Kinetics and Thermodynamics of Au Colloid Monolayer Self-Assembly

Undergraduate Experiments in Surface and Nanomaterials Chemistry

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To a surprising extent, the properties of many important materials are determined by the properties of only a small percentage of their atoms: those on the surface. Surface chemistry controls the properties of heterogeneous catalysts, semiconductor devices, bioimplants, and a host of other materials. It is this realization that literally drives the fields of surface and interfacial chemistry. Functionally significant surface features can be found on the scale of atoms (catalysis at single crystal defect sites such as step edges), molecules (biocompatible polymers), or larger (nanometer- to micron-sized particulate or supramolecular assemblies). Through a detailed understanding of surface morphology from the angstrom scale to the micron scale, chemists hope to create new materials with tailor-made surface features for new applications (or improvements of old ones).

One method for control of surface morphology is through assembly of small, spherical, uniformly sized particles at an interface, imparting a repeating feature size that is dependent upon the particles used. Colloidal Au nanoparticles have several properties making them suitable for use as these surface building blocks. (i) Monodisperse colloidal Au solutions can be prepared with average particle diameters from 3 nm to 150 nm (*1*), thus allowing a wide range of repeating feature sizes. A transmission electron microscope (TEM) image of 12-nm-diameter Au nanoparticles is shown in Figure 1. (ii) Metal nanoparticles have surface reactivity amenable to immobilization at chemically functionalized surfaces: for example, Au or Ag particles bind strongly to positively charged polymers via electrostatic attractions (*2*), and to amine, thiol, and phosphine functional groups through covalent bonding (*3*). (iii) Colloid-based surfaces can be prepared by simple wet-chemical methods, without the need for any expensive or sophisticated equipment. (iv) Aqueous solutions of Au nanoparticles are an intense burgundy color, enabling surface formation to be followed by eye or with a UV–vis spectrophotometer. In addition, optical spectra can give information about interparticle spacing.

The unique optical properties of colloidal Au solutions have been recognized for centuries. In ancient times, the intense red hue of colloidal Au led to its use as a dye and, because of its superficial resemblance to blood, to popularity as a veritable cure-all. In 1857, Michael Faraday prepared Au hydrosols (solutions of nanoparticles in water); some of these solutions still exist at the Royal Institute in London. More recently, colloidal Au has been used as an electron-dense stain for TEM (*1*) and investigated for its novel optical and electronic properties (*4*), and has generated interest as a materials building block (*5*).

Colloidal Au is red owing to the absorption of light by free electron oscillations (the surface plasmon). The position of the surface plasmon λ_{max} for colloidal Au is located between 500 and 600 nm, depending on particle size and shape, solvent or adsorbate refractive index, and interparticle distance (*4*). For hydrosols of spherical, 12-nm-diameter Au, the absorbance maximum (λ_{max}) is around 518 nm. For smaller colloids, a slight (few nanometer) blue shift is expected, whereas for larger colloids, red-shifting of this band is observed. The peak width gives information about the polydispersity of the colloidal solution, with broader peaks indicating a greater distribution in particle size. The surface plasmon absorbance is also sensitive to the spacing of the colloidal particles: upon flocculation (or clumping together) of the nanoparticles, a second absorbance feature grows in around 650–750 nm, causing the solution to turn a deep blue (*6*). The appearance of this dark blue color is indicative of aggregation in Au hydrosols (*4*).

The optical properties and stability of colloidal Au are investigated by students in section A of this experiment. In sections B and C, students study the kinetics and thermodynamics of Au colloid self-assembly on glass microscope slides coated with a bifunctional organosilane. Schemes I and II illustrate the general method of Au colloid surface self-assembly. First, a hydroxyl or oxide-bearing substrate (glass, quartz, SnO_2, etc.) is coated with a thin film of bifunctional organosilane having a terminal functional group that will bind Au (−NH_2, −SH, −NC, etc). A representation of this step is shown in Scheme I (the actual bonding between the silane and the surface can take several forms, depending upon reaction conditions). The organosilane loses −OR groups and forms siloxane bonds to the substrate, leaving the unreacted

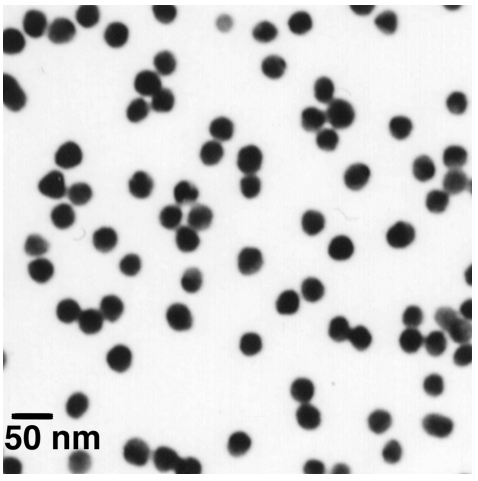
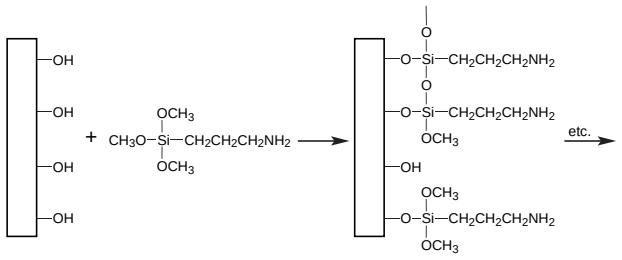
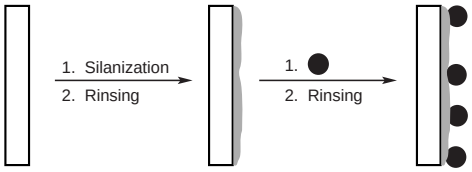


Figure 1. TEM image of 12-nm-diameter colloidal Au.

terminal group oriented toward solution. The silanized surface is then rinsed and soaked in colloidal Au solution, where Au particles self-assemble to form monolayers on the surface (Scheme II). Because of the intense wine-red color of colloidal Au particles, optical spectra can be used to follow surface formation on transparent substrates. Moreover, this process can be understood using the same formalism developed for adsorption of gas molecules to atomically flat surfaces (*7*).



Scheme I



Scheme II

Experimental Section

Materials

The purest H_2O available should be used. The H_2O used in these experiments was purified by a Barnstead Nanopure system; it had a resistance of 18 MΩ and had been passed through a 0.2-mm-diameter filter. Substrates were glass microscope slides (Fisher) cut into 0.9×2.5-cm strips using a diamond scribe glass-cutting pen (Fisher). Derivatizations were carried out in 1-dram glass vials (Kimble), in which glass substrates "stand up" and thus can be derivatized on both sides at once. Protein A (Sigma), 3-aminopropyltrimethoxysilane (APTMS [United Chemical Technologies]), HPLC-grade CH_3OH (EM Science), $HAuCl_4$ (Acros), and Na_3citrate (Aldrich) were used as received. Acids and bases (H_2SO_4, HNO_3 HCl, NaOH) were from J. T. Baker or EM Science. Optical spectra were acquired in H_2O, using an HP 8452A UV–visible spectrophotometer. Small Teflon blocks (1/3 sample height, with slits in the center to hold slides upright) were used to hold slides in 3-mL plastic cuvettes; these are optional.

Glassware Cleaning

All glassware involved in preparation of colloidal Au must be scrupulously clean and well rinsed. Cleaning procedures used successfully include (i) soaking in base bath (1 L 95% CH_3CH_2OH/120 mL H_2O/120 g KOH) for several days, or (ii) treating with "piranha" solution (4 parts concentrated H_2SO_4/1 part 30% H_2O_2) to remove organics and then aqua regia (3 parts concentrated HCl/1 part concentrated HNO_3) for removal of metals (for most *unsilanized* glassware, aqua regia washing is sufficient and piranha solution can be avoided.) CAUTION: Great care must be taken in using these dangerous cleaning reagents, particularly the piranha solution (*8*).

Preparation of Au Sol[1]

Twelve- to 13-nm-diameter Au colloid was prepared as follows. In a 1-L Erlenmeyer flask, 500 mL of 1 mM $HAuCl_4$ was brought to a boil, with vigorous stirring on a magnetic stirring hot-plate. Fifty milliliters of 38.8 mM Na_3citrate was added to the solution all at once, with vigorous stirring. The yellow solution turned clear, dark blue and then a deep red-burgundy color within a few minutes. Stirring and boiling was continued for 10–15 min after the burgundy color was observed. The solution was then removed from heat and kept stirring for 15 min. After the Au colloid solution had cooled, the volume was adjusted to 500 mL with H_2O. Considerable volume loss (due to evaporation) can occur during colloid preparation; this can be avoided if colloid is synthesized under refluxing conditions. Colloidal solutions were stored in clean brown glass bottles until used (shelf life is several weeks to months). Au nanoparticles prepared according to this protocol were nearly spherical, with average diameters of 12–13 nm (standard deviations about ±1.5 nm), as determined by TEM image analysis using NIH Image.[2] The concentration of Au particles in this solution is ~17 nM. The solutions exhibited λ_{max} at 516–518 nm, with an intense absorbance of 0.7–0.9 after dilution (1 part colloid/4 parts H_2O), and a full peak width at half max (PWHM)[3] between 80 and 90 nm. If the colloid preparation did not meet these requirements, it was discarded.

Protein A Flocculation

The pH of colloidal Au solutions (0.500 mL) was brought to 5.5–6.0 with 0.020 mL of 0.020 M NaOH to optimize binding. The pH of the colloid was determined with pH test strips (colorpHast Indicator strips, pH 2–9; EM Science).[4] Solutions of protein A were added to 0.500-mL aliquots of 17 nM, 12-nm-diameter Au nanoparticles to produce final protein concentrations of 1–20 μg/mL. Next, 0.100 mL of 1.5 M NaCl was added to each solution with rapid mixing to induce aggregation. Optical spectra were taken 5 min after addition of aggregant.

Preparation of Au Colloid Monolayer Surfaces

Clean glass slides were rinsed in CH_3OH and derivatized for 10 min in 1% 3-aminopropyltrimethoxysilane (APTMS) in CH_3OH. After silanization, surfaces were rinsed well several times with CH_3OH and then H_2O before being placed into solutions of Au colloid. If necessary, silanized substrates may be stored in water for a short time (up to 1–2 h) before exposure to colloidal Au. All manipulations of clean or derivatized glass slides should be done using tweezers, to avoid damaging the surfaces.

Experiment A. Investigation of Au Colloidal Solution and Preparation of Protein A–Au Conjugates

Colloidal solutions are thermodynamically unstable with respect to precipitation. However, Au particles are negatively charged, so they repel each other and remain in solution. Colloids can be flocculated and ultimately precipitated by addition of electrolytes (NaCl, KI, etc.). This occurs because the ions shield the charges on the particles, allowing them to approach closely and aggregate into larger and larger clumps, eventually settling to the bottom of the vessel containing them. Optical excitation of aggregates results in collective-

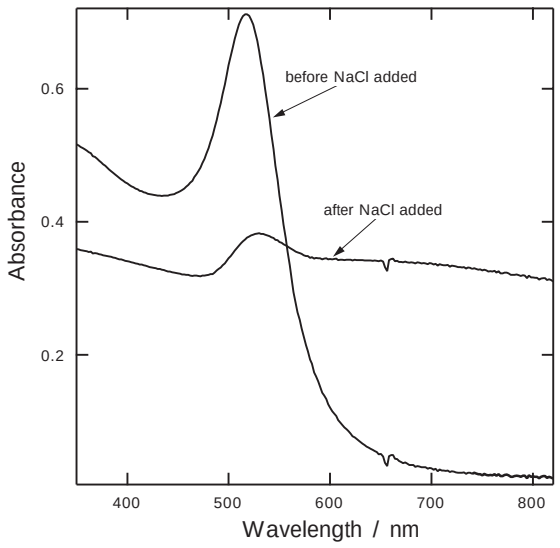


Figure 2. Optical spectra for colloidal Au solution before and 6 min after aggregation by addition of 1 M NaCl.

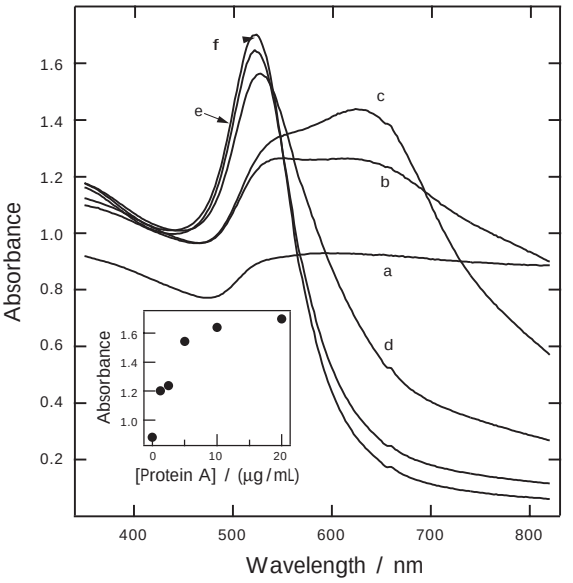


Figure 3. Optical spectra for colloidal Au solutions (12-nm-diameter particles, 1.00 mL, 8.9 nM in Au particles) 5 min after addition 0.10 mL of 1.5 M NaCl. Spectra are shown for solutions containing (a) 0; (b) 1.25; (c) 2.5; (d) 5.0; (e) 10.0; and (f) 20.0 mg/mL of protein A. Inset: Absorbance at λ_{max}, plotted against concentration of added protein A.

particle electron oscillations, giving rise to a new feature in the optical spectrum (the collective-particle plasmon band) at longer wavelengths (~600 nm). Students should add a few drops of 1 M NaCl(aq) to their Au colloid, mix well, and observe the results over several seconds and then several minutes. An optical spectrum can be taken of the aggregated sol to compare with a spectrum of the solution before addition of salt. Figure 2 shows optical spectra for 12-nm-diameter Au colloid diluted 1:5 with H_2O before and 6 min after addition of 0.100 mL of 1 M NaCl.

Au colloidal solutions can be stabilized by the addition of high molecular weight adsorbates such as polyethylene glycol or proteins. These adsorbates inhibit colloid aggregation, even at high salt concentrations, by causing steric repulsion of particles and by screening particles from the high ionic strengths that facilitate flocculation (*9*). The amount of adsorbate necessary to stabilize a colloidal solution is the amount required to coat the surface of the nanoparticles (*10*). For proteins, the optimal pH for stabilization is affected by the protein's isoelectric point (the pH at which the net charge on the molecule is 0; for protein A, this value is 5.1) and by the presence of charged patches on the protein surface (multiple positively charged patches can lead to particle cross-linking and aggregation of the negatively charged particles). The amount of stabilizer necessary to prevent salt-induced aggregation can be determined by adding increasing amounts of stabilizer to different aliquots of Au colloid and then adding NaCl to each. Solutions to which enough stabilizer has been added to coat the colloidal particles will remain red after addition of NaCl, whereas those having too little stabilizer will turn blue owing to particle aggregation, and will eventually sediment. This procedure for determination of the minimum stabilizing concentration of adsorbate is referred to as a flocculation assay (*1*).

Figure 3 shows flocculation data for stabilization of 12-nm-diameter Au particles by addition of protein A, an antibody-binding protein isolated from bacterial cell walls (*11*). Several optical spectra are shown, each taken 5 min after addition of NaCl(aq) to aliquots of Au sol containing different protein A concentrations. In this experiment, 20 mg/mL was found to be the minimum stabilizing concentration of protein A; at this concentration, no aggregation was induced by addition of NaCl. The range of concentrations tested was chosen by estimating the number of protein molecules capable of fitting around each 12-nm-diameter Au sphere and using this ratio as a starting point. To do this, we make the simplifying assumption that our proteins are spherical. This assumption holds best for water-soluble proteins, which tend to "ball up" to protect hydrophobic amino acid residues from interacting with solvent H_2O. The specific volume for such globular proteins is approximately 0.74 cm^3/g (*12*). This allows us to calculate the volume per individual protein molecule from the radius of the protein sphere, and then the cross-sectional area, or "footprint" of the protein molecules. The ratio between the protein footprint and the surface area of the 12-nm-diameter Au spheres gives us a rough estimate for the maximum number of protein molecules that can be expected to bind per particle. For protein A (MW 42,000 g/mol) (*11*) and 12-nm-diameter Au particles, this ratio is approximately 27:1.

The data in Figure 3 indicate that stabilization is not achieved until 20 mg/mL protein A is present in the solution, which works out to twice the predicted value. This discrepancy is due not only to the approximations made in the calculations, but also to the presence of an equilibrium between adsorbed and free protein A in solution. Protein–colloid complexes can be separated from free protein by centrifugation and resuspension of the pellet in H_2O or buffer (free protein will remain in the supernatant). Protein-coated Au colloid particles are used as stains for localizing protein binding sites in tissue sections for both optical microscopy and transmission electron microscopy (TEM) because of their high extinction coefficient and electron density. Protein A, in particular, has a high binding affinity for a large class of immunoglobulins (IgG's),

which allows researchers to use protein A–Au conjugates in labeling many different biomolecules in tissue samples (*13*).

Experiment B. Kinetics of Au Colloid Surface Assembly

If 12-nm-diameter Au particles as described in the experimental section are used, the surface coverage of Au particles, Γ, can be estimated from eq 1

$$A_{surf} = \frac{\Gamma \varepsilon_{surf}}{6.02 \times 10^{20}} \quad (1)$$

where A_{surf} is the absorbance of a surface-confined species, Γ is the colloid coverage (number of particles/cm^2), and ε_{surf} is the surface extinction coefficient (2.8×10^8 M^{-1} cm^{-1} at 524 nm for 12-nm-diameter Au) (*14*). This equation is the surface equivalent of Beer's law, which has been previously shown to hold for colloidal Au in solution and on surfaces (*15*).[5] The measured absorbance at λ_{max} must be divided by two to get A_{surf}, since only half of the surface-bound particles are on each side of the glass slide.

The rate of adsorption of nanoparticles from an unstirred solution to a planar substrate is given by eq 2

$$\Gamma = pC\left(\frac{Dt}{2}\right)^{1/2} \quad (2)$$

where Γ is defined above, p is the probability that a particle reaching the surface will adsorb, C is the solution colloid concentration (number of particles/cm^3), D is the diffusion coefficient of the particles, and t is the time, in seconds (*15*, *17*). For spherical particles (such as Au colloid), the diffusion coefficient, D, is given by eq 3

$$D = \frac{kT}{6\pi\gamma r} \quad (3)$$

where k is the Boltzmann constant (1.38×10^{-16} g cm^2 s^{-1} K^{-1}), T is the temperature (K), γ is the viscosity of the dispersed medium (taken to be that of water; 0.010 g cm^{-1} s^{-1}) (*18*), and r is the particle radius (in cm) (*17*). Equation 2 describes systems where the adsorbing particles are noninteracting. In the case of Au colloid surface self-assembly, the negative charges on Au particles cause some interparticle repulsions, a phenomenon that has been observed at high surface coverages. Because all the terms in eq 1 are constants except Γ and t, data may be fit to a $t^{1/2}$ plot using nonlinear least squares analysis. Experimental data can be plotted as absorbance at λ_{max} vs time, and the points fit to $y = kt^{1/2}$, where $k = pC(D/2)^{1/2}$ and y is absorbance, which is proportional to coverage. Thus, it can be determined whether data follow $t^{1/2}$ kinetics without knowledge of particle coverage, concentration, or diffusion coefficient.

In this experiment, APTMS-derivatized glass surfaces were placed into Au colloid at time $t = 0$, and then removed from the solution at various times (t) during surface assembly to measure the absorbance at λ_{max} (note that the λ_{max} on the surface, 524–526 nm, is slightly to the red of that in solution, 518 nm). The simplest way to perform this experiment is by acquiring an entire data set for one surface, by repeated exposure to Au colloid, followed by rinsing in H_2O and acquisition of the optical spectra (in H_2O) at various exposure times. However, to better meet the assumptions of eq 2, separate surfaces may be prepared for each data point; the data shown here were collected using this method. The absorbance at λ_{max} was then plotted versus (total) time in Au colloid. Optical spectra and absorbance-vs-time data are shown in Figures 4 and 5, respectively. The first several points of these data were then fit to a $t^{1/2}$ plot using nonlinear least squares,[6] shown in Figure 5 as the solid line. In the absence of a curve-fitting routine, the log of the absorbance can be plotted versus the log of time to produce a linear plot with slope equal to 0.5. This $t^{1/2}$ behavior assumes no interparticle interactions. The inset to Figure 5 shows absorbance-vs-time data for longer times. Data acquired over the course of 30–45 min will follow $t^{1/2}$ behavior reasonably well; it is at longer times (higher cov-

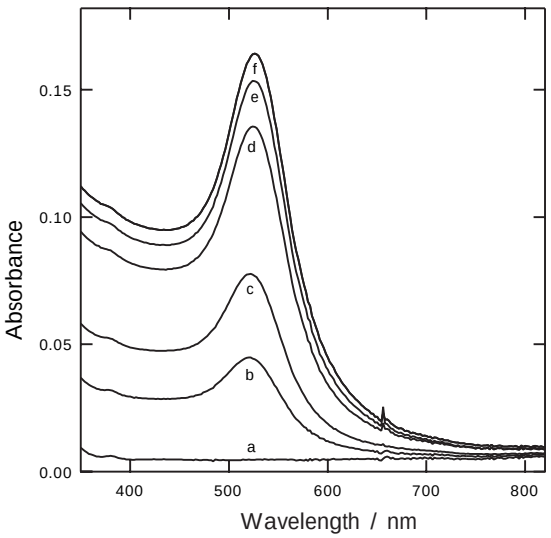


Figure 4. Optical spectra of an APTMS-derivatized glass slide after immersion in 12-nm diameter colloidal Au solution for (a) 2 min; (b) 4 min; (c) 10 min; (d) 15 min; (e) 150 min.

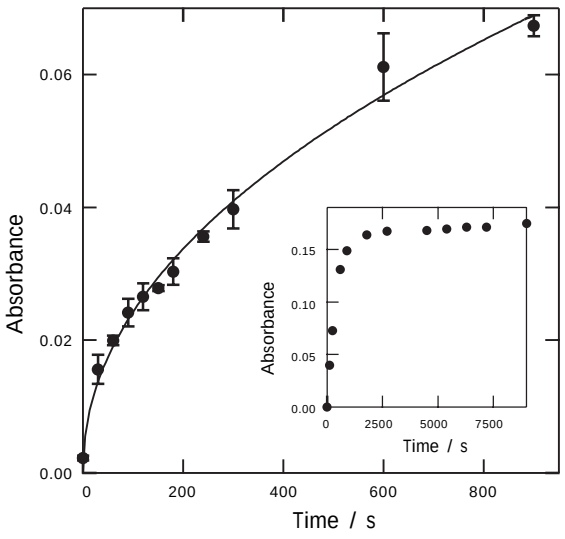


Figure 5. Absorbance vs time plot for an APTMS-derivatized glass slide after immersion in 12-nm Au solution. The solid line is the nonlinear least squares fit to eq 2. Inset: Absorbance vs time plot showing data points at longer times.

erages) that significant deviations are most apparent. To observe this behavior, students can leave a slide in Au colloid for several hours or longer (i.e., until the next laboratory period). The negative deviation from $t^{1/2}$ behavior at longer times indicates that, as colloid coverage increased, colloidal particles diffusing to the surface were less and less likely to bind.[7]

There are many possible sources of error in this experiment. Small changes in particle size impact the extinction coefficient and particle concentration greatly, and also impact the diffusion coefficient. In addition, eq 1 is valid only for unstirred solutions; this is a poor assumption for solutions of Au sol into which glass slides are repeatedly inserted and removed. Other sources of error are present as well. It may be instructive to have students investigate the effect of several error sources on the magnitude of the sticking probability calculated. For further work, changes in p as a function of temperature, particle radius, and concentration can be investigated and compared to predictions from eqs 2 and 3.

Experiment C. Thermodynamics of Au Colloid Surface Assembly

The adsorption of molecules or particles to a surface from solution is described by the Langmuir equation (eq 4),

$$KC' = \frac{\theta}{(1-\theta)} \tag{4}$$

where θ is the fractional surface coverage of Au particles, K is the equilibrium constant for surface binding, and C' is the concentration of colloidal particles in solution (M) (*19*). Thus, the equilibrium constant for adsorption of colloidal Au to the amino-derivatized surface can be calculated from a knowledge of the fractional surface coverage and solution concentration. This equation makes two assumptions: (i) that particles cannot bind at locations where there are already particles present (thus, there can be no multilayers); and (ii) that there are no interactions between particles. The first of these assumptions is reasonable for Au colloid deposition, since NH_2- groups are present only at the glass surface, and there is nothing to cause the Au particles to bind if they cannot reach the functionalized silane film. The second assumption, however, is not valid for the negatively charged particles used in this study. Thus, we will use another isotherm, the Frumkin isotherm, to fit the adsorption data generated in this experiment.[8]

The Frumkin isotherm describes adsorption when there are interactive forces between particles (eq 5).

$$KC' = \left(\frac{\theta}{1-\theta}\right)e^{f\theta} \tag{5}$$

The Frumkin parameter, f, takes into account interparticle repulsion (or attraction) by assuming that the apparent free energy of binding to the substrate changes linearly with particle coverage. Equation 5 describes systems with repulsive forces between particles when f is positive, and attractive forces when f is negative. When there are no long-range interactive forces between adsorbates ($f = 0$), eq 5 simplifies to eq 4.

For this experiment, students will prepare solutions of various concentrations of Au colloid. The concentration of the 12-nm diameter Au preparation described above is typically around 17 nM if there has been no noticeable plating or precipitation and any volume lost to evaporation has been replaced.[9] Note that for different colloid preparations (i.e., those with different diameters), the particle concentration changes, and a new surface extinction coefficient must also be calculated. This can be done from a knowledge of particle size and of the number of particles per unit area on a surface having a given absorbance (surfaces can be digested in strong acid for analysis by atomic absorption spectroscopy or can be imaged at the nanometer scale). Note that these calculations all involve the assumption that all particles are identical; errors will be large for polydisperse colloidal solutions.

For the equilibrium constant to be meaningful, the surface assembly reaction must be allowed to reach equilibrium. Thus, once silanized surfaces have been placed into Au colloid solutions of various concentrations (prepared by dilution of the stock colloid solution with H_2O), they must be left at least overnight. To avoid colloid aggregation and precipitation, vials should be tightly sealed to prevent evaporation and stored in the dark until the next laboratory period. Surfaces are then removed from colloid, rinsed with H_2O, and stored in H_2O until optical spectra can be measured. Data may then be plotted as absorbance (which is proportional to coverage) vs particle concentration and fit to eqs 4 and 5. This will enable students to determine which equation better describes their experimental results.

For calculation of K and f, particle coverages must be known for each surface. Particle coverages may be calculated as in the previous section, using the absorbance at 524 nm (or λ_{max}) measured for each surface. θ is obtained from Γ by dividing by the surface coverage for a cubic close-packed monolayer of 12 nm Au particles [6.94×10^{11} particles/cm^2]. θ is plotted against colloid concentration (in M). A nonlinear least-squares (NLS) fit to eq 5 can then be carried out to find the coefficients f and K. Figure 6 shows representative data for thermodynamics of 12-nm diameter Au binding to NH_2-terminated surfaces (Keating, C. D.; Musick, M. D.; Trumbly, R. E.; Natan, M. J.; manuscript in preparation). For these data, the equilibrium constant, K, was determined to be 1.7 ± 0.3

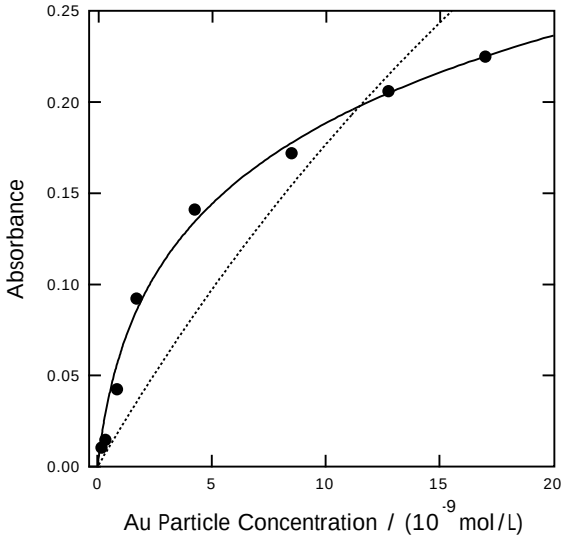


Figure 6. Absorbance at equilibrium versus colloid concentration. The solid line is the NLS fit to a Frumpkin isotherm (eq 4); the dotted line shows a fit to the Langmuir isotherm.

$\times 10^8$ M^{-1}, and the Frumkin parameter, f, was 4.7 ± 0.4.[10] The positive value of f indicates the presence of interparticle repulsive forces. The large equilibrium constant observed for Au particle binding is unsurprising, owing to the strong attraction between the amine functionalities and colloidal Au and to the large number of bonds formed between each particle and the surface.

Conclusions

The experiments described here incorporate several fundamental concepts (diffusion, equilibrium, kinetics, thermodynamics) covered in undergraduate physical chemistry lecture within the context of the rapidly expanding field of nanomaterials. This is accomplished with a visible spectrophotometer as the only required instrumentation. Additionally, students can see particle adsorption with their bare eyes as it occurs, which helps them to envision what is happening on the nanometer scale.

These experiments can act as a springboard for further experiments and are ideal for individual or group student projects. For example, the effect of temperature, solution viscosity, particle size, pH, silane functionality, etc. on thermodynamics and kinetics of Au colloid adsorption to silanized glass can be investigated. Protein–Au binding studies can be performed on any number of commercially available proteins and could be investigated as a function of protein molecular weight or isoelectric point, or solution pH. The materials produced in these laboratory exercises can be used in further experiments. For example, colloid monolayers are active substrates for surface enhanced Raman scattering and can be imaged by atomic force microscopy (making evident the effects of tip convolution in images of colloids having known dimensions), and monolayers prepared on In-doped SnO_2 electrodes can be used in electrochemical experiments. Finally, biologically oriented students might isolate protein–colloid complexes by centrifugation and use them to stain tissue samples for light or electron microscopy.

Tips for Instructors

Handling Silanes

Buy new silanes each semester. Other silanes can be used in these experiments; however, we have found that APTMS is noticeably more forgiving (of silane age, purity, water content, ambient humidity, air oxidation—all of which are evidenced by poor surface formation [splotchy surfaces or no binding at all]). We have had the best results with silanes purchased from United Chemical Technologies; APTMS is also available from other vendors (e.g., Sigma).

Do not mix large quantities of diluted silane prior to the laboratory experiment. Although this is convenient from the instructor's standpoint, the diluted silane has a much reduced "shelf-life" compared to the neat solution. Note that silanes will bind to any oxide surface (e.g., glass) they come into contact with, lowering the solution concentration of silane and permanently binding to the glass. Silanized glass can be cleaned by soaking in "base bath", which dissolves away the outermost layer of glass and takes the silane with it.

Glassware that will be used to hold or measure silane solutions (or to distill old silanes) can be thoroughly cleaned and then intentionally silanized with a methyl-terminated silane. This will prevent loss of other silanes from solution to coat the walls of the container.

Mercaptosilanes air-oxidize. This renders them less capable of binding Au particles over time. Humidity seems to hasten the process.

Gloves should be worn when dealing with silane–methanol solutions.

Handling Colloidal Au

Au colloid solutions should be stored in clean, brown glass bottles away from heat or light (alternately, clear glass bottles may be wrapped in aluminum foil). If Au colloid solutions are to be stored for long periods, they can be kept in the refrigerator to prevent bacterial and fungal growth. Colloidal solutions can be inspected visually for precipitates or fungal growth ("fuzzy globs") before use and should be discarded if either is found.

Handling Au Colloid-Coated Surfaces

Note that to determine the particle coverage on one side of the glass slide, absorbance must be divided by 2 (because silane and particles will be bound to both sides).

Colloidal particles can be mechanically removed. That is, tweezers, fingers, Kimwipes, etc. will wipe off the colloidal particles. The silane film will remain behind, and a new colloidal Au film can be put onto the wiped-off surface (Bright, R. B.; Keating, C. D.; Baker, B. E.; Grabar, K. C.; Smith, P. C.; Natan, M. J. submitted to *Chem. Mater.*). For the experiments in this laboratory, damage to the colloidal film due to tweezer scratches at the top or bottom of the slides will do no harm so long as they don't reach the part of the surface interrogated by the UV–vis beam.

Au colloid monolayers tend to turn blue or purple upon drying. This occurs to a greater extent for surfaces with high Au particle coverage. The color change is due to changes in interparticle spacing upon drying of the polymer film, a process similar to aggregation in solution (see part A). Part of the effect is due to refractive index change from water to air, but this effect is minor compared to that due to motion of the particles. Refractive index changes can be investigated by placing surfaces into solvents of different refractive index (e.g., water, glycerol, CS_2). A paper describing similar experiments for polymer-stabilized Au particles in solution has been published (*20*).

Larger particles tend to settle with time. If students do thermodynamics experiments as a function of particle size and use larger particles, this will be a problem. One solution is to put samples on an orbital shaker (as is used in biology to mix cell cultures or stain gels) or to agitate frequently in some other way. Note that large-particle preparations (whether purchased or synthesized according to a literature protocol) yield low concentrations of particles. We have noticed in thermodynamics experiments that *all* of the 45-nm Au in a 2.5-mL volume had adsorbed to the silanized slide; this made fitting to the Frumkin equation invalid.

Web Sites Related to Protein–Colloidal Au Conjugates

The home page for British Biocell International (BBI), a manufacturer of protein–Au conjugates for electron microscopy, is *http://www.british-biocell.co.uk/goldbp.html*. The Web site for AndCare, a company which uses protein-coated colloidal Au particles in electrochemical sensors, is *http://andcare.com*.

Acknowledgments

Support from NSF (CHE-9256692, CHE-9627338, CHE-9626326), NIH (GM55312-01), and the Henkel Corporation/ACS Division of Colloid and Surface Chemistry (through a graduate fellowship to CDK) is gratefully acknowledged. Acknowledgment is also made to the Electron Microscopy Facility for the Life Sciences in the Biotechnology Institute at The Pennsylvania State University.

Notes

1. Au colloidal solutions are commercially available from several sources; however, use of commercial sols for these experiments is strongly discouraged. The protocol described here produces a more concentrated solution (hence faster kinetics and a larger range of concentrations available for adsorption isotherms) and can be used to prepare large quantities of Au at a fraction of the cost.

2. Available by anonymous FTP from *http://zippy.nimh.nih.gov* or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part number PB93-504868.

3. PWHM was measured for Au colloid solutions by doubling the distance between the λ_{max} and the right-hand half-max position. This was necessary owing to the nonzero absorbance on the left-hand side of the Au particle plasmon band.

4. pH meters should *not* be used to determine the pH of unstabilized colloidal solutions, since irreparable damage to the electrode is likely to result.

5. Note that calculation of ε_{surf} is made possible only by an *independent* measurement of Γ (in this case, TEM, atomic force microscopy, atomic absorption, or field emission scanning electron microscopy) (*14*). Note also that ε_{surf} for other colloid diameters may be roughly estimated by assuming that all particles are spheres and that the value of ε_{surf} scales with cross-sectional area (*15*).

6. These data were fit using the built-in nonlinear least squares subroutine in the software package IgorPro (which makes use of the Marquardt algorithm). A good description of this procedure—which could be incorporated as part of the material to be studied for this lab—can be found in Bevington, P. B. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.

7. The surface coverage of Au particles can be estimated, using a value for *p* calculated from eqs 1–3. The viscosity, γ, for the Au colloidal solution can be taken as that of pure H_2O. The sticking probability calculated for the data at short times (first 5 points) shown in Figure 5 was 0.5 or 1.2, depending on whether ε_{surf} was obtained by scaling with cross-sectional area to the published value for 15-nm diameter Au (thus, 5.9×10^8 M^{-1} cm^{-1}) or from 9 FE-SEM images of different spots on one 12-nm Au submonolayer surface (2.8×10^8 M^{-1} cm^{-1}). The latter value implies that particles stick more than 100% of the times they hit the surface, which seems unreasonable; however, this does not mean that the former value is correct. Rather, there is clearly a large uncertainty in *p* associated with uncertainties in ε_{surf}. Nevertheless, it is imperative to point out to the students that even an imperfect determination of *p* is possible only because Γ can be accurately estimated by measurement of absorbance. This, in turn, is true because Beer's law holds for colloidal Au.

8. However, it is a useful exercise to have the students *try* to fit the data to the Langmuir equation.

9. A good exercise in dimensional analysis is to have students calculate the concentration of particles assuming (i) all the particles are spheres; (ii) all particles have the same size; (iii) all added Au^{3+} is reduced to colloidal Au; and (iv) the density of colloidal Au is that of the bulk material (18 g/cm^3). From the number of moles of Au^{3+} initially present in the solution, a mass of Au is calculated (Au atomic weight is 196.97 g/mol). Using the density of bulk Au metal, a volume of Au is calculated; this volume is divided by the volume of an individual Au particle (for 12-nm spheres, $v = 4/3\pi r^3 = 256$ nm^3/particle) to give the number of Au particles in solution. Knowing the volume of the final solution (note that it may have decreased owing to evaporation during preparation of the colloid), the final concentration in moles of particles/L can be calculated using Avogadro's number.

10. These values were obtained using $\varepsilon_{surf} = 2.8 \times 10^8$ M^{-1} cm^{-1}. As previously noted, uncertainties in ε_{surf} are a significant source of error in these calculations.

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