEARCH FINDINGS

In order to assess the stability of these suspensions to unwanted changes, such as flocculation and aggregation, solutions of colloidal gold nanoparticles were analyzed over a period of up to 1 month. The stability of these particles is primarily dictated by their surface chemistry. This interface between the particles and their surroundings determines how the particles interact with each other, as well as with any molecules or ions in the solution. Damage to the surface coatings could result from chemical attack, or destabilization of the interactions between the capping molecules and the surfaces of the

nanoparticles. Our goal was to determine the correlation between long-term stability of the nanoparticles with respect to the methods and reagents used to prepare the surface coatings on these particles.

Gold nanoparticles were chosen in this study because of their widespread use in research and development projects in many different laboratories. These particles have a better chemical stability relative to many other materials.[11, 19] The surfaces of gold nanoparticles can also be easily decorated with organic molecules through specific interactions (e.g., thiol-gold bonds).[11, 19, 32, 33] The surface chemistry of gold nanoparticles are easily modified through small structural changes to the molecules within the monolayers.[60] However, the quality of these monolayers can be inconsistent from sample to sample. One measure of this quality is the ability of a capping layer to stabilize colloidal particles against aggregation and other undesirable interactions with the surrounding solution.

The interactions of a nanoparticle with its surrounding environment are determined, in part, by the terminal groups of individual molecules within its monolayers. The design of these terminal groups is dictated by their desired interactions with the solution. Terminal groups such as carboxylic acids, sulfonates, amines, and amino acids are typically used to disperse gold nanoparticles in aqueous solutions. The linkers between the terminal groups and the surface bound sulfur head groups are also important in determining the interactions of a nanoparticle with its surroundings. Polar linkers, such as polyethylene glycols or polypeptides, can impart increased solubility of a nanoparticle in aqueous solutions. The length and composition of the linkers can also influence the stability of the monolayers. Increased intermolecular interactions within the monolayers, such as through van der Waals forces, can improve the stability of the SAMs. A balance of all of these interactions must be considered when choosing the composition of the capping molecules. The molecule of choice for our experiments is mercaptohexadecanoic acid (MHDA). It is a long-chain alkanethiol with a terminal carboxylic acid group. The interactions of a nanoparticle with its surroundings are also determined by the methods used to form the SAMs and their density on the surfaces of the gold.

The processes used to form SAMs on the surfaces of gold nanoparticles are wide-ranging in the literature. Directly coating gold nanoparticles with alkanethiols can result in the instability of these nanoparticles.[56, 57] To avoid this instability, additional surfactants are added to the solution to further stabilize the nanoparticles during the formation of the SAMs. These extra surfactants are displaced during assembly of the alkanethiols (e.g., MHDA) onto the gold surfaces. This displacement process can also destabilize the nanoparticles if the excess surfactant does not prevent particle-to-particle interactions that result in aggregation. In our study, monolayers are prepared in the presence of polysorbate 20, a secondary surfactant that has been used in previous studies to stabilize citrate capped gold nanoparticles during the formation of alkanethiolate SAMs.[56, 58, 59] After formation of the alkanethiolate monolayers, these nanoparticles were purified by a series of steps that include repeated centrifugation and rinsing to remove the excess polysorbate 20 surfactants and alkanethiols from solution. The purified particles were then suspended in a phosphate buffered solution at pH 7.2.

Another key aspect to form high quality SAMs on gold nanoparticles is the time permitted for the formation of the monolayers. It has been previously established that the duration of the molecular assembly process can influence the quality of SAMs protecting planar films of gold. These studies suggest that for ambient conditions an exchange reaction over a period of at least 2 days is required to achieve high quality monolayers.[19, 22, 61] The time reported for the assembly of alkanethiols onto the surfaces of gold nanoparticles varies and is typically for periods less than 2 days.[6, 62-64] These relatively short periods of time suggest that the particles might be unstable due to low or variable quality monolayers. We suspect that a period of 2 days or more is required for alkanethiolates to assemble into dense, well-packed monolayers covering the gold nanoparticles. Therefore, we compared the quality of MHDA monolayers formed over periods of 30 min, 4 h, or 2 days. The quality of these monolayers was evaluated using a wide range of experimental conditions.

The stability of gold nanoparticles in solution provides an indication of the quality of the monolayers protecting these particles. Changing the composition of the solution is one approach to probe the

presence of defects in the assembled monolayers. These compositional changes include additives that bind to, or otherwise strongly interact with the gold surfaces. In our study, we added phosphate salts to the nanoparticle solutions in an attempt to destabilize these particles. Another method of testing the quality of the monolayer is varying the solution temperature. Higher solution temperatures could increase mass transport to and from the surfaces of the nanoparticles,[65, 66] as well as promote oxidative damage to the alkanethiolates within the monolayers. Temperatures chosen for our study include ambient laboratory conditions (~22°C), near physiological conditions (~37°C), and a higher temperature (45°C). The influences of these conditions on stability of the gold particles were monitored on a regular basis through out each experiment. Instability of the colloidal nanoparticles could manifest itself within a period of a few hours or days in proportion to the size and nature of defects present within the monolayers. The ultimate quality of the monolayers is, however, measured by the ability of the capping molecules to stabilize the particles over periods of time longer than a few days. We evaluated the stability of colloidal solutions of gold nanoparticles held at each temperature over the course of one month. Any instability of the nanoparticles during this time can be the result of a kinetically slow or hindered degradation of the monolayers. These relatively slow processes could be attributed to oxidative damage at defects in monolayers that are initially protected by physically adsorbed species. A number of simple techniques were used over the one month period to assess the quality of the monolayers protecting the nanoparticles.

One of our criteria when choosing techniques to monitor the quality of SAMs on gold nanoparticles was the relative accessibility of the techniques to other researchers. The techniques chosen for our studies included UV-Vis absorption spectroscopy, transmission electron microscopy (TEM) analysis, as well as zeta potential (ZP) and particle size analysis (PSA) by dynamic light scattering. The UV-Vis absorbance spectroscopy, zeta potential and PSA measurements were made on a regular basis (e.g., daily). These techniques are relatively simple to implement, require less time than TEM analysis, and monitor the interactions of the solution (e.g., solvent, surfactants, and salts) with the nanoparticles.

Electron microscopy was, however, used as a complementary technique to monitor changes in size and shape of the nanoparticles. The zeta potential and PSA measurements were performed to determine changes in the surface charge density and hydrodynamic diameter of the nanoparticles. A decrease in zeta potential values would suggest instability of the suspended particles. An increase in hydrodynamic diameter would suggest flocculation of the colloidal particles. In addition, light scattering from 600 to 900 nm observed by absorbance spectroscopy also provided a measure of nanoparticle flocculation within the colloidal solutions.[67, 68] Absorbance spectroscopy measurements also monitored particle stability by observing any changes in the position, width and intensity of the localized surface plasmon resonance band. These spectral features are correlated to the composition of the dielectric layers on the surfaces of the nanoparticles and the concentration of gold colloids.

In our study, the UV-Vis absorbance data indicated a wide variation in nanoparticle stability between those particles capped with monolayers formed over a period of 30 min, 4 h, or 2 days. For example, a large variation in particle stability was observed for solutions of these gold nanoparticles suspended in phosphate buffered solutions held at 37°C (a physiologically relevant temperature) for ~1 month (Figure 1a). The nanoparticle solutions were shaken prior to each UV-Vis absorption measurement in order to differentiate between sedimentation and aggregation of the particles. This agitation dispersed particles that had settled from solution, but aggregated particles are more likely to remain adhered to the bottom or sides of the glass vial. A portion of the suspended particles were transferred to another container to measure the absorbance spectra. The intensity of these spectra correlates to the concentrations of nanoparticles in solution, and a decrease in absorbance indicates precipitation of nanoparticles from solution. A reference spectrum for the as-synthesized citrate capped gold nanoparticles is included for comparison (Figure 1a). The particles capped with SAMs prepared over a period of 30 min had the largest decrease in spectral intensity after being held at 37°C for 1 month. The solutions with the least spectral change, relative to the as-synthesized particles, were those prepared with monolayers formed over a period of 2 days. The spectra for these solutions had the smallest decrease in the peak absorbance.

All UV-Vis spectra for MHDA capped nanoparticles stored at 37°C for 1 month had a plasmon peak centered at 523 nm. These spectral peaks are slightly shifted from the 521 nm peak position of the citrate capped gold nanoparticles. This spectral shift is attributed to the formation of the MHDA monolayers on the gold particles. In addition, a minimal change in light scattering at wavelengths higher than 600 nm suggests there is no change in the concentration of suspended flocculates or aggregates within these solutions.

The most stable colloids after 1 month at 37°C, as determined from the UV-Vis spectra, were those particles capped with monolayers formed over a period of 2 days. After one month, these gold nanoparticles suspended in phosphate buffered solutions at 22°C have similar spectral properties to the same colloidal suspensions held at 37°C (Figure 1b). A more dramatic difference is observed for those colloids held at 45°C. A large decrease in spectral intensity is observed for these particles, but without an increase in spectral scattering or a shift in the position of the surface plasmon peak. An increase in temperature of the solutions can lead to an increased mass transport of solutes to and from the surfaces of the nanoparticles. The impact of this increased mass transport is detrimental for the samples held at 45°C for ~1 month. The maximum absorbance intensity of the plasmon peak gradually decreases over the one month period, suggesting that some of these nanoparticles are precipitating out of solution (Figure 1c). The UV-Vis spectra were acquired at regular intervals during this one month period (i.e., 0 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, and ~1 month). There is a negligible spectral change from 24 to 72 h. A more substantial change in maximum absorbance intensity is observed after 1 week. The peak intensity continues to decrease with an increased time of holding these colloidal samples at 45°C. The trends for these UV-Vis absorbance spectra are easily observed, but other methods for analyzing the data can provide a more quantitative measure of the spectral changes.

A simple approach to analyze changes in the dispersion of gold colloids is to plot the maximum spectral absorbance after each time interval during the one month period. This approach provides a quick comparison of the relative rates of change in nanoparticle stability for different solutions. The

surface plasmon peaks for these solutions of MHDA capped nanoparticles are consistently centered at 523 nm (Figure 1), but the intensity of these peaks decreases at different rates depending on the method used to prepare the SAMs and the conditions of each experiment. Distinct differences are observed when comparing the changes in maximum absorbance for solutions of gold nanoparticles held at 22, 37 and 45°C for a period of up to 672 h (Figure 2). Error bars on these plots indicate the reproducibility of each trend as determined from four independent experiments. All samples held at 22°C exhibited a similar trend over the 1 month period (Figure 2a). The maximum change at 22°C approached 7%, and most of this spectral change is observed during the initial stage of the experiment (i.e., from 1 h to 168 h). The monolayers of alkanethiolates are susceptible to oxidative damage of the sulfur head group.[30, 69, 70] It is possible when storing the gold nanoparticles for extended periods of time that the alkanethiolates desorb from the gold surfaces through oxidative damage or other processes. It is likely that the initial period of instability observed for the samples at 22°C is due to desorption from the nanoparticles' surfaces of physically adhered alkanethiolates. This diffusion of molecules away from the surfaces increases the size and number of defects within the monolayers, decreasing the overall stability of the nanoparticles.

A further increase of solution temperature has a dramatic influence on the stability of the gold colloids. An increase in temperature can promote oxidative damage of the stabilizing ligands, as well as an increase of mass transfer to and from these surfaces. On the other hand, temperatures above 22°C can increase the mobility of protecting groups on the gold surfaces, which can lead to a reduction of defects in the monolayers through an annealing process.[71-73] Colloidal solutions held at 37°C for 1 month exhibited both an increased and decreased stability (Figure 2b). Particles capped with SAMs formed over 30 min were the most destabilized with a decrease of ~40% in maximum absorbance at 523 nm after being held for 1 month at 37°C. The nanoparticles capped with SAMs formed over 4 h had a 15% decrease in maximum absorbance over the same period of time. The most stable particles were those capped with monolayers formed over a period of 2 days. These particles had ~1% decrease in maximum

absorbance. Nanoparticles capped with monolayers prepared over shorter periods of time (e.g., 30 min and 4 h) most likely have a lower density of MHDA covering their surfaces, which leads to a greater instability of these particles. Particles stabilized with denser monolayers of MHDA, such as those with SAMs formed over a period of 2 days, are more stable. The later type of particle also had an increased stability at 37°C (Figure 2b) in comparison to solutions held at 22°C (Figure 2a). This increased stability is attributed to the annealing of the SAMs protecting these particles. Further increases in solution temperature may lead to instability of these particles due to increased oxidative damage to the surfactants.

Increasing the solution temperature to 45°C significantly decreases the stability of the monolayer capped gold colloids. The decrease in maximum peak absorbance (for all suspensions of nanoparticles) was substantial after 2 days of being held at 45°C (Figure 2c). The capping groups continued to degrade over the course of 1 month. Solutions of gold nanoparticles capped with SAMs formed over 30 min decreased to ~15% of their original absorbance values. Colloids capped with monolayers assembled over 4 h had a similar decrease in particle stability. These solutions decreased to ~25% of their original absorbance values over the 1 month period at 45°C. In contrast, nanoparticles stabilized with SAMs formed over 2 days maintained ~50% of their original maximum peak absorbance values. For all of these samples, after 2 days of being held at 45°C the average spectral change is between 0.08% and 0.13% per hour. The instability of the monolayer protected gold colloids at 45°C could be due to an increased oxidation of the alkanethiolates and the polysorbate 20 surfactants adsorbed onto the gold surfaces.

Instability of the capping monolayers protecting the colloidal gold can lead to flocculation and aggregation of these particles. Flocculation of nanoparticles would increase their hydrodynamic diameter as measured by dynamic light scattering analysis. All solutions of gold nanoparticles had a consistent hydrodynamic diameter is ~35 nm while suspended in a phosphate buffer solution for ~1 month at 22°C (Figure 3a). Error bars in these plots are calculated as one standard deviation from the mean of four

independent experiments. This distribution in the measured diameters is most likely attributable to variations in the diameters of gold nanoparticles in solution, as well as a non-uniform distribution of adsorbed polysorbate 20 surfactants between different nanoparticles. Average diameter of the as-prepared gold nanoparticles is 21±2 nm by TEM analysis (Figure 4). The larger diameters reported from the PSA measurements are attributed to the electrostatic double layer on the alkanethiol-capped gold nanoparticles and the adsorbed polysorbate 20 surfactants. The majority of particle diameters, whether measured by PSA or TEM analysis, are consistent within the calculated errors for each data point over the one month period (Figures 3 and 4, respectively). Solutions of nanoparticles held at both 22°C and 37°C have a consistent hydrodynamic diameter of ~33 nm throughout these studies. A slight deviation from this trend is observed for nanoparticles capped with SAMs formed over 4 h after being held at 37°C for 1 month (Figure 3b). In addition, the hydrodynamic diameter increased to ~47 nm for particles stabilized with SAMs formed over either 4 h or 2 days when held at 45°C. A significantly larger increase in hydrodynamic diameter is observed for particles stabilized with SAMs prepared over 30 min and stored at 45°C over the course of 1 month (Figure 3c). Aggregates were not observed in the TEM analysis for samples stored at 45°C for one month. After this period of time, black precipitates were observed in each of the solutions held at 45°C. The presence of precipitates and the increased hydrodynamic diameters of the particles held at 45°C indicate a degradation of the capping layer on the surfaces of the gold. Another indication of the quality of this stabilizing layer is the surface charge on the nanoparticles.

A simple technique to measure average charge on the surfaces of the nanoparticles is through zeta potential analysis. Nanoparticles decorated with MHDA and polysorbate 20 surfactants in phosphate buffered solution at pH 7.2 have an average ZP of –30 mV. This value is in the range of acceptable values for stable dispersions of nanoparticles. A deviation in ZP value can indicate instability of the colloidal particles. This value progressively changes over the course of a month for the colloids stored at 22°C (Figure 5a). Those nanoparticles stabilized with MHDA monolayers assembled over 30 min have a

ZP value of –18 mV after 72 h and –9 mV after ~1 month. Similarly, after 72 h those nanoparticles capped with SAMs prepared over 4 h displayed a change in ZP to –20 mV, which continued to decrease to –9 mV after 1 month. In contrast, the particles capped with SAMs formed over 2 days steadily decreased to –18 mV over the course of one month when held at 22°C. These results, in combination with the observation that these solutions exhibit little precipitation or flocculation (Figures 2a, 3a), suggest that the observed changes in ZP values are due to alterations of the surface chemistry or double layer of the gold colloids. One possibility for these observed changes is a decrease in density of the alkanethiolates on the surfaces of the nanoparticles due to desorption and/or oxidative damage. Another possibility is a re-distribution of the loosely adsorbed polysorbate 20 surfactants, or its degradation products, over the surfaces of the colloids.[74] Polysorbate 20 surfactants degrade by oxidative damage as a function of pH and temperature.[75] The loss of alkanethiolates from the SAMs is less probable for these experiments, as this would have led to a wide-spread destabilization and aggregation of the colloids. The minimal precipitation in these solutions (Figures 2a, 3a) suggests that the more likely mechanism for decreased ZP values is screening of surface charges by reorganization and potential degradation of polysorbate 20 surfactants. The ZP value for nanoparticles protected with SAMs formed over 2 days (–18 mV after 1 month at 22°C) is approximately twice that of the other two types of nanoparticles. It is likely that the particles protected with SAMs prepared over 2 days had less polysorbate 20 surfactants adsorbed on their surfaces at the beginning of the experiments because of more densely packed monolayers (Figure 6). These defects might be repaired through annealing the monolayers when heating the solutions to higher temperatures, but an increased temperature will also accelerate the rate of polysorbate 20 surfactant degradation.[75]

A significant increase in solution temperature is necessary before observing a significant change in the average ZP values. Solutions of gold colloids maintained at 37°C for up to ~1 month (Figure 5b) displayed a similar trend in ZP values to those colloids held at 22°C. A different trend in ZP values was observed for nanoparticles maintained at 45°C (Figure 5c). The average ZP value remained around –30

mV for up to 72 h. At 1 week the average ZP values changed to –40 mV. Those nanoparticles capped with SAMs formed over 30 min have a ZP of –22 mV after 1 month at 45°C. In contrast, the gold nanoparticles capped with monolayers assembled over 4 h or 2 days have a ZP value of approximately –30 mV after being held at the same conditions. Increasing the solution temperature to 45°C promotes the degradation of polysorbate 20 surfactants, as well as an increase in mass transport to and from the surfaces of the nanoparticles. Both of these processes result in a decreased shielding of the surface charges by the polysorbate 20 surfactants, and a subsequent decrease in ZP to –40 mV for the particles remaining in solution. The degradation of the polysorbate 20 surfactants also promotes further interactions between the gold nanoparticles. Oxidative damage of the alkanethiolates is also promoted at the higher solution temperature. This oxidative damage promotes an increase in the size of defects in the monolayers.[31] The increased precipitation within the samples held at 45°C is probably due to the increased interactions of particles (e.g., aggregation) at exposed defect sites within the SAMs. These interactions are proportional to the quantity and size of defects within the monolayers. Hence, the monolayers assembled over 2 days provide the most stabilization for the gold nanoparticles in these experiments because these SAMs are the densest and contain the least amount of adsorbed polysorbate 20 surfactants.

The thermal degradation of polysorbate 20 surfactants suggests that the absence of this surfactant could increase the long-term stability of the SAMs and the gold colloids. The addition of polysorbate 20 surfactants has, however, improved the stability of gold nanoparticles during the process of capping these particles with SAMs of alkanethiolates.[56] To further assess the impact of polysorbate surfactants on the results of our study, we prepared solutions of gold colloids capped with MHDA monolayers without polysorbate surfactants, and studied these solutions over the course of 1 month while keeping the solutions at 22, 37 or 45°C. These solutions were also prepared with phosphate buffer at a pH of 7.2. Our studies determined that the most stable particles are those capped with SAMs formed over a period of 2 days in the presence of polysorbate 20 surfactants. Therefore, all of the nanoparticles in these later

studies were also capped with monolayers formed over a period of 2 days. A larger decrease in maximum absorbance is observed for the nanoparticles prepared without polysorbate surfactants (Figure 7a). The maximum absorbance for these solutions decreased by 2x to 4x more over the first 72 h than that previously observed for colloids capped with both MHDA and polysorbates. After a period of one month at 22°C, the colloids capped with SAMs prepared in the absence of polysorbate surfactants had a decrease in maximum absorbance nearly twice that for nanoparticles prepared with polysorbate 20 surfactants. In addition, those nanoparticles prepared without polysorbate 20 surfactants and held at 37°C for 1 month decreased in maximum absorbance by ~21% in comparison to 2% for those particles capped with alkanethiolates and polysorbates. In contrast, at higher solution temperatures (i.e., 45°C), the polysorbate 20 surfactants stabilized nanoparticles decreased in maximum absorbance by ~50% in comparison to ~16% for those particles capped with only alkanethiolate SAMs. At 22°C and 37°C, stability of the MHDA capped nanoparticles increased with addition of the polysorbate 20 surfactants. This data suggests that the polysorbate surfactants incorporate into the monolayers during their formation, or adsorb onto defects within the SAMs. At higher temperatures the polysorbate 20 surfactants diffuse away from the nanoparticles and expose defects within the SAMs to the surrounding solution. At higher solution temperatures the polysorbate 20 surfactants are also prone to oxidative degradation and produce peroxides.[75] The degradation of the polysorbates may also promote an increased oxidation of the alkanethiolates within the SAMs. Therefore, the increased temperature can significantly decrease the stability of the MHDA and polysorbate surfactant stabilized colloids.

The measured changes in hydrodynamic diameter and zeta potential values further support our conclusions regarding the influences of adsorbed polysorbate 20 surfactants on the stability of the MHDA capped gold nanoparticles. The PSA measurements suggest an increased flocculation of nanoparticles prepared without polysorbate surfactants when held at 22, 37 and 45°C over the course of 1 month (Figure 7b). The ZP values are also relatively consistent over the 1 month period for the same solutions of particles (Figure 7c). A notable difference from those results reported above is the more

negative ZP values for nanoparticles prepared in the absence of polysorbate surfactants. The MHDA stabilized particles have an initial ZP of approximately –38 mV, in comparison to a ZP value of –30 mV for particles capped with MHDA and polysorbate 20 surfactants. This difference is attributed to a screening of surface charges by adsorbed polysorbate surfactants.

The colloids capped with polysorbate 20 surfactants and MHDA are more stable than those without polysorbate surfactants when held in pH 7.2 phosphate buffered solutions either at or below physiologically relevant temperatures. The stabilization of gold nanoparticles by the polysorbate 20 surfactants is further supported by varying the pH of the solution. A series of solutions were prepared with gold nanoparticles capped with SAMs formed over 2 days in either the absence or presence of polysorbate surfactants. Each solution was partitioned into five equal volumes and each portion mixed with a specific buffer to achieve solutions at pH values of ~3, ~5, ~7, ~9 and ~11. Particle stability at different pH's was characterized by both UV-Vis spectroscopy and PSA measurements. A change in near-infrared absorbance within a spectrum can also indicate flocculation of nanoparticles in solution.[67, 68] In the absence of polysorbate surfactants, a comparison of the solutions at different pH values indicates that the solutions at pH 7.2 and 9.1 were the most stable with the least increase in flocculation (Figure 8a). In contrast, all of those colloids with SAMs formed in the presence of polysorbate 20 surfactants are more stable. The adsorbed polysorbate 20 surfactants stabilize the nanoparticles from pH values of 3.0 to 9.1. Some flocculation is observed for these particles at pH 11.2, which is attributed to the instability of polysorbate surfactants under basic conditions.[75] Similar trends are observed for the hydrodynamic diameters of particles with and without polysorbate surfactants at the various pH values (Figure 8b). These results further support the stabilization of the gold colloids by adsorbed polysorbate 20 surfactants.

The stability of the suspended nanoparticles is also correlated to the composition of the solution. For example, gold nanoparticles capped only with MHDA and suspended in a tris borate EDTA (TBE) buffered solution at pH 8.3 only had a small decrease in maximum UV-Vis absorbance over a period of

1 month at 22, 37, and 45°C. In comparison, those nanoparticles capped with MHDA and polysorbate 20 surfactants in phosphate buffered (PB) solution at pH 7.2 are more stable at 22°C, but less stable at 45°C (Figure 9 and Table 1). The phosphate buffer was chosen for the studies discussed herein because of its high affinity to the gold surfaces and its ability to destabilize the nanoparticles. The phosphate buffered solution at pH 7.2 was also chosen for its physiological relevance. It is important that nanoparticles and the quality of their capping groups be investigated under conditions relevant to the intended use of the nanoparticles. It is also important to study the long-term stability, and the stability under extreme conditions that might also be relevant for the intended use of the nanoparticles.

## IMPLICATIONS FOR FUTURE RESEARCH ON OCCUPATIONAL HEALTH

It is important that nanoparticles remain stable during storage and transport, as well as when used in their intended applications. Shelf-life and stability of nanoparticles is of increasing importance as these particles are considered for biological applications, where gold nanoparticles could take days before clearing out of the biological system. These applications include fluorescence imaging,[76, 77] photoacoustic imaging,[36] specific cell or tissue targeting,[1] as well as photothermal[38-40] and photodynamic[78] therapies. These gold nanoparticles must remain well dispersed within aqueous solutions of a wide range of compositions in order to be useful for these intended applications. Further work is necessary to continue to improve the quality of capping groups on particles and to continue to understand the correlation of nanoparticle stability with reproducibility of results under physiologically relevant conditions. A high variability in the quality of the nanoparticle stability could have significant implications in long-term studies, such as multiyear programs evaluating the use of nanoparticles for *in vivo* biological studies. These studies are also essential to anyone handling or otherwise working with nanoparticles.

In the studies reported herein, suspensions of gold nanoparticles were analyzed for colloidal stability as a function of solution composition and temperature, as well as quality of SAMs capping the nanoparticles. Instabilities are correlated to defects in the capping layers that stabilize these particles.

Some particles initially appear to be stable, but after a period of being held at elevated temperatures demonstrate significant aggregation and precipitation of particles. For these experiments, particles with monolayers formed over a period of 2 days displayed the highest colloidal stability, with minimal changes in UV-Vis spectral absorbance, particle size, and surface charges. This colloidal stability is attributed to the high quality of monolayers formed on these particles because adequate time was allowed for the MHDA molecules to organize into well-packed monolayers. Further work is required to extend these studies to other types of nanoparticles. Complementary techniques might also have to be developed for monitoring the stability of nanoparticles of other compositions, especially those without a localized surface plasmon resonance (e.g., oxides, carbon nanostructures, and semiconducting nanoparticles). Extension to other materials might depend more on the use of light scattering techniques and other optical properties of the nanoparticles (e.g., fluorescence to quantify the number of particles in solution). The studies presented herein reflect the need to analyze the stability of nanoparticles.

In conclusion, we have established simple protocols for evaluating the stability of colloidal solutions of nanoparticles. We demonstrated these techniques on alkanethiolate capped gold nanoparticles, creating an understanding of the quality of these monolayers. The stability of these nanoparticles over a period of 1 month was assessed by monitoring changes in the concentration, hydrodynamic size and particle surface charges. Stability of the nanoparticles and their surface stabilizing agents were investigated as a function of the quality of monolayers capping these particles, as well as the solution temperature, and composition (e.g. the choice of buffer and pH). These studies were carried out over the course of 1 month in order to understand the long-term stability of the nanoparticles. The time and temperature variability of these studies is essential to evaluating the nanoparticle solutions for relevant storage conditions, transport to or use in their intended application. For example, nanoparticles introduced into biological systems, whether intentionally or not, must maintain their stability for durations longer than that required to clear from these systems. The stability of gold nanoparticles is dependent on the conditions in which these nanoparticles are prepared. The presence of both non-

specific and specifically bound surfactants improves the stability of gold nanoparticles during formation of self-assembled monolayers on these particle surfaces. Under certain conditions, the non-specifically adsorbed species/surfactants improve the long-term stability of the nanoparticles. The ultimate stability of the nanoparticles does, however, depend on the quality of the covalently bound self-assembled monolayers. The capping groups of the highest quality on gold colloids are prepared through the formation of self-assembled monolayers over ≥2 days. Sufficient time is necessary for the formation of densely packed monolayers of alkanethiolates on the surfaces of gold colloids to adequately stabilize these particles against aggregation and other unwanted changes.

## POLICY AND PREVENTION

Avoidance is the best measure of prevention, but when contact (or the potential of contact) with nanoparticles is unavoidable then appropriate actions are necessary to ensure the safety of workers. These actions should include developing an understanding of how best to protect workers using nanoparticles or materials containing nanoparticles. Our suggestion is to assess the stability of nanoparticles using conditions that are relevant to potential worker exposure. The focus of our studies is on the stability of nanoparticles in solution because workers exposed to nanoparticles through contact (whether initially in solution, dry or aerosolized forms) will be transported through the systems of the human worker by solution (e.g., bloodstream). These suspended nanoparticles would be exposed to variations in pH, temperature, and composition of the solution. The results presented here are a preliminary study suggesting further work is required to determine the stability of nanoparticles before assessing biological toxicity of the nanoparticles. A nanoparticle that changes over the course of its use may or may not present a toxic or otherwise harmful product to the worker. Instability of the nanoparticles implies a change in the properties of these particles, such that the initial understanding of the nanoparticle composition, shape, size, and solubility might no longer be relevant. Without screening for long-term stability, the results of toxicity and other types of stability tests could be in question. We recommend a standardized approach be implemented for both preparing and assessing the short-term

(e.g., hours to days) and long-term (e.g., months to years) stability of nanoparticles. As demonstrated in our study, the approach should be simple and relatively inexpensive to implement, as well as relying upon an integrated set of appropriately informative techniques. Our recommendation is to implement a policy of caution in the use and exposure of workers to nanoparticles, as well as implementing a rigorous testing and labeling of nanoparticles. As an example, a proposed label for the gold nanoparticles studied herein could look like Figure B. A rating of 1 has been chosen for the quantity of material, corresponding to a nM concentration of suspended gold nanoparticles. A value of 0 is chosen for flammability since these solutions are aqueous dispersions of gold particles, and a rating of 2 assigned for the reactivity of these particles. The later was chosen for the oxidative susceptibility of their surface chemistry that stabilizes the nanoparticles. Further work, such as cellular toxicity assays, would be necessary to properly assign the health rating of these nanoparticles, but a value of 1 has been assigned to particles prepared with SAMs of MHDA formed over 2 days in the presence of polysorbate 20 surfactants. A standardized policy should be implemented for nanoparticle signage in the work place.

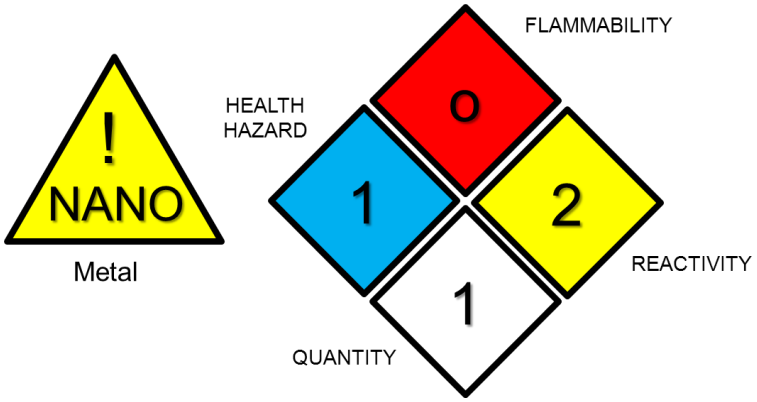


**Figure B.** Proposed label for indicating the presence of metal nanoparticles (i.e., gold) and the hazards associated with these nanoparticles when stabilized with SAMs formed over 2 days in the presence of polysorbate 20 surfactants and dispersed in phosphate buffered solution at a pH of 7.2.

We appreciate that further work will be necessary to understand the full implications of nanoparticle stability and for the creation of new labels as they pertain to the use and hazards of nanoparticles in the work place. However, work place policies should require workers to implement a standardized process to assess nanoparticle stability. Policies and methods for determining particle stability currently vary between work places, and are often not as rigorous as the methods presented here. Further work is needed to understand the implications of these results to other studies on gold nanoparticles, the stability

of nanoparticles of other compositions, and the need for more extensive changes in policy. Nanoparticles are being pursued for a wide range of applications that include catalysis, improved performance of window coatings, mechanical robustness of plastics and composite materials, enhanced antimicrobial agents in soaps and clothing, and products for use in enhanced imaging within biological systems. The implications of this work extend to businesses and research laboratories around the world with implications to consumers of products containing nanoparticles.

## DISSEMINATION AND KNOWLEDGE TRANSFER

This research was supported in part with funds from WorkSafeBC (Workers' Compensation Board of British Columbia) and the Workers' Compensation Board of Nova Scotia, the Natural Sciences and Engineering Research Council (NSERC) of Canada, and Simon Fraser University through the Community Trust Endowment Fund. This work will be submitted for publication in a peer reviewed journal with the permission of our primary funding sources, WorkSafeBC and the Workers' Compensation Board of Nova Scotia. Other aspects of knowledge transfer and dissemination of these results will include presentation of the findings at up-coming national and international conferences. The goal is to educate others on the importance of appropriately screening for and labeling the stability of their nanoparticles. Simple methods will be introduced that can be utilized to provide workers with the necessary information on nanoparticle stability. Further work is necessary to understand the full implications of the observed instabilities in gold colloids as a function of the quality of their surface chemistry and to extend these studies to other materials (e.g., oxides, semiconductors, and carbon). Contributions from other scientists world-wide is essential to achieve these studies, given the enormity of nanoscale materials that are being made. This work will serve as both a caution and a guideline to other scientists to assist in understanding nanoparticle instabilities and to design processes that improve the quality of their nanoparticles and safety of handling these materials.

**REFERENCES**


1. Giljohann, D. A.; Seferos, D. S.; Daniel, W. L.; Massich, M. D.; Patel, P. C.; Mirkin, C. A. *Angew. Chem. Int. Ed.* **2010,** *49*, 3280-3294.
2. Storm, G.; Belliot, S. O.; Daemen, T.; Lasic, D. D. *Adv. Drug Deliv. Rev.* **1995,** *17*, 31-48.
3. Rana, S.; Yeh, Y.-C.; Rotello, V. M. *Curr. Opin. Chem. Biol.* **2010,** *14*, 828-834.
4. Nel, A. E.; Mädler, L.; Velegol, D.; Xia, T.; Hoek, E. M. V.; Somasundaran, P.; Klaessig, F.; Castranova, V.; Thompson, M. *Nat. Mater.* **2009,** *8*, 543-557.
5. Aubin-Tam, M.-E.; Hamad-Schifferli, K. *Biomed. Mater.* **2008,** *3*, 034001.
6. Lévy, R.; Thanh, N. T. K.; Doty, R. C.; Hussain, I.; Nichols, R. J.; Schiffrin, D. J.; Brust, M.; Fernig, D. G. *J. Am. Chem. Soc.* **2004,** *126*, 10076-10084 .
7. Wang, H.; Chen, S.; Li, L.; Jiang, S. *Langmuir* **2005,** *21*, 2633-2636.
8. Witt, D.; Klajn, R.; Barski, P.; Grybowski, B. A. *Curr. Org. Chem.* **2004,** *8*, 1763-1797.
9. Subramani, C.; Bajaj, A.; Miranda, O. R.; Rotello, V. M. *Adv. Mater.* **2010,** *22*, 5420-5423.
10. Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001,** *17*, 2841-2850.
11. Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2004,** *104*, 293-346.
12. Eustis, S. El-Sayed, M. A. *Chem. Soc. Rev.* **2006**, *35*, 209-217.
13. Smyder, J. A; Krauss, T. D. *Mater. Today*, **2011,** *14*, 382-387.
14. Burda, C.; Chen, X.; Narayanan, R.; El-Sayed, M. A. *Chem. Rev.* **2005**, *105*, 1025-1102.
15. Sau, T. K.; Rogach, A. L.; Jackel, F.; Klar, T. A.; Feldmann, J. *Adv. Mater.* **2010,** *22*, 1805-1825.
16. Grassian, V. H. *J. Phys. Chem. C* **2008,** *112*, 18303-18313
17. Bratlie, K. M.; Lee, H.; Komvopoulos, K.; Yang, P.; Somorjai, G. A. *Nano Lett.* **2007,** *7*, 3097-3101.

18. Viñes, F.; Lykhach, Y.; Staudt, T.; Lorenz, M. P. A.; Papp, C.; Steinrück, H. P.; Libuda, J.; Neyman, K. M.; Görling, A. *Chem. Eur. J.* **2010,** *16*, 6530-6539.
19. Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005,** *105*, 1103-1169.
20. Bumm, L. A.; Arnold, J. J.; Charles, L. F.; Dunbar, T. D.; Allara, D. L. Weiss, P. S. *J. Am. Chem. Soc.* **1999,** *121*, 8017-8021.
21. Cossaro, A.; Mazzarello, R.; Rousseau, R.; Casalis,L.; Verdini, A.; Kohlmeyer, A.; Floreano, L.; Scandolo, S.; Morgante, A.; Klein, M. L.; Scoles, G. *Science*, **2008**, *321*, 943-946.
22. Vericat, C.; Vela, M. E.; Salvarezza, R. C. *Phys. Chem. Chem. Phys.* **2005,** *7*, 3258-3268.
23. Geissler, M.; Schmid, H.; Bietsch, A.; Michel, B.; Delamarche, E. *Langmuir* **2002,** *18*, 2374-2377.
24. Sun, L.; Crooks, R. M. *Langmuir* **1993**, *9*, 1951-1954.
25. Lee, L. Y. S.; Lennox, R. B.; *Phys. Chem. Chem. Phys.* **2007,** *9*, 1013-1020.
26. Maoz, R.; Sagiv, J. *Langmuir* **1987,** *3*, 1034-1044.
27. Kim, M.; Hohman, J. N.; Cao, Y.; Houk, K. N.; Ma, H.; Jen, A. K.-Y.; Weiss, P. S. *Science*, **2011,** *331*, 1312-1315.
28. Noh, J.; Hara, M. *Langmuir* **2001,** *17*, 7280-7285.
29. Jalali, H.; Gates, B. D. *Langmuir* **2009**, *25*, 9078-9084.
30. Dasog, M.; Scott, R. W. J. *Langmuir* **2007**, *23*, 3381-3387.
31. Srisombat, L.; Jamison, A. C.; Lee, T. R. *Colloids Surf. A* **2011,** *390*, 1-19.
32. Raha, S.; Paunesku, T.; Woloschak, G. *Nanomed. Nanobiotechnol.* **2011,** *3*, 269-281.
33. Niemeyer, C. M. *Angew. Chem. Int. Ed.* **2001,** *40*, 4128-4158.
34. Zhu, Z.; Meng, H.; Liu, W.; Liu, X.; Gong, J.; Qiu, X.; Jiang, L.; Wang, D.; Tang, Z. *Angew. Chem. Int. Ed.* **2011,** *50*, 1593-1596.

35. Lemelle, A.; Veksler, B.; Kozhevnikov, I. S.; Akchurin, G. G.; Piletsky, S. A.; Meglinski, I. *Laser Phys. Lett.* **2009,** *6*, 71-75.

36. Eghtedari, M.; Oraevsky, A.; Copland, J. A.; Kotov, N. A.; Conjusteau, A.; Motamedi, M. *Nano Lett.* **2007,** *7*, 1914-1918.

37. Yang, X.; Skrabalak, S. E.; Li, Z.-Y.; Xia, Y.; Wang, L. V. *Nano Lett.* **2007,** *7*, 3798-3802.

38. Yamashita, S.; Fukushima, H.; Niidome,Y.; Mori, T.; Katayama, Y.; Niidome, T. *Langmuir* **2011**, *27*, 14621-14626.

39. Poon, L.; Zandberg,W.; Hsiao,D.; Erno, Z.; Sen, D.; Gates, B. D.; Branda, N. R. *ACS Nano,* **2010,** *4*, 6395-6403.

40. Bakhtiari, A. B. S.; Hsiao, D.; Jin, G.; Gates, B. D.; Branda, N. R. *Angew. Chem. Int. Ed.* **2009,** *48*, 4166-4169.

41. Huschka, R.; Neumann, O.; Barhoumi, A.; Halas, N. J. *Nano Lett.*, **2010**, *10*, 4117-4122.

42. Li, J. L.; Day, D.; Gu, M. *Adv. Mater.* **2008,** *20*, 3866-3871.

43. Tong, L.; Zhao, Y.; Huff, T. B.; Hansen, M. N.; Wei, A.; Cheng, J.-X. *Adv. Mater.* **2007,** *19*, 3136-3141.

44. Takahashi, H.; Niidome, T.; Nariai, A.; Niidome, Y.; Yamada, S. *Chem. Lett.* **2006,** *35*, 500-501.

45. Huang, X.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. *Photochem. Photobiol.* **2006,** *82*, 412-417.

46. Huang, H.-C.; Barua, S.; Kay, D. B.; Rege, K. *ACS Nano,* **2009,** *3*, 2941-2952.

47. von Maltzahn, G.; Park, J.-H.; Agrawal, A.; Bandaru, N. K.; Das, S. K.; Sailor, M. J.; Bhatia, S. N. *Cancer Res.* **2009,** *69*, 3892-3900.

48. Schrand, A. M.; Rahman, M. F.; Hussain, S. M.; Schlager, J. J.; Smith, D. A.; Syed, A. F. *Nanomed Nanobiotechnol.* **2010,** *2*, 544-568.

49. Niidome, T.; Yamagata, M.; Okamoto, Y.; Akiyama, Y.; Takahashi, H.; Kawano, T.; Katayama, Y.; Niidome, Y. *J. Controlled Release* **2006,** *114*, 343-347.

50. Khlebtsov, N.; Dykman, L. *Chem. Soc. Rev.* **2011,** *40*, 1647-1671.

51. Li, S.-D.; Huang, L. *Mol. Pharmaceutics* **2008,** *5*, 496-504.

52. Owens, D. E. III, Peppas, N. A. *Int. J. Pharm.***2006,** *307*, 93-102.

53. Dickerson, E. B.; Dreaden, E. C.; Huang, X.; El-Sayed, I. H.; Chu, H.; Pushpanketh, S.; McDonald, J. F.; El-Sayed, M. A. *Cancer Lett.* **2008,** *269*, 57-66.

54. Kimling, J.; Maier, M.; Okenve, B.; Kotaidis, V.; Ballot, H.; Plech, A. *J. Phys. Chem. B* **2006,** *110*, 15700-15707.

55. Jiang, C.; Markutsya, S.; Tsukruk, V. V. *Langmuir* **2004,** *20*, 882-890.

56. Aslan, K.; Pérez-Luna, V. H. *Langmuir* **2002**, *18*, 6059-6065.

57. Duy, J.; Connell, L. B.; Eck, W.; Collins, S. D.; Smith, R. L. *J. Nanopart. Res.* **2010**, *12*, 2363-2369.

58. Duchesne, L.; Gentili, D.; Comes-Franchini, M.; Fernig, D. G.; *Langmuir* **2008**, *24*, 13572-13580.

59. Lin, S.-Y.; Tsai, Y.-T.; Chen, C.-C.; Lin, C.-M.; Chen, C.-H. J. *Phys. Chem. B* **2004**, *108*, 2134-2139.

60. Klajn, R.; Stoddart, J. F.; Grzybowski, B. A. *Chem. Soc. Rev.* **2010,** *39*, 2203-2237.

61. Vericat, C.; Vela, M. E.; Benitez, G. A.; J A Gago, J. A. M.; Torrelles, X.; Salvarezza, R. C. *J. Phys. Condens. Matter* **2006,** *18*, R867-R900.

62. Zhang, S.; Leem, G.; Srisombat, L.-O.; Lee, T. R. *J. Am. Chem. Soc.* **2008,** *130*, 113-120.

63. Zhu, T.; Vasilev, K.; Kreiter, M.; Mittler, S.; Knoll, W. *Langmuir* **2003,** *19*, 9518-9525.

64. Roux, S.; Garcia, B.; Bridot, J.-L.; Salomé, M.; Marquette, C.; Lemelle, L.; Gillet, P.; Blum, L.; Perriat, P.; Tillement, O. *Langmuir* **2005,** *21*, 2526-2536.

65. Schwartz, D. K. *Annu. Rev. Phys. Chem.* **2001**, *52*, 107-137.

66. Harada, Y.; Girolami, G. S.; Nuzzo, R. G. *Langmuir* **2004,** *20*, 10878-10888.

67. Boisselier, E.; Astruc, D. *Chem. Soc. Rev.* **2009,** *38*, 1759-1782.

68. Janib, S. M.; Moses, A. S.; MacKay, J. A. *Adv. Drug Delivery Rev.* **2010,** *62*, 1052-1063.

69. Huang, X.; Jain, P. K.; El-Sayed, I. H.; El-Sayed, M. A. *Lasers Med. Sci.* **2008,** *23*, 217-228.

70. Gao, D.; Tian, Y.; Liang, F.; Ding, L.; Bi, S.; Chen, Y.; Zhang, H.; Yu, A. *Colloids Surf. B* **2006,** *47*, 71-77.

71. Delamarche, E.; Michel, B.; Biebuyck, H. A; Gerber, C. *Adv. Mater.* **1996,** *8*, 719-729.

72. Camillone, N.; Eisenberger, P.; Leung, T. Y. B.; Schwartz, P.; Scoles, G.; Poirier, G. E.; Tarlov, M. J. *J. Chem. Phys.* **1994,** *101*, 11031-11036.

73. Schöenenberger, C.; Jorritsma, J.; Sondag-Huethorst, J. A. M.;Fokkink, L. G. J. *J. Phys. Chem.* **1995**, *99*, 3259-3271.

74. Mayya, K. S.; Patil, V.; Sastry, S. *Langmuir* **1997**, *13*, 3944-3947.

75. Sandhyarani, N.; Pradeep, T. *Chem. Phys. Lett* .**2001**, *338*, 33-36.

76. Lee, M.-T.; Hsueh, C.-C.; Freund, M.-S.; Ferguson, G. S. *Langmuir* **1998**, *14*, 6419-6423.

77. Kerwin, B. A. *J. Pharm. Sci.* **2008**, *97*, 2924-2935.

78. Donbrow, M.; Azaz, E.; Pillersdorf, A. *J. Pharm. Sci.* **1978**, *67*, 1676-1681.

**FIGURES AND DATA TABLES**

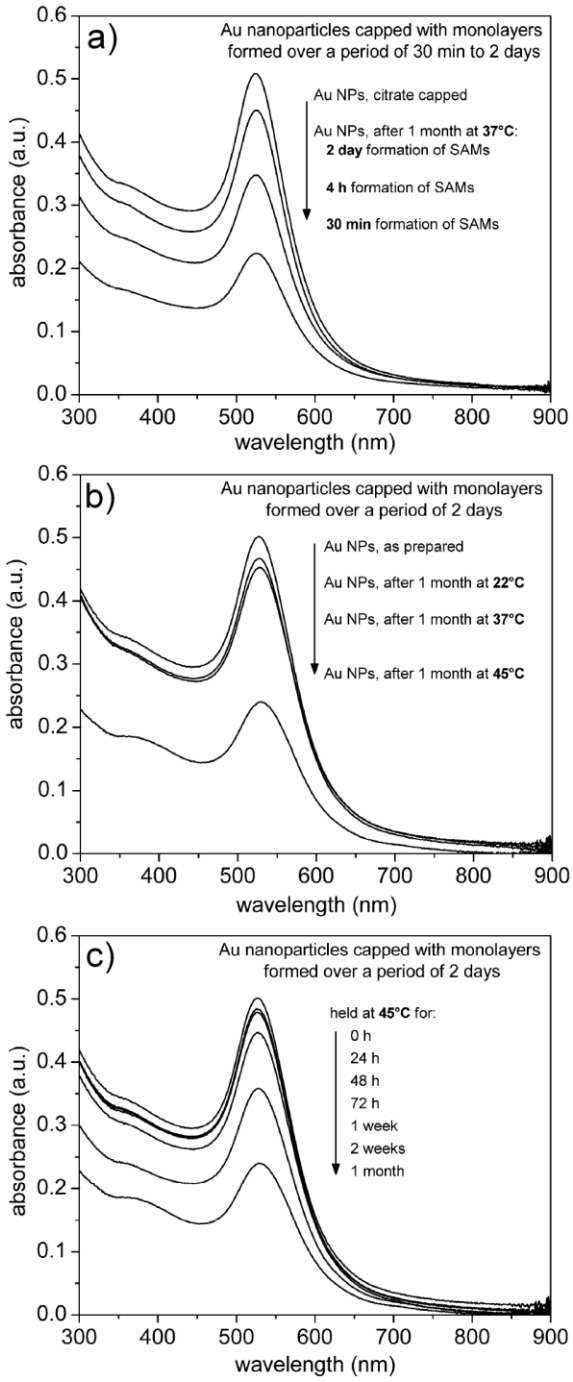


**Figure 1.** Stability of gold nanoparticles in phosphate buffered solutions was monitored by UV-Vis absorption spectroscopy at regular intervals over a period of 1 month. These gold nanoparticles are capped with self-assembled monolayers (SAMs) of 16-mercaptohexadecanoic acid (MHDA) and Tween® 20 (polysorbate surfactants). (a) Spectra of nanoparticles held at 37°C for 1 month. These particles are capped with SAMs formed over 30 min, 4 h, or 2 days. (b) Absorption spectra taken after 1 month for solutions of gold nanoparticles held at 22, 37, or 45°C. The nanoparticles are capped with monolayers that were assembled over a period of 2 days. (c) Spectra taken over a period of 1 month for a solution of nanoparticles held at 45°C. The monolayers on these particles were assembled over 2 days.

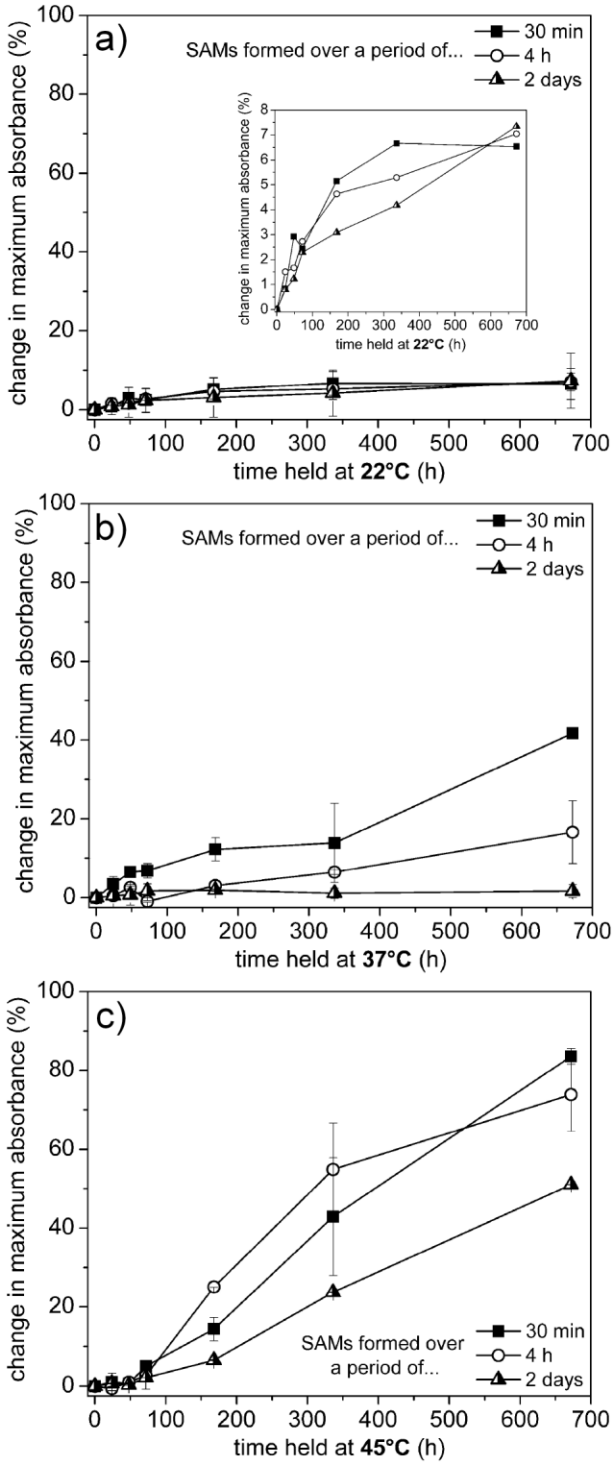


**Figure 2.** The change in maximum UV-Vis absorbance at 523 nm for gold nanoparticles suspended in phosphate buffered solutions at pH 7.0 was determined as a function of time held at (a) 22°C, (b) 37°C, or (c) 45°C. Particles are capped with SAMs of MHDA assembled in the presence of polysorbate surfactants. These monolayers were formed over 30 min, 4 h or 2 days, and subsequently purified and suspended in the phosphate buffered solutions for the duration of the experiments.

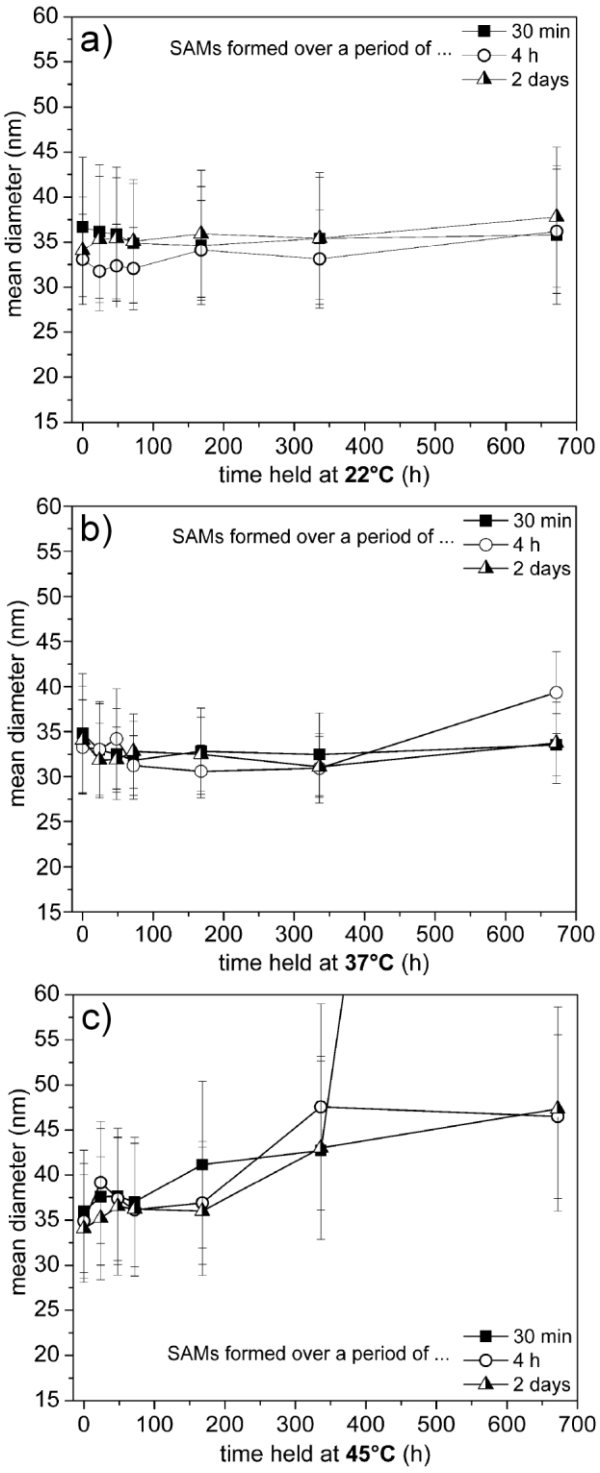


**Figure 3.** Diameters of gold nanoparticles suspended in phosphate buffered solutions measured by dynamic light scattering measurements. Particle size was monitored at regular intervals over a period of 1 month for particles held at (a) 22°C, (b) 37°C, or (c) 45°C. The nanoparticles are capped with monolayers of MHDA assembled in the presence of polysorbate surfactants over periods of 30 min, 4 h or 2 days.

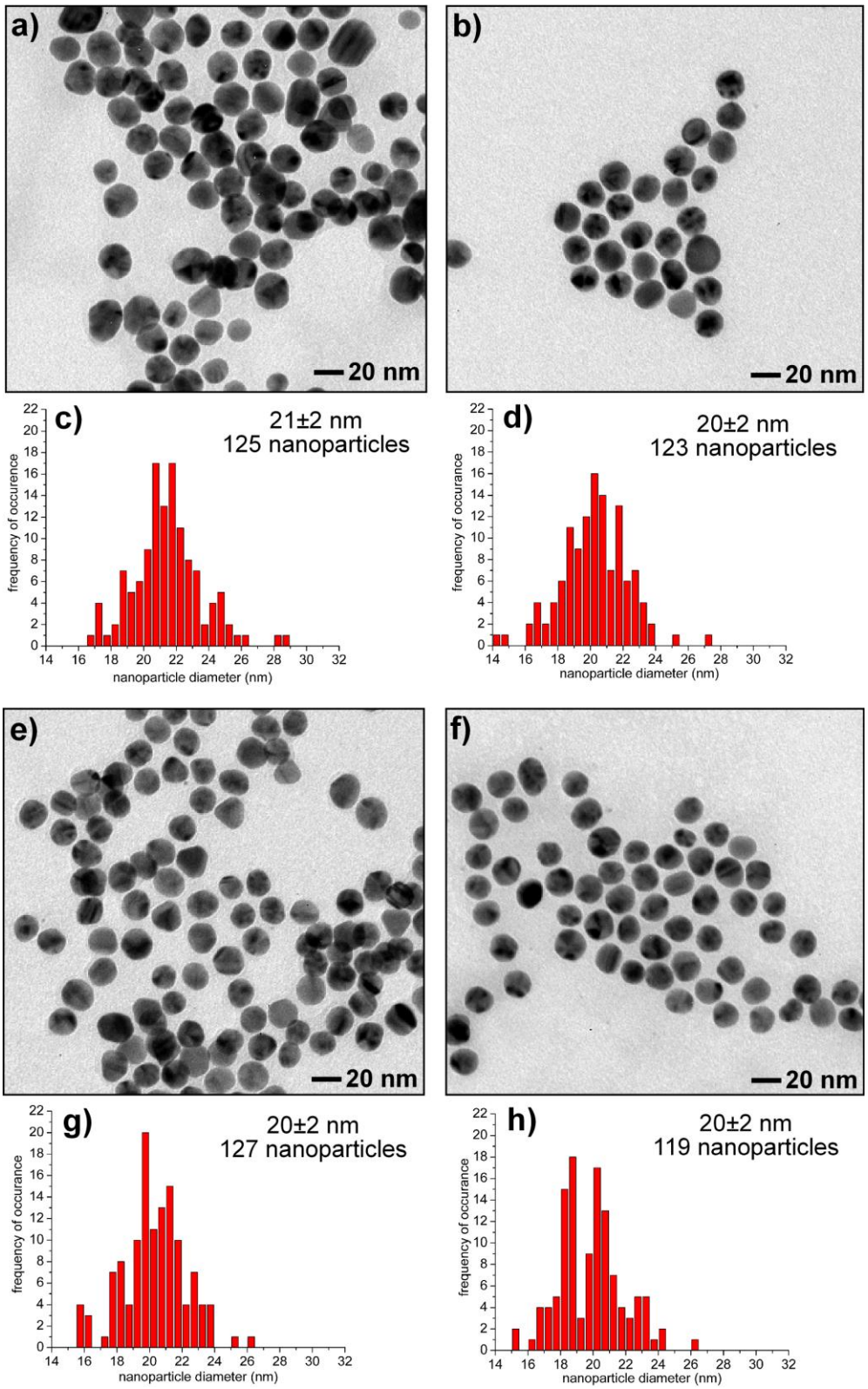


**Figure 4.** (a) Transmission electron microscopy (TEM) images of gold nanoparticles capped with SAMs of MHDA and polysorbate 20 surfactants formed over 2 days. These particles were reanalyzed by TEM after suspension in a phosphate buffered solutions for 1 month at (b) 22°C, (e) 37°C, and (f) 45°C. The average diameters of the particles have been determined from measuring the dimensions of over 100 nanoparticles. Histograms and the mean diameters are reported for the (c) as prepared nanoparticles, as well as those particles held at (d) 22°C, (g) 37°C, and (h) 45°C for one month.

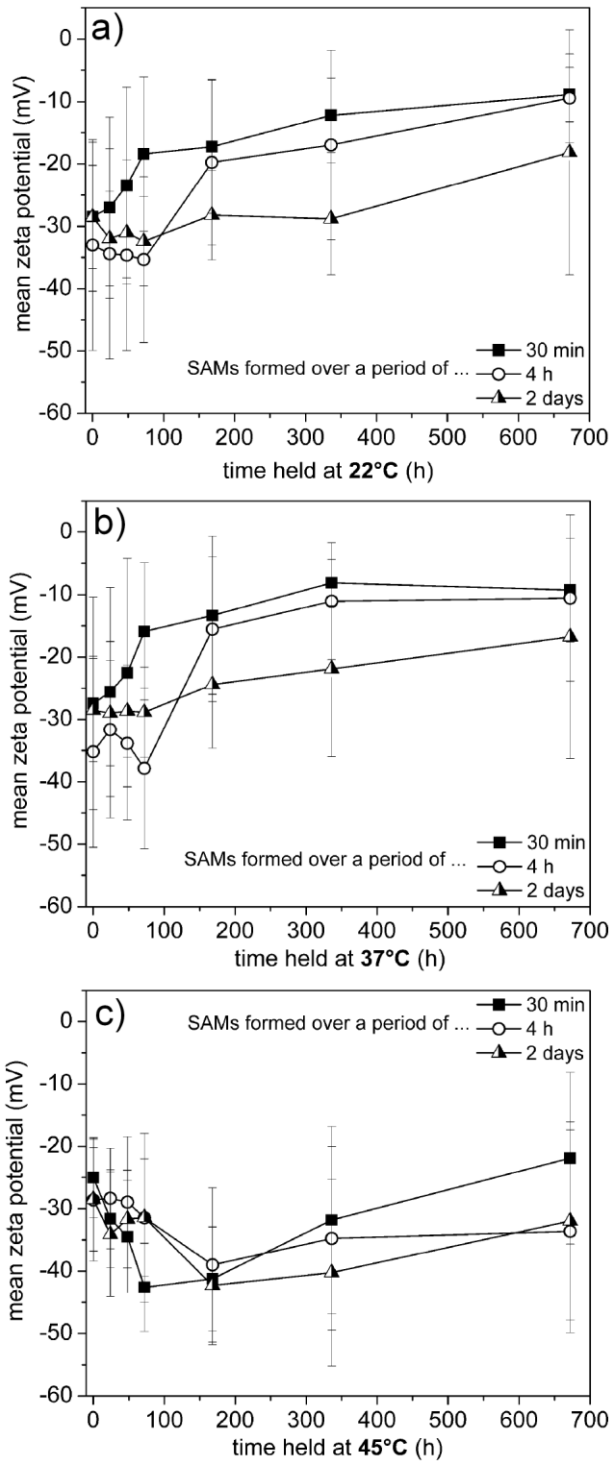


**Figure 5.** Mean zeta potential values were measured at regular intervals over the course of 1 month for gold nanoparticles suspended in phosphate buffered solutions that were held at (a) 22°C, (b) 37°C, or (c) 45°C. The MHDA monolayers capping these nanoparticles formed in the presence of polysorbate surfactants over 30 min, 4 h or 2 days before the particles were purified and suspended in the buffered solutions.

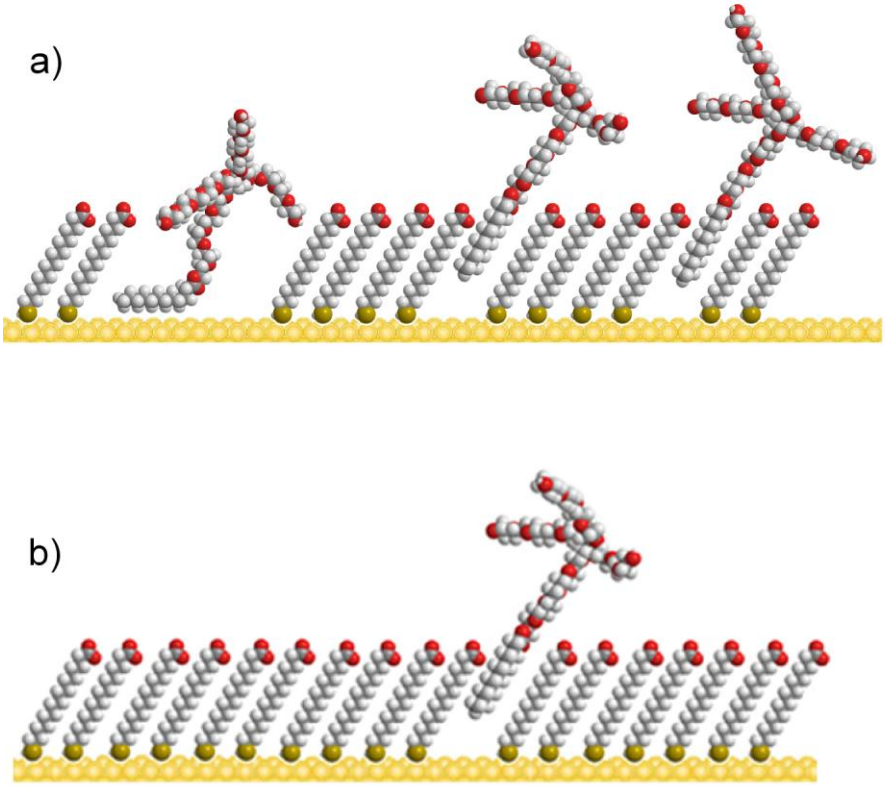


**Figure 6.** Schematic depiction of covalently attached MHDA molecules forming SAMs on gold surfaces, along with adsorbed Tween® 20, or polysorbate 20 surfactants. The adsorbed polysorbate surfactants assist in the stabilization of the gold surfaces by blocking defects within the alkanethiolate SAMs, as depicted for (a) low density MHDA SAMs, and (b) higher density MHDA SAMs.

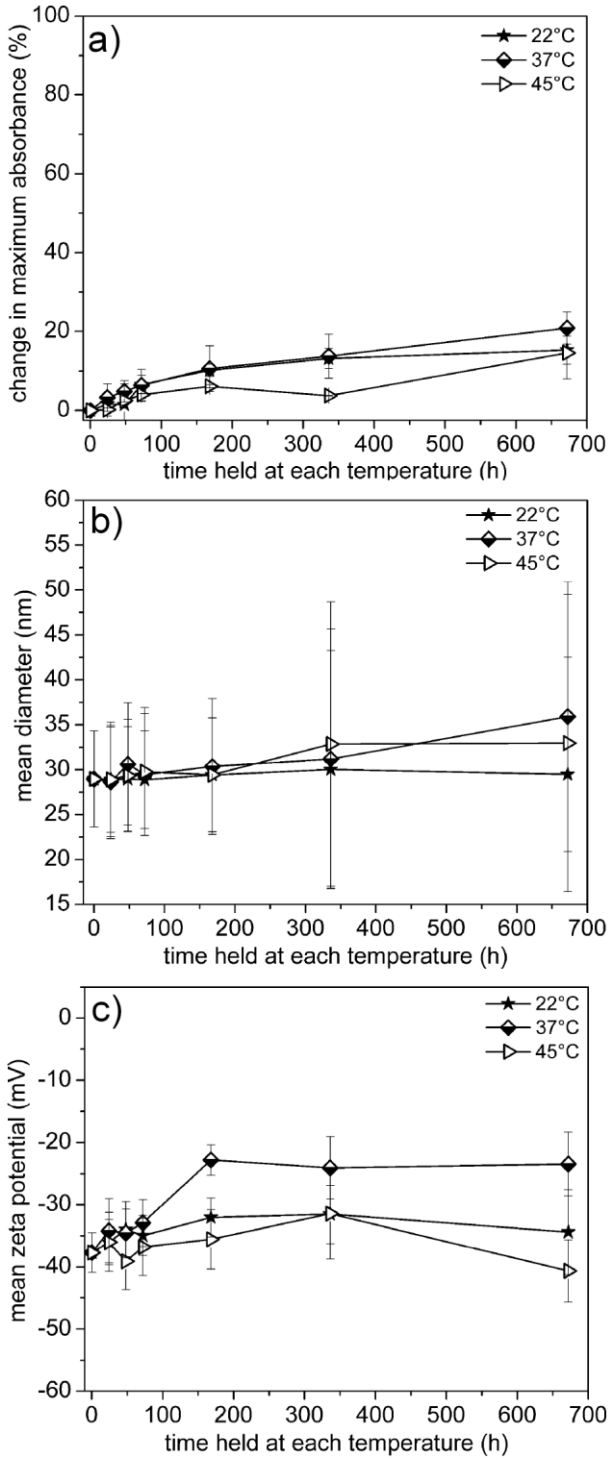


**Figure 7.** Gold nanoparticles were capped with SAMs of MHDA assembled over 2 days in the absence of polysorbate surfactants. These particles were held at 22°C, 37°C, or 45°C, and monitored at regular intervals over a period of 1 month by changes in (a) UV-Vis spectral absorbance at 523 nm, (b) particle diameter, and (c) mean zeta potential.

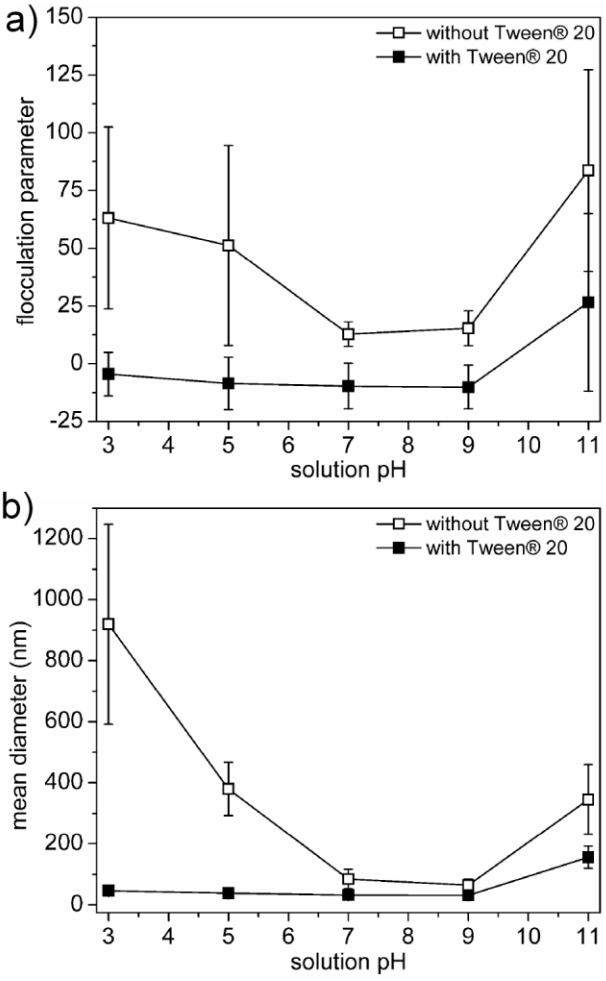


**Figure 8.** Stability of gold nanoparticles is assessed as a function of changes in solution pH. These particles are capped with SAMs of MHDA prepared either in the presence or absence of polysorbate 20 surfactants. These monolayers were formed over a period of 2 days, and the purified particles were suspended in various buffers to achieve a solution pH from 3.0 to 11.2. Stability of the suspended nanoparticles is measured by their (a) flocculation parameter, and (b) mean diameter. The flocculation parameter is calculated from integrated extinction between 600 and 800 nm in the UV-Vis absorption spectra.[70,74] The particle size is measured by dynamic light scattering. The associated error bars are derived from the standard deviation of these measurements.

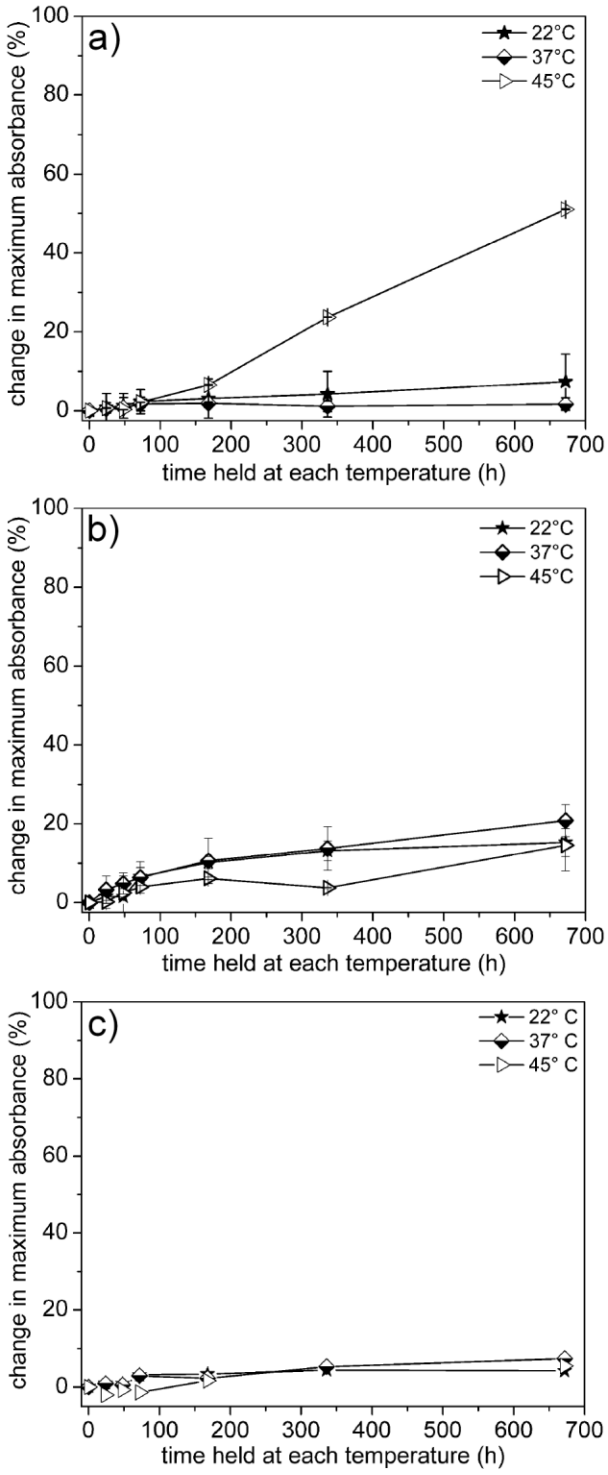


**Figure 9.** Change in maximum UV-Vis absorbance at 523 nm for gold nanoparticles suspended in either (a) 10 mM phosphate buffered (PB) solutions at pH 7.2 with polysorbate 20 surfactants, (b) 10 mM phosphate buffered solutions at pH 7.2 without polysorbate 20 surfactants, or (c) 1x tris borate EDTA (TBE) buffered solutions at pH 8.3 without polysorbate 20 surfactants. These spectral changes are plotted as a function of time over which the solutions have been held at 22, 37, or 45°C. In these studies, all nanoparticles are capped with monolayers of MHDA formed over 2 days.

**Table 1.** Comparison of Nanoparticle Stability after One Month in Buffered Solutions

| time allotted to formation of MHDA SAMs and solution composition | decrease in maximum absorbance at 523 nm after 1 month | | |
|---|---|---|---|
| | 22°C | 37°C | 45°C |
| 30 min SAMs + Tween® 20 + PB | 7 % | ~40 % | 85 % |
| 4 h SAMs + Tween® 20 + PB | 7 % | 15 % | 75 % |
| 2 day SAMs + Tween® 20 + PB | 7 % | ~2 % | 50 % |
| 2 day SAMs + PB | 15.6 % | 21 % | 16 % |
| 2 day SAMs + TBE | 7 % | 7 % | 7 % |