NUCLEATION AND GROWTH OF GOLD NANOPARTICLES

Chapter 4
The unusual nucleation and growth kinetics of gold nanoparticles synthesized in the presence of the antibiotic cephalexin using in situ static and dynamic light scattering in conjunction with in situ UV-vis absorption measurements and transmission electron microscopy is reported in this chapter. Following a brief latency period, two well-separated populations of gold nanoparticles, with average sizes of 1-2 nm versus several tens of nanometer, respectively, nucleate simultaneously. For either size regime, the total number of particles increased rapidly, while their mean size and relative frequency remained essentially constant over a growth period of 5 h. With increasing temperature, the morphology of the larger nanoparticles became increasingly spherical, as indicated by the noticeable blue shift of the plasmon frequency for these particles. The detailed morphology for either particle population was confirmed with TEM. This is the first observation of simultaneous growth of two distinct populations of crystalline nanoparticles in the solution phase. The coupled growth of two distinct populations of nuclei, their tight control of size but rapid increase in overall numbers present novel and intriguing facets of this antibiotic-mediated solution-growth of gold nanoparticles. The size evolution of the Mie-frequency exhibits a noticeable blue shift as the temperature increases, correlating with the roundness of the particle/bimodal distribution.

Part of the work presented in this chapter has been published:

4.1. Introduction

The properties of colloidal gold, silver and other such similar metals have been of interest for centuries with an extensive scientific research going back to Michael Faraday in 1857 on the colloidal gold samples.\(^1\) In 1908, Mie presented a solution to Maxwell’s equations that describes the extinction (absorption and scattering) of spherical particles of arbitrary sizes.\(^2\) The exact theory of light scattering based on Mie theory is very complicated and limited to a small number of particle shapes. The calculated extinction, scattering and absorption spectra of a particle of size, \(d = 16\) nm, are shown in Figure 4.1(a, b & c respectively).\(^3\) In this chapter, the scattering and the absorption of gold nanoparticles are studied.

![Figure 4.1](image)

**Figure 4.1.** Calculated extinction, scattering and absorption spectra of a particle of size, \(d = 16\) nm. Adapted from ref \(^3\).

Ever since the Mie theory, there have been various models and approximations to study nanoparticles systems.\(^4\) \(^5\) Over the years, it has been realized that the morphology and the growth-rate of these nanostructures in the solution phase can be controlled and designed by tuning the reaction parameters. The wet chemical synthesis of nanomaterials has advanced to the level where it is possible to tailor make particle shapes, sizes, and their distributions. Different parameters and factors
affecting the process are varied to achieve a tight particle size control.\textsuperscript{6,7} However, to achieve control over the synthesis, it is important to understand the process of nucleation and growth of crystallites from the cluster level upward. This includes the specific roles played by various physical and chemical parameters such as temperature, concentration, pH, stirring, osmotic potential, incubation time, and so on. The mechanisms involved in the growth of nanoparticles follow different rules than those applicable to bulk materials. Over the last several decades, the mechanism of nucleation and growth processes of colloidal particles synthesized by various methods has been researched in detail. The initial swell in the nucleation and growth studies began predominantly with condensation\textsuperscript{8,9} and crystallization\textsuperscript{10,11} studies during the early twentieth century. However, mechanistic studies of colloid and cluster formation began when LaMer and Dinegar synthesized sulfur hydrosols nucleating from supersaturated solutions.\textsuperscript{12} Uniform particle size was achieved by short nucleation and relatively long growth periods. Studies on kinetics and mechanisms of particle formation showed incompatibility with Lamer’s supersaturation theory.\textsuperscript{13,14} Models and statistical theories began to be developed for understanding the formation of the critical nucleus and spontaneous growth which gives rises to particular sizes.\textsuperscript{15,16} Overbeek did extensive studies on the particle growth rate and the particle size distribution citing the possible rate-determining steps.\textsuperscript{17} Analysis of the activation barrier in the nucleation process, studies on the parameters relevant for kinetic or thermodynamic control and factors controlling the growth process have improved our understanding of the overall process. However, the process of growth is quite complicated and difficult to study experimentally. It depends on numerous factors like:

- nucleation rates,
- cluster mobility,
- maximum cluster density,
- spatial and size distribution of clusters and
- modes of growth.

To fully understand the formation of particles at various levels, it is essential to capture and investigate the early stages of nucleation of the nanoparticles, their growth kinetics and the effect of various parameters. The chemistry and physics of gold nanoparticles has emerged as a broad new sub-discipline in the domain of
colloids. Specifically, the unique optical properties of gold nanoparticles have made this model system more useful in exploring a wide range phenomenon. This chapter describes the study undertaken during the initial stages of the reaction of chloroauric acid and cephalaxin to form gold nanoparticles, the synthesis procedure and characterization of which is described in the previous chapter (Chapter 3).

Among the experimental techniques used to study and understand the kinetic and thermodynamic nature of the nanocrystal nucleation and growth, in general, are small angle X-ray scattering (SAXS), UV-visible spectroscopy, X-ray photoelectron absorption spectroscopy (XPS), time-dependent transmission electron microscopy (TEM), and dynamic light scattering (DLS). Though these techniques are highly efficient for the in situ measurements of particle size and shape determination, there are few problems, such as:

i. All methods obtain information about larger clusters (around >1-2 nm).

ii. The limitation on the time scale of the measurements; since nucleation and growth of the nanoparticles during laboratory syntheses proceeds quite fast.

iii. The nucleation events, in particular, are difficult to resolve since they represent a transient, meta-stable state.

iv. Complications due to the reaction setup (multistep synthesis processes, high temperature-pressure, etc.) prevent combination of light scattering with X-ray scattering for simultaneous in situ measurements.

Therefore, a careful investigation of nucleation and growth of nanocrystallites in the solution phase demands the synthesis process that is (1) single-step, (2) can be coupled with standard light/X-ray scattering set-ups, and is (3) slow enough reaction rate to capture the growth process.

The synthesis of gold nanoparticles using a biomolecules, such as cephalaxin proved to be a suitable system for such a study due to the following reasons:

i. reaction ends over a large period (approximately 1.5 h at 28 °C),

ii. establishes the mechanism of metal synthesis by a biomolecule, specifically an antibiotic,

iii. scattering and absorption studies done using a simple system with no auxiliary chemicals or processes needed and

iv. no interference in the DLS study from micelle formation as happens in the reverse micelle-based methods.
This chapter discusses the results on the \textit{in situ} light scattering and UV-vis measurements in combination with the transmission electron microscopy at various controlled temperatures to understand the growth mechanism. The \textit{in situ} observation of antibiotic mediated concurrent growth of two distinct homogeneous populations of gold nanoparticles in solution phase is reported.

4.1.1. DLS theory

There are two basic methods of Light Scattering: Static Light Scattering (SLS) and Dynamic Light Scattering (DLS). “Classical” light scattering (also known as “static” or “Rayleigh” scattering\textsuperscript{22, 23}) provides a direct measure of molecular mass. SLS is a powerful technique to estimate sizes, average molar masses of various single or assembled structures and their interaction with environment. SLS can be used to study various systems like colloids, synthetic and natural polymers in solution, including proteins and polysaccharides and to investigate oligomerization, complex formation, aggregation, stability, conformation and 2\textsuperscript{nd} virial coefficient.\textsuperscript{21}

Leon Brillouin was the first one to describe a short theoretical note describing the frequency distribution of the light scattered from thermally excited density fluctuations in an isotropic body.\textsuperscript{24, 25} When an electromagnetic radiation is incident upon a molecule, the molecule constitutes an oscillating dipole or electric oscillator depending upon the effect of the magnitude of the polarizability of the molecule, i.e. the ease of shifting charges within the molecule. In an elastic scattering, the oscillating dipole acts as an emitter of an electromagnetic wave of the same wavelength as the incident wavelength, emitting isotropically in all directions perpendicular to the oscillator. Now, for molecules or particles larger than 20 nm, most of the oscillating dipoles created within one given particle possess a significant phase difference. Accordingly, interference of the scattered light emitted from such an individual particle of size larger than 20 nm leads to a non-isotropic angular dependence of the scattered light intensity. The interference pattern of intra-particular scattered light, also called particle form factor, is characteristic for size and shape of the scattering particle. As a consequence, it provides the quantitative means for the characterization of particles in very dilute solution by light scattering. For particles smaller than $\lambda/20$, only a negligible phase difference exists between light emitted from the various scattering centers within the given particle (See Figure
Particles in solution, however, usually show a random motion (Brownian motion) caused by thermal density fluctuations of the solvent. As a consequence of the temporal changes in inter-particle positions and the corresponding temporal concentration fluctuations, the interference pattern and the resulting scattered intensity detected at a given scattering angle also change with time, reflecting the Brownian motion of the scattering particles. This phenomenon provides the basis for dynamic light scattering, an experimental procedure which yields a quantitative measure for the mobility of scattering particles in solution as characterized by their self diffusion coefficient. Most modern particle sizers, frequently used both in industry and academia nowadays to determine the (hydrodynamic) size of particles in solution, are based on this principle. The theory behind this principle is explained in the following section with reference to the system used in this study.

![Interference pattern of light scattered from two scattering centers of small particles (A) and from larger particles (B). (C) & (D) shows the change in the interference pattern of](image)
scattered intensity with time, caused by Brownian motion of two scattering particles. [Figure adapted from ref 26]

The size distribution of the gold colloids scattering light and diffusing through the solution is related to the field correlation function $g_1(\tau)$ of scattered light$^{27-29}$ via

$$g_1(\tau) = \int_0^\infty F(\Gamma) \exp(-\Gamma \tau) d\Gamma \quad \text{......................................... (1)}$$

indicating that the field correlation function is a linear superposition of exponential decays with decay rate $\Gamma$ and relative amplitude $F(\Gamma)$.$^{28,29}$

The field correlation function $g_1(\tau)$, in turn, is obtained from the experimentally measured intensity correlation function via the Siegert relation$^{28,29}$

$$g_2(\tau) = |g_1(\tau)|^2 + 1 \quad \text{................................. (2)}$$

Equations 1 and 2 are used to obtain the distribution of decay rates $F(\Gamma)$ from the measured correlations of the light intensity fluctuations. The decay rates $\Gamma$ themselves are related to the diffusion constants $D$ of the diffusing particles via

$$\Gamma = D q^2 \quad \text{................................................. (3)}$$

where, $D$ is the diffusion constant of the scattering particle and $q$ is the magnitude of the scattering vector given by

$$q = (4\pi n / \lambda) \sin(\Theta/2) \quad \text{........................................... (4)}$$

where $\lambda$ is the wavelength of the incident light, $n$ is the refractive index of the medium and $\Theta$ is the angle at which the scattered light is detected with respect to the direction of the incident radiation.

For this system, parameters are $n = 1.33$ (aqueous solutions), and $q = 2.64 \text{ nm}^{-1}$. Hence, particles with hydrodynamic radii $r_h$ close to or below $q^{-1} \approx 38 \text{ nm}$ could be treated simply as isotropic Rayleigh scatterers. Finally, the distribution of diffusion coefficients can be converted into particle size distributions using the Stokes-Einstein relation

$$D = (k_B T) / (6\pi \eta r_h) \quad \text{........................................... (5)}$$

where $k_B$ is the Boltzmann factor, $T$ is the absolute solution temperature, $\eta$ the temperature-dependent solution viscosity and $r_h$ is the hydrodynamic radius of the diffusing particle species.

During the nucleation studies, the amplitude of the autocorrelation functions steadily increased as nucleation and aggregation of the gold sol progressed. Correlation
functions with intercepts at \( t \to 0 \) smaller than 0.2 were excluded from our analysis due to their intrinsic noisiness. Otherwise, correlation functions were converted into particle size distributions using the “general purpose” inversion algorithm provided with the Zetasizer Nano S software. Particle size distribution obtained from alternative inversion algorithms yielded comparable results. There are few options for performing accurate and repeatable particle size analysis on materials in the nanoparticle range. DLS provides several advantages over electron microscopy since many more particles are actually analyzed, which provides improved precision and ability to detect small numbers of larger particles. Improvements in detector technology have led to detections of very small particles (measurements have been made up to 1 nm), particles that scatter low amounts of light and low concentrations of particles.

4.1.2. Surface Plasmon Resonance

The appearance of the ruby color in the tinted glass is explained due to the finally divided gold\(^1\) and later on theoretically explained by Mie\(^2\). With this pioneering work he could trace the origin of the colours back to a resonant absorption of light by small metal spheres. The interaction of metals with the electromagnetic radiation is largely dictated by the free conduction electrons in the metal, also known as the Drude metal. This resonant absorption, which is caused by a collective excitation of the conduction band electrons in metal nanoparticles, is called localised surface plasmon polariton resonance (LSPPR).

According to the simple Drude model, the free electrons oscillate 180° out of phase relative to the driving electric field.

\[
m_e \frac{\partial^2 r}{\partial t^2} + m_e \Gamma \frac{\partial r}{\partial t} = eE_0 e^{-i\omega t} \quad \text{(6)}
\]

where \( e \) and \( m_e \) are the charge and the effective mass of the free electrons and \( E_0 \) and \( \omega \) are the amplitude and frequency of the applied electric field.

Due to this, most metals possess a negative dielectric constant at optical frequencies which causes, for example, a very high reflectivity. At optical frequencies, the metal’s free electron gas sustains surface and volume change density oscillations called as plasma polaritons or plasmons with distinct resonance frequencies.
By definition, surface plasmons are the quanta of surface charge-density oscillations. The term “surface” stems from the fact that although all electrons are oscillating with respect to the positive-ion background, the main effect producing the restoring force is the surface polarization. The surface, thus, plays a very important role for the observation of surface plasmon resonance because it alters the boundary condition for the polarizability of the metal and therefore, shifts the resonance to optical frequencies, contributing to the beautiful color of gold nanoparticles.

The total extinction coefficient of nanoparticles is given in Mie’s theory as the summation over all electric and magnetic multipole oscillations contributing to the absorption and scattering of the interacting electromagnetic radiation. The existence of plasmon is characteristics of the interaction of metal nanostructures with light. At the interface between a metal and a dielectric, the surface charge density oscillations give rise to strongly enhanced optical near-fields which are spatially confined near the metal surface. The localization at the interface is characterized by electromagnetic fields that exponentially decay with increasing distance from the interface into both half-spaces (media and the metal).

When the dimensions are reduced, boundary and surface effects become very important and thus the optical properties of small metal nanoparticles are dominated by such a collective oscillation of conduction electrons in resonance with incident electromagnetic radiation. In this case of a sub-wavelength scale particle, the overall displacement of the electrons with respect to the positively charged lattice leads to a restoring force, which in turn gives rise to specific particle-plasmon resonances depending on the geometry of the particle. (see Figure 4.3 below)

![Figure 4.3. Schematic representation of the plasmon oscillation (shown in green) for spherical nanoparticles. (Image adapted from ref. 4)](image-url)
In case of sharp edges or pointed shapes, extreme local accumulations occur that are accompanied by strongly enhanced optical fields. Ellipsoids and nanorods display two distinct plasmon bands related to transverse and longitudinal electron oscillations as shown in Figure 4.4, giving rise to the quadrupole and octopole absorbances. The longitudinal oscillation is very sensitive to the aspect ratio of the particles.

![Figure 4.4. Schematic representation of transverse and longitudinal oscillations in an anisotropic particle.](image)

Metals, especially noble metals such as gold and silver have a large negative real part of the dielectric constant along with a small imaginary part. To account losses associated with electron scattering (ohmic losses) the imaginary part of the metals’ dielectric function has to be considered. Since these resonances arise from the particular dielectric properties of the metals, they can be easily modeled using the equations derived by Mie for the resolution of Maxwell equations for the absorption and scattering of electromagnetic radiation by small spheres and their modification by Gans for ellipsoids. According to the prediction by Mie theory, the expressions for the extinction cross section $C_{ext}$ for very small particles with a frequency dependent, complex dielectric function, $\varepsilon = \varepsilon' + i\varepsilon''$, embedded in a medium of dielectric constant $\varepsilon_m$, this can be expressed as:

$$C_{ext} = \frac{24 \pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon''}{\varepsilon'' + (\varepsilon' + 2\varepsilon_m)^2} \hspace{1cm} (7)$$

where R is the radius of the particle.
When condition $\epsilon' = -2\epsilon_m$ is fulfilled, the long wavelength absorption by the bulk metal is condensed into a single surface plasmon band. Mie theory holds true for dilute and non absorbing media as the condition expressed; for concentrated systems, the nanoparticles become closer to each other, and interactions between neighboring particles can arise, so that the models for isolated particles do not hold any longer, and different behavior of the particles are observed.

In this chapter, we observe the scattering and the absorption arising from the reaction solution comprising of chloro auric acid and cephalexin, as the gold nanoparticles are synthesized at various temperatures. The most striking finding is the evolution of a tight bimodal particle distribution. The spectra are supported by TEM micrographs where the morphology of the particles is exhibited.

### 4.2. Experimental Details

The one-step synthesis protocol is used as described in the previous chapter $^3$ to synthesize the antibiotic functionalized gold nanoparticles. A slight modification is made in this work, where $1 \times 10^{-4}$ M chloroauric acid (Aldrich Inc.) was reduced by $10^{-5}$ M of the antibiotic cephalexin (a kind gift to us from Lupin Laboratories, India). Here, both the antibiotic and the chloroauric acid were first diluted to twice of their final concentrations into the distilled water before mixing them for the formation of nanoparticles. For the DLS studies, both the stock solutions are filtered through 0.22 μm syringe filters to remove any unwanted particles. Using DLS, both the stock solutions were checked carefully for the presence of pre-existing particle clusters (or dust particles) that might interfere with subsequent nucleation studies.$^4$ The 2× stock solutions were cooled to 5 °C, mixed in equal proportion to their final concentration and then placed into a quartz cuvette for light scattering measurements. The pH of the solution was monitored by using a digital pH meter (Cyberscan, Eutech Instruments). The pH was stable around ~3.7. Following the particle synthesis, the resulting colloidal gold nanoparticles suspensions remained stable without aggregation or precipitation. Glass cuvettes containing the mixed chloroauric acid/cephalexin solutions were placed inside the thermostatted sample holder of the DLS unit and were allowed to equilibrate to their set temperature (15, 25, or 35 °C) for 5 min. From then on, intensity autocorrelation functions of scattered light were collected continuously using acquisition times of 60 s per
correlation function. Throughout the experiment, the total intensity of scattered light changed dramatically due to the incessant nucleation and growth of strongly scattering gold colloids. Therefore, the measurement software protocol was set up to first measure the total scattering intensity and to adjust a variable neutral density filter in the detection arm accordingly to keep the avalanche photodiode count well below saturation. Relative scattering intensities were corrected for this variable attenuation.

4.3. Results and Discussions

4.3.1. DLS study of the system and its data analysis

In Figure 4.5A, we show the temporal evolution of the intensity correlation function of light scattered from the solution undergoing the synthesis of gold colloids at 15 °C. Due to the reduced synthesis rate, the early stages of the nucleation and growth of the colloidal gold particles are more readily resolved at T = 15 °C. Initially, no correlations are detected since the concentration fluctuations of the gold sol alone are too fast to be resolved by DLS.

Figure 4.5B displays the temporal evolution of the intercept of the intensity correlation function g₁(τ) versus the incubation time of the sample. A fit through the intercepts of g₁(τ) vs. incubation time with a simple sigmoidal functions faithfully reproduces the experimentally observed behavior, as expected for an “activated process” such as nucleation. Notice also the significant lag of the total scattering intensity compared to the upswing in the amplitude of the correlation function. This implies that dynamic light scattering is a much more sensitive indicator of the nucleation event than static light scattering. There is a significant latency period of approximately 30 min before the onset of nucleation and growth of gold particles as detected by DLS. This latency period decreases significantly as the solution temperature is raised to 25 °C or to 35 °C. After a period of rapid increase, the g₁(τ) intercept eventually levels off around 0.78, below the theoretical limit of 1. The lower plateau value of 0.78 arises from contributions to the dynamic signal from purely static scattering off the various interfaces (air/glass/solution). Together with the zero intercepts of the intensity correlation functions, Figure 4.5B also shows the total intensity of scattered light during the synthesis of the gold colloids.
Figure 4.5. (A) Normalized temporal correlations of the intensity of scattered light, $g_2(\tau) - 1$, vs. delay time $\tau$ obtained at different time points (see label on curve) during the synthesis of colloidal gold particles from chloroauric acid solution ($10^{-4}$ M) in the presence of the antibiotic cephalxin ($10^{-5}$ M), incubated at 15 °C. With increasing incubation period, the correlations of the scattered light arising from the gold colloids nucleating and diffusing in the aqueous suspension increases significantly. (B) Intercepts of the intensity correlation function of scattered light (O) and the overall intensity of scattered light ($\Delta$) vs. the incubation time of the sample. The solid squares highlight the time points for most of the correlation functions displayed in (A) and their corresponding particle size distributions shown in (B).

Obviously, the rapid increase in the temporal correlations of scattered light (~30 min) significantly precedes the upswing in overall scattering intensity (~80 min), both of which are associated with the nucleation and growth of the gold colloid particles. This is an intriguing observation since increases in static scattering intensity are frequently used as indicators for the onset of nucleation events in supersaturated solutions. Our observations suggest that the correlation amplitude of dynamically scattered light is a much more sensitive and reliable indicator for nucleation events than “kinks” in static light scattering data. Nevertheless, DLS is unlikely to capture the actual nucleation event due to at least two complicating factors. First, the dynamic signal during the very early phases of nucleation is contaminated by contributions from residual dust and air inclusions. In addition, the shot noise of the photon detector limits resolution of very small populations of particles.
Figure 4.6A displays the particle size distributions obtained from the autocorrelation functions during the early stages of the nucleation and aggregation process (see also open squares in Figure 4.5B). Noticeably, the larger particle peak around 20-30 nm emerges ahead of the smaller aggregates near 1-2 nm, which appear only at 69 min. The particle distributions at all temperatures eventually showed two well-resolved particle peaks centered around 25 and 1 nm, respectively. At higher temperatures, the apparent delay between the emergence of the larger and smaller peak was much less pronounced. Notice that the minor contribution from the smaller colloids, which are well resolved in the correlation functions at later times, might not be readily resolved for the small intercepts and weak scattering intensities during the early stages of nucleation and growth (see Figure 4.5B).

**Figure 4.6.** (A) Examples of particle size distributions obtained during the early phases of the synthesis of gold colloids in the presence of cephalexin during incubation at 15 °C. Figure 4.1B indicates at what point in the nucleation process these particle size distributions were obtained. In the display we also excluded third, larger peak (>500 nm) associated with dust and air inclusions present in the solutions. (B) TEM micrographs of the gold nanoparticles synthesized at 15 °C after 1 hour reaction time.

To further investigate this, in Figure 4.6B we have shown the TEM images on the particles pre-69 min, which essentially captures the growth behavior before 69 min. However, caution is required when interpreting this DLS result. First, as indicated by
the Stokes-Einstein relation (eq 5) and eq 4, the autocorrelation of light scattered by small 1-2 nm aggregates decays at rates of only few microseconds. Unfortunately, the correlation functions g1(t) remain rather noisy, particularly at these short delay times, until the amplitude of the zero-intercept is well above 0.5. In addition, the scattering intensity of the particles increases approximately quadratic with particle volume. As a result, a single particle of radius 25 nm will scatter one million times more light than a 2 nm particle. As is apparent from Figure 4.5B, the overall scattering intensity from the solutions remains rather weak prior to approximately 85 min into the experiment. In addition, the contribution to the scattering intensity from the small particles never exceeds 20% of the total scattering intensity (see Figure 4.8B). All these factors might collude to minimize the contributions of smaller particles to the dynamic light scattering signal during the very early stages of nucleation. In spite of these speculations, surprisingly, in Figure 4.6B, we observe that the larger particles have already formed within 60 min of reaction whereas the smaller particles (as observed in Figure 4.12B after the reaction is complete) are absent. The background spotting, observed as a lighter contrast compared to gold nanoparticles is probably attributed from the unreacted antibiotic. To confirm this we focused on the lighter contrast spots using HRTEM and we did not find any indication of crystallites unlike gold nanoparticles where strong lattice fringes were observed (inset in Figure 4.6B). The temporal evolution of the two peaks in the particle size distribution vs. the incubation time for samples at 15, 25, and 35 °C, respectively is summarized in Figure 4.7. Most strikingly, the particle distribution is bimodal with two narrow peaks located around ≈25 nm and 0.5-1.5 nm. Following a brief latency period, the two well-separated populations of gold nanoparticles emerge from the supersaturated solutions nearly simultaneously, with the larger particles slightly preceding the smaller particles particularly at the lowest reaction temperature of 15 °C. As discussed above, it is not obvious whether this apparent difference in latency of nucleation is not just a consequence of the limited detection sensitivity for the smaller aggregates. Two well-separated populations of gold colloids with surprisingly tight limits on their particle distributions emerged at all incubation temperatures. The radius for the peak of either population of gold colloids remained essentially unchanged throughout the entire observation period of several hours.
We are, therefore, cautiously concluding that both populations of gold nanoparticles nucleate essentially simultaneously. The observation of two different particle populations of distinct mean size are consistent with earlier observations made by the authors in a separate study (as discussed above) where the TEM micrographs showed the presence of larger particles surrounded by a large number of smaller particles.33

Figure 4.8A summarizes the temporal evolution of the relative scattering intensity from the solutions at $T = 15^\circ C$ and $25^\circ C$, respectively. In contrast to the relative distribution of gold colloids (Figure 4.7), the total number of colloidal gold particles rapidly increases throughout the incubation period. In addition, the synthesis clearly proceeds significantly faster at $T = 25^\circ C$ than at $T = 15^\circ C$.

Following the initial lag-time for nucleation, the overall scattering intensity from these solutions rapidly increases with time, closely following a power law with exponents around 1.7. Figure 4.8B shows the corresponding changes in relative scattering intensity for the small vs the large colloidal particles over the same time.
period. Similar to the overall sizes of the two colloidal gold particles, the relative populations for either peak does not appear to change throughout the nucleation and growth period shown in our data.

Figure 4.8. (A) Changes in the total intensity of scattered light during the synthesis of colloidal gold particles at $T = 15$ and 25 °C. Intensity data shown here have been corrected to account for neutral density filters inserted in front of the detector to prevent saturation. (B) Percentage of total light scattered by either population of colloidal gold particles during synthesis at $T = 15$ °C.

The results at $T = 25$ and 35 °C are comparable but have been omitted here for clarity. In stark contrast to the rapid increase in total scattering intensity, the relative contributions to the scattering intensity from either particle population remain nearly fixed at a ratio of approximately 20% for the small colloids versus 80% for the larger colloids. Again, this suggests the remarkable feature that both populations are growing at identical rates throughout the synthesis process. The chemical origin of the coexistence of two different size ranges with tight control over particle size, nucleation, and growth rates is not obvious to us, but does suggest that all three components of the synthesis are somehow tightly coupled to one another. We believe that this is the first report of simultaneous growth of two size ranges. It seems that various functional groups on the antibiotic molecules (cephalexin) might be playing a significant role in this process. We reported earlier, based on our NMR and XPS analysis, that as-synthesized particles are stabilized by the antibiotic molecules which get coated at the gold nanoparticle surfaces, helped by their sulfur moiety (Chapter 3).
4.3.2. UV-vis absorption spectroscopy data

The UV-vis absorption spectroscopy is another easy and established technique to characterize the gold nanoparticles due to their strong absorption in the visible to IR range due to the surface plasmon resonance (SPR) where the absorption peak width, intensity, and the wavelength are highly sensitive to the size, shape, and chemical environment around the gold nanoparticles. By solving Maxwell’s equations with the appropriate boundary conditions for spherical particles, Gustav Mie quantitatively described the plasmon absorbance.\(^2\) The origin of these “surface plasmon resonances” is attributed to the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field.

The observation of the optical properties thus provides information about the particle size and shape. However, the measured values do not coincide quantitatively with Mie’s theory.\(^{37-39}\) These discrepancies are due to the fact that the idealizing assumptions are not met in reality.\(^{40}\) Deriving an approximation for quasi-spherical particles and anisotropic particles obtained in a single system, therefore, is difficult.

The shape-factor, \(L\), changes with both the size and shape of the crystals and is strongly influenced by surface defects, roughness, and the quality of facets. Gold nanoparticles made of less than 300 atoms display distinct optical and electronic properties compared to bulk. Size confinement results in new electronic and optical properties, which is a characteristic feature of noble metal nanoparticles.

![Image of gold nanoparticles](image)

**Figure 4.9.** Image of the vials containing gold nanoparticles synthesized by the cephalexin mediated method in aqueous medium at temperatures 15, 25, and 35 °C (vials A, B, and C), respectively.
In Figure 4.9, we have shown the image of the vials containing the gold nanoparticles synthesized at temperatures 15, 25, or 35 °C, labeled as A, B, and C respectively. The vial labeled A shows a weak purple color, whereas vials B and C show increasing trend toward the ruby red. The color of these suspensions confirms the presence of stable particles. The origin of the variation in the colors will be described in detail in the coming paragraphs. By monitoring absorption spectra, the growth and changes in the morphology of crystals can be monitored in real time\(^4\). There are various complicating factors affecting the optical properties of the nanoparticles like the presence of a substrate, the solvent layer and the particles that are close enough together that their electromagnetic coupling changes the spectra\(^4\). To understand further the growth dynamics of these particles, we carried out *in situ* UV-vis spectroscopy studies using a Cary 50 UV-vis spectrophotometer. Similar to the light scattering measurements discussed above, the absorption data was also obtained *in situ* at temperatures (15, 25, or 35 °C) at the intervals of each 15 min after placing the gold salt and antibiotic (mixed together as mentioned in the experimental section), inside the thermostatted sample holder. In this study, we showed the temporal evolution of the SPR peak of such particles at the different temperatures. Earlier studies on the temperature dependence of the SPR absorbance showed that the effect of temperature on the width, intensity and wavelength of the SPR peak is negligible, consistent with the fact that dephasing of the surface plasmon electron motion is a result of the electron-electron repulsion rather than electron-phonon interaction.\(^4,2\) Hence, any difference that we observe in the SPR peak characteristic will be purely due to the change in the particle morphology itself and/or the chemical environment around the nanoparticles. In Figure 4.10, we have compared the temporal evolution of the SPR peaks in the solution phase at various temperatures (15, 25, and 35 °C) as shown in panels A, B, and C, respectively. In each panel, we have followed the evolution of the absorbance spectra as a function of reaction time where the intensity of the surface plasmon resonance peaks increases as the number of particles grows in the solution at their respective temperatures. In panel A, we see a quite broad absorption band instead of well defined peak even after 6 h of reaction at 15 °C which is consistent with the light purple color seen in Figure 4.10A.
Figure 4.10. *In situ* UV-visible absorption spectroscopy measurements to demonstrate the temporal evolution of peaks due to the surface plasmon resonance absorption as the population of the nanoparticles increases in the solution. The panels A, B, and C show a group of spectra taken during the synthesis at temperatures 15, 25, and 35 °C, respectively, taken at an interval of every 15 min.

However, the DLS data shows the evolution of the two different size ranges of particles beyond 60 min (Figure 4.6A). As mentioned earlier, even though the gold nanoparticles are formed at this temperature, the reaction rate is quite slow. Here the limitation of conventional UV-vis spectroscopy in detecting the presence of lower population of the particles becomes clear. It is well known that the Rayleigh scattering is size-dependent and shape-dependent. It scales as the sixth power of the particle diameter (for a sphere); this places a lower practical limit on the size of particles that can be detected of 40-50 nm. Absorption techniques are able to measure the optical properties of particles as small as 2.5 nm. Most of the studies in reporting the synthesis of gold nanoparticles rely on the UV-vis spectroscopy alone. However, the results here clearly indicate the difference in the sensitivity of both techniques. It is known that absorption spectroscopy is insensitive to the presence of gold nanoparticles less than 3 nm in size due to the quantum size effects as determined by the Kubo criterion. Only when the size of the particles increase beyond this limit, gold nanoparticles develop observable absorbance. Therefore, we do not expect the smaller particles shown in our DLS data to contribute to the absorption spectra in the UV visible range. In contrast, these particles are readily detected not only by DLS, as shown here, but also by TEM in our earlier published paper by Jagannathan et al. Comparing the spectrum with the TEM micrographs obtained for particles at 15 °C temperature, note that for $2R > 25$
nm, the extinction spectrum is dominated by quadrupole and octopole absorbance as well as scattering.\textsuperscript{32,46} Though the population density of smaller particles is high, they appear to have aggregated/coalesced into larger, mostly anisotropic particles giving rise to the longitudinal mode of anisotropic absorbance in the range from 700 to 1000 nm (light purple color), which decreases as the reaction temperature is raised to 25 °C, as seen in the Figures 4.9 and 4.10 (panel B). At 35 °C this absorbance is almost absent owing to the fast reaction of the system at this temperature, consistent with the quasi-spherical particles shown in our previous studies.\textsuperscript{33}

In Figure 4.11, we have plotted the time-dependent change in the $\lambda_{\text{max}}$ (panel A) and intensity (panel B) of the transverse mode of the SPR peaks at the reaction temperatures 25 and 35 °C (obtained from Figure 4.10B and 4.10C) with the error bars (due to the broadness of the peaks). Due to the absence of the well defined transverse/longitudinal modes of the SPR peaks at 15 °C, we could not plot the peak-shift and the peak-intensity with respect to time.

![Figure 4.11](image)

**Figure 4.11.** (A) Time-dependent hypsochromic shift in the wavelength of the surface plasmon resonance absorption peaks during the synthesis for reactions at temperatures 25 and 35 °C. (B) Temporal evolution in the absorption peak intensity for the reactions at 25 and 35 °C.

In panel A, we observe that the $\lambda_{\text{max}}$ shifts toward higher energies as the reaction proceed (for both 25 °C and 35 °C) which is quite significant and remarkable. At 35 °C, this hypsochromic shift is quicker and larger, which is perhaps due to the particle morphology shifting toward quasi-spherical geometry\textsuperscript{4,47} as the growth of the
particle proceeds at 25 and 35 °C. The increase in the SPR peak intensity with reaction time shows an increase in the population of nanoparticles, which is much faster for the reaction at 35 °C, as expected, and the growth curve reaches the flatness quite fast.

4.3.3. TEM and SAED measurements

To confirm the morphology of the particles and the presence of the bimodal distribution as predicted by the dynamic light scattering results, we obtained TEM micrographs of the samples. For this purpose, we prepared the samples by drop coating diluted cephalexin bound gold nanoparticles solution on carbon-coated copper TEM grids. The results for various temperatures are presented in the Figures 4.12, 4.13, and 4.14, where the panels represent the typical morphologies at different length scales.

Figure 4.12. TEM micrographs of the gold nanoparticles synthesized at 15 °C (A and B represent images at different scales).

Figure 4.12A and B represents the particle morphology for the synthesis at 15 °C, where the reaction rate is quite slow. In this panel we can clearly see two different size ranges of particles. The big clusters (∼30 nm) are surrounded by a large number of smaller particles. The larger clusters (average diameter ∼ 30 nm) at this temperature seems to be a collection of smaller particles (∼1-3 nm) and show the
broad SPR peak and light purple color due to their non-spherical morphologies. The observation of two different size ranges is consistent with the DLS results. However, at 25 °C (Figure 4.13), the clusters of particles appear to fuse to form the particles of increasingly quasi-spherical geometrical as shown by a relatively lower intense longitudinal SPR peak and red color. At this temperature also the larger particles coexist with the smaller particles (1-2 nm). The particles though appear to be facetted and still contribute to the Near Infra-Red (NIR) peak observed in Figure 4.10B.

![Figure 4.13](image)

Figure 4.13. TEM micrographs of the gold nanoparticles synthesized at 25 °C (A and B represent images at different scales).

In Figure 4.14, we have shown the particles grown at 35 °C. In comparison to the particles grown at 15 and 25 °C, here the majority of the particles are spherical in shape due to faster reaction rate, which leads to the spherical shape and complete suppression of the longitudinal modes.14
We did not pursue synthesis at temperatures higher than 35 °C as it would degrade the antibiotic. To check the crystalline nature of the as-synthesized particles, we also took the snap-shots of the selected area electron diffraction (SAED) patterns of the gold nanoparticles at respective temperatures. The results are shown in Figure 4.15, which demonstrates nice spot and ring patterns for the particles confirming the nice crystalline structure.
Figure 4.15. Selected area electron diffraction (SAED) patterns of gold nanoparticles synthesized at temperatures 15 °C (A), 25 °C (B), and 35 °C (C), respectively, showing nice crystallinity in all the three cases confirming the formation of crystalline gold.
4.4. Conclusion

Overall, the TEM results in combination with light scattering and absorption studies exhibit a highly unusual and surprising nucleation and growth processes for gold nanoparticles synthesis mediated by the presence of cephalexin. In addition, these two nanoparticle populations reach their respective final sizes very rapidly and then cease growth altogether. At the same time, the total number of gold colloid continues to grow rapidly, and their rate of formation is a sensitive function of incubation temperature. It is intriguing to note that, at all the temperatures, we observe bimodal distribution of particles in a homogeneous system. These observations raise important fundamental questions relating to the nucleation and growth mechanisms. Additionally, this study shows the relative importance of absorption and scattering processes, and how these affect the data acquirements. This study can be very useful to determine and optimize some of the physical properties of nanoparticles by controlling their shape and size during and after a growth process.
4.5. References

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