Electronic Supplemental Information for:

Implications of citrate concentration during the seeded growth synthesis of gold nanoparticles

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S1. Calculation of ionic strength

Ionic strengths for the second generation gold nanoparticle solutions were calculated using the following equation:

$$\text{Ionic strength} = \mu = 0.5 \sum c_i z_i^2 \qquad (1)$$

where c_i is the concentration of the ith species and z_i is its charge.

The ion concentrations were determined using the following pertinent equilibria for sodium citrate in water:

$$H_3cit \leftrightarrow H_2cit^- + H^+ \qquad K_{a_1} = 7.44x10^{-4}$$

$$H_2cit^- \leftrightarrow Hcit^{2-} + H^+ \qquad K_{a_2} = 1.73x10^{-5}$$

$$Hcit^{2-} \leftrightarrow cit^{3-} + H^+ \qquad K_{a_3} = 4.02x10^{-7}$$

The concentration ratio between the predominant citrate species at the measured pH for each second generation sample was determined using the Henderson – Hasselbalch equation. Next, the concentration of the predominant citrate species was estimated by considering the total sodium citrate concentrations added (and diluted). The calculated concentrations and ion charges were used in equation 2.

When determining the concentrations for NH_3^+OH, Cl^-, Na^+, H^+ and OH^-, the following equilibria were considered.

$$NH_3^+OH \leftrightarrow NH_2OH + H^+ \quad K_a = 1.10x10^{-6}$$

The Henderson – Hasselbalch equation was used to calculate the concentration ratio between the acid and base forms of the molecule. The concentration of each species was then calculated using the total NH_3^+OH present in the second generation samples. Both the NH_3^+OH remaining after the reduction reaction in the first generation synthesis and NH_3^+OH added during the second generation were included. Total Cl^- concentration was calculated by accounting for the Cl^- from $HAuCl_4$ in the seeds and first generation synthesis and $NH_3^+OH{\cdot}HCl$ added in each step. This concentration of chloride remained constant at 1.90_2 mM for all of the samples studied. Total Na^+ concentration was determined by accounting for the sodium citrate added during each synthesis. Finally, the measured pH was used to calculate the H^+ concentration and the dissociation of water equilibrium was applied to estimate the OH^- concentration.

As a result, the ionic strength for the second generation samples is as follows:

$$\mu = 0.5[c_{H_3cit} \cdot z_{H_3cit}^2 + c_{H_2cit^-} \cdot z_{H_2cit^-}^2 + c_{Hcit^{2-}} \cdot z_{Hcit^{2-}}^2 + c_{cit^{3-}} \cdot z_{cit^{3-}}^2 + c_{NH_3^+OH} \cdot z_{NH_3^+OH}^2 + c_{Cl^-} \cdot z_{Cl^-}^2 + c_{Na^+} \cdot z_{Na^+}^2 + c_{H^+} \cdot z_{H^+}^2 + c_{OH^-} \cdot z_{OH^-}^2] \qquad (2)$$

Using equation 2, the ionic strength was calculated for the second generation samples (Table S1). Throughout nucleation and growth, no ions were removed (until optical and stability characterization was performed).

Table S1. Measured pH and calculated ionic strengths for the various nanoparticle samples.

Sample	[Citrate] (mM)	pH	Ionic Strength (mM)
1	0.10	3.47	2.07
2	0.30	4.31	2.45
3	0.50	4.64	3.05
4	0.70	4.97	3.77
5	0.90	5.13	4.46
6	1.10	5.55	5.34

S2. TEM and optical analysis

Table S2. TEM analysis of the minimum and maximum diameters measured for second generation nanoparticles grown in the presence of 0.10 – 1.10 mM citrate.

Sample	[Citrate] (mM)	Minimum Diameter (nm)	Maximum Diameter (nm)
1	0.10	27.6 ± 3.3 (12 %)	34.9 ± 6.6 (19 %)
2	0.30	28.2 ± 3.3 (12 %)	35.2 ± 5.6 (16 %)
3	0.50	27.3 ± 2.7 (10 %)	33.1 ± 4.0 (12 %)
4	0.70	28.2 ± 3.0 (11 %)	32.6 ± 3.3 (10 %)
5	0.90	28.4 ± 3.6 (12 %)	31.5 ± 3.7 (12 %)
6	1.10	29.4 ± 4.0 (14 %)	32.8 ± 4.1 (13 %)
Control*	0.10	29.0 ± 3.8 (13 %)	35.9 ± 6.3 (18 %)

* The control sample was grown in the presence of 0.10 mM citrate at pH 4.97.

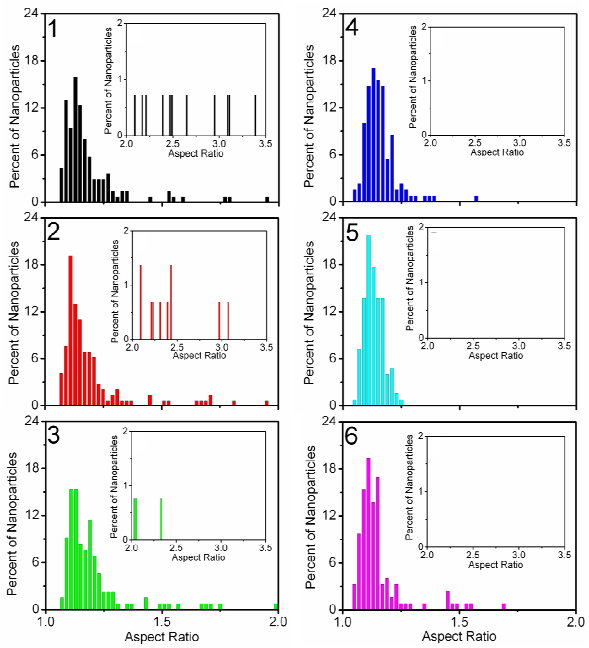

Figure S1. Histograms containing the aspect ratios for nanoparticles grown in the presence of 0.10, 0.30, 0.50, 0.70, 0.90, or 1.10 mM citrate for samples 1 – 6, respectively. The insets reveal the distribution of nanoparticles with an aspect ratio value greater than 2 which are (1) 8 %, (2) 7 %, (3) 2 %, (4) 0 %, (5) 0 %, and (6) 0%.

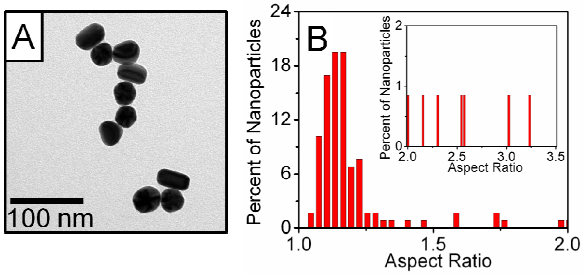

Figure S2. Characterization of the control sample. (A) Representative TEM image and (B) aspect ratio data (1.27 ± 0.37, N = 119) for the sample. The percent of nanoparticles with an aspect ratio greater than 2 is 6 %.

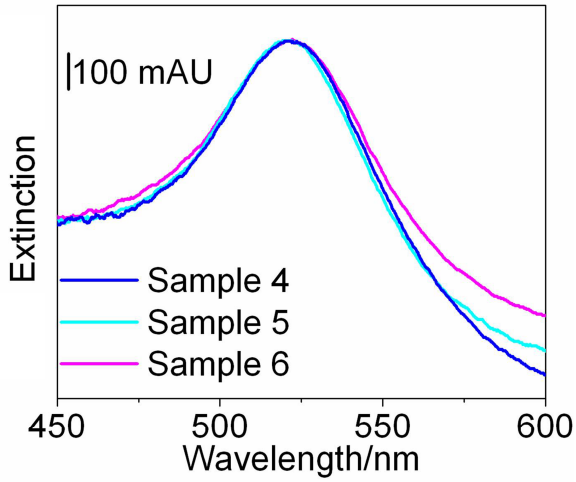

Figure S3. Extinction spectra for Au nanoparticles grown in the presence of (4) 0.70, (5) 0.90, (6) 1.10 mM citrate. Sample 4 (0.70 mM citrate): λ_{max} = 519.7 nm, Γ = 102.3 nm; Sample 5 (0.90 mM citrate): λ_{max} = 519.1 nm, Γ = 105.8 nm; Sample 6 (1.10 mM citrate): λ_{max} = 522.1 nm, Γ = 113.6 nm.

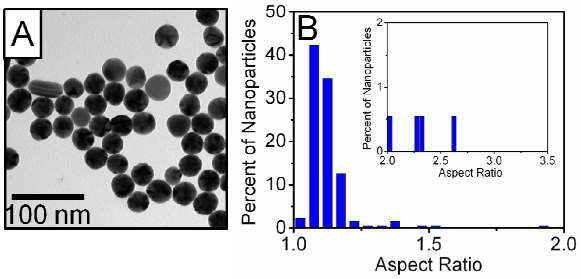

Figure S4. Characterization of second generation gold nanoparticles grown in the presence of 0.10 mM citrate after centrifuging at 2773 x g for 25 minutes. (A) Representative TEM image (d_{max} = 33.1 ± 3.8 nm, d_{min} = 29.0 ± 2.9 nm, d_{mean} = 31.0 ± 2.5 nm, N = 182) and (B) aspect ratio analysis (1.15 ± 0.20, N = 182) for this sample. The percent of nanoparticles with an aspect ratio greater than 2 is 2 %.

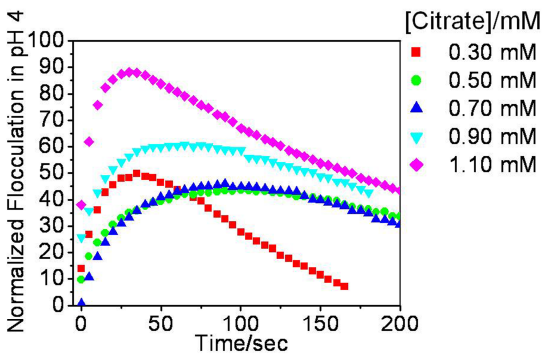

Figure S5. Normalized flocculation parameters for the various nanoparticle samples incubated in pH 4 buffer as a function of time.