Published in final edited form as:

J Phys Chem Lett. 2018 April 05; 9(7): 1516–1521. doi:10.1021/acs.jpclett.8b00327.

Electrophoretic Mechanism of $\text{Au}_{25}(\text{SR})_{18}$ Heating in Radiofrequency Fields

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Abstract

Gold nanoparticles in radiofrequency fields (RF) have been observed to heat. There is some debate over the mechanism of heating. $\text{Au}_{25}(\text{SR})_{18}$ in RF is studied for the mechanistic insights obtainable from precise synthetic control over exact charge, size, and spin for this nanoparticle. An electrophoretic mechanism can adequately account for the observed heat. This study adds a new level of understanding to gold particle heating experiments, allowing for the first time a conclusive connection between theoretical and experimentally observed heating rates.

Graphical Abstract

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Author Contributions

The manuscript was written through contributions of all authors.

Supporting Information

Experimental procedures, CE data, Heating data, Furgeson analysis (PDF)

The interaction of gold nanoparticles with radiofrequency radiation has been observed to generate heat. Applications of this heat include cancer hyperthermia,^{1–4} amyloid dissolution,^{5,6} aptamer control,⁷ and enzyme activation.^{8,9} Prompted by the promising applications of gold nanoparticle heat generation, the physical mechanisms by which nanomaterials may convert RF energy into thermal energy is an area of active inquiry.

Initial investigation into the observed heating rates of gold nanoparticle solutions in radiofrequency electric fields concluded that inductive currents caused joule heating of the electrons within the particle, resulting in a hot particle.^{10–12} Consistent with joule-heating was the observation that particles smaller than 50 nm in diameter heat at nearly twice the rate of larger diameter gold nanoparticles, due to the higher resistivity of the structures.¹² After several follow up reports, the validity of the joule heating mechanism was questioned experimentally and theoretically.^{13–16}

Two reports have concluded that observed heat could be attributed almost completely to background electrolyte resistive heating. They showed that AuNP resuspended in rigorously deionized water produce negligible observable heat or absorb negligible amounts of RF energy until protein corona formation increases the surface charge.^{17,18} An additional report suggesting that oxidation of Au₁₀₂(pMBA)₄₄ clusters results in paramagnetism. Such paramagnetic clusters could heat by a mechanism similar to superparamagnetic iron oxide particles.¹⁹ In general reproducing heating rates experimentally observed in different labs was difficult and confounded by differences in RF antenna design, field properties and sample container geometry. We recently published a critical review of gold nanoparticle heating.²⁰

An emerging consensus for mechanism of gold nanoparticle heating phenomena is that observed heating rates arise primarily from electrophoretic motion of charged particles in an alternating RF electric field. This is in addition to a small, likely negligible, contribution from the inductive heating of the particles.^{13,14,21} This electrophoretic motion of the particles results in inelastic collisions with the surrounding solvent molecules, producing measurable heat.

Heat that arises from electrophoretic motion of particles is proportional to the net charge of the nanoparticle (including any double-layer) and inversely proportional to the hydrodynamic (Stokes-Einstein) radius. It is independent of the core composition and the same mechanism that applies to all charged species, including salts, at these frequencies. Observed heat depends not only on the size and charge of the dissolved species, but also on the surrounding medium. Notably, the permittivity (and conductivity) of the solution, which are influenced by solvent structuring and ionic strength, have large effects on the ‘background heating’ rates of samples of nanoparticles in RF.^{22–24}

The heating rates of silica nanoparticles and gold nanoparticles in RF are similar,^{25,26} indicating that the composition of the particles does not substantially affect the observed heating rate. Still, a quantitative understanding of the heating phenomena is lacking. Uncertainties in assigning particle net charge and Stokes-Einstein radius may confound comparisons between experimentally observed and calculated heating rates.

Herein, we quantify the net charge and Stokes-Einstein radius of Au₂₅(SR)₁₈ nanoclusters (figure 1), and measure the heating rate of solutions of variants of this nanocluster in a 13.56 MHz radiofrequency field. The net charge of Au₂₅(SR)₁₈ clusters arises from a combination of the charge on the core (which is stable in +1, 0 and -1 oxidation states) and any charge arising from ionizable functional groups in the 18 ligands comprising the ligand shell.

Thus, net ionic charge of the cluster depends on the pK_as of the ionizable groups and the pH of the surrounding solution, as well as any charge shielding arising from co-dissolved ions. The ligands protecting Au₂₅(SR)₁₈ in this study are either the tripeptide glutathione (γ-glutamyl cysteinyl glycine, abbreviated GSH) or phenylethanethiol (PET). Both ligands are shown schematically in figure 1. GSH ligation allows the quantification of ligand net charge on observed heat, whereas PET ligated clusters allow quantification of core net charge and any paramagnetic contributions to observed heat.

Each GSH ligand contains 3 ionizable functional groups: two carboxylic acids and the N-terminus of the peptide, with approximate pK_as of pK₁ = 2.12 (COOH), pK₂ = 3.59 (COOH), pK₃ = 8.75 (NH₂).²⁷ When ligated to a cluster, the dense packing of ionizable groups modifies their pK_a.^{28,29} For instance, the pK_a of the carboxylic acid group of p-mercaptobenzoic acid increases by 2 pK_a units when incorporated in the monolayer of Au₁₀₂(pMBA)₄₄.²⁹

Because of the difficulty in predicting the magnitude of the effects of altered ligand pK_a and any counterion shielding, we experimentally determined the net effective charge on the clusters as a function of pH. Experimentally, to ascertain the effective charge of the Au₂₅(GSH)₁₈ particles, we determined their electrophoretic mobility and Stokes-Einstein radius. These properties are related to effective charge by the Stokes-Einstein relation:

$$\mu_{EP} = \frac{q}{6\pi\eta a} \quad (1)$$

Where μ_{EP} is the electrophoretic mobility in m²V⁻¹s⁻¹ and a is the radius q is the particle charge and η is the viscosity of the solution.

We determined the electrophoretic mobility of Au₂₅(GSH)₁₈ as a function of pH by capillary electrophoresis. Figure 2, left panel, shows this at pH values 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. In all cases, buffer concentrations were 10 mM. Different buffers were used to buffer at each pH, and this leads to differing values of ionic strength and conductivity (SI Table S1). The deviations in viscosity from deionized H₂O were assumed to be negligible for the different buffer conditions due to all of them being rather dilute (10 mM). Ferguson analysis of the mobility³⁰ of Au₂₅(SG)₁₈ clusters in polyacrylamide gel electrophoresis at pH 8.3 is similar to the high pH CE results at 2.2*10⁻⁴ in cm² V⁻¹s⁻¹ (see SI Table S3).

We determined a hydrodynamic radius value of 1.65 nm by dynamic light scattering (Zetasizer Nano ZS, Malvern, see SI Figures S3 and S4). This value is in good agreement with literature values³¹ and theoretical calculations using hydro pro and the theoretical structure of Akola.³² We could not observe differences in hydrodynamic diameter at

different pH values within the resolution of this experiment. For particles with a larger or more compressible ligand shell, pH and ionic strength may underlie dramatic changes in observed Stoke-Einstein radius.³³ If the protecting ligand layer is a flexible polymer such as polyethylene glycol (PEG), then the compression of the double layer can further change the hydrodynamic radius by allowing for compression or expansion of the ligand layer as well as the surrounding double layer. This can result in drastic changes in hydrodynamic radius for some particles.³⁴ For instance, in deionized H₂O, the Stokes-Einstein radius of particles may become much larger, and this effective increase in size may explain why gold nanoparticles show less heating in deionized water compared to solutions that are not rigorously deionized.

Using the measured electrophoretic mobilities and Stokes-Einstein radius, we calculate using equation 1 the charges on the clusters as -0.35 , -5.87 , -6.76 , and -7.43 in units of electron charge for pHs 3, 5, 7, and 9 respectively. This is significantly less than the net charges assumed from estimates based on the pK_a of the ligands at those pHs, where net charges ranging from -4.6 at pH 3 to -36 at pH 9 are expected (see SI Table S2). To ascertain the relationship between heat evolved by clusters in RF fields and the net charge on the cluster, we measured the heating rates of Au₂₅(GSH)₁₈ at the same pH/ionic strength as used in the experiments for charge determination in a custom-built RF generator. This instrument, operating at 13.56 MHz under 50W power is fully described in a prior publication.¹⁹ We observe a clear dependence of heating rate on net charge, as shown in figure 2, right panel.

We found that modelling the heat produced by the Au₂₅(SR)₁₈/counterion/buffer solutions in the electrophoretic heating model proposed by Sassaroli could account well for the observed heating rates.¹⁴ In the Sassaroli model, the absorption of power as a result of electrophoretic movement is described by the imaginary component of the dielectric constant for the particles.¹⁴ The power absorption due to dielectric loss is quantified by the following:

$$P = \frac{\phi}{\frac{4\pi}{3}a^3} \frac{E^2 q^2}{\beta} \left(\frac{1}{1 + \delta^2 \omega^2} \right) \quad (2)$$

Where $\delta = \frac{2\rho a^2}{3\eta}$ and $\beta = 6\pi\eta a$. Here q is the particle charge, a is the hydrodynamic radius of the particle, η is the dynamic shear viscosity of the solvent, E is the electric field strength, ρ is the particle density, and ϕ is the volume fraction of the particles.

The electric field strength needed in this equation is difficult to measure accurately. For instance, it is unclear whether a measured field strength in an ‘empty antenna’ is representative of the field strength inside the solution/sample containing vessel that is inside the antenna.^{15,22} (In other words, the shape of the sample container can modify the electric field strength in ways that are difficult to predict or measure.) Therefore, instead of attempting to measure ‘specific absorption rates’ (which would require a meaningful measurement of electric field strength) we instead compared normalized heating rates, relative to the highest power-absorbing sample. This makes the heating rate independent of

the field strength. This approach is especially useful as it allows ratiometric comparison of experimental to calculated heating rates.

Figure 2, right panel, shows the theoretical heating rates for $\text{Au}_{25}(\text{SG})_{18}$, measured at 4 different pH (e.g. particle charge) values. Theoretical heating rates are shown as hatched bars, just adjacent to the experimental heating rates (solid bars). We observe theoretical agreement within error, suggesting that observed, modest heating rates can be completely accounted for in these experiments by the Sassaroli proposed electrophoretic mechanisms. This mechanism is also in agreement with the results of Suchalkin in that they didn't observe any significant RF absorption until the surface charge of the particle was significantly increased via corona formation.¹⁸ Additionally the mechanism shows a potential reason that groups observe attenuated RF heating after dialysis;^{17,18} the hydrodynamic radius increases when ionic strength is reduced,³³ resulting in a lower charge to hydrodynamic volume ratio and attenuated electrophoretic motion.

In addition to studying the water-soluble $\text{Au}_{25}(\text{SG})_{18}$ cluster, we also studied the organosoluble $\text{Au}_{25}(\text{PET})_{18}$ cluster. This cluster is a useful point of study for examining the effects of not only charge, but also paramagnetism/diamagnetism and solvent polarity. Solvent polarity is a useful parameter in understanding heating mechanisms, because nonpolar solvents such as toluene do not stabilize ions effectively and should quench heating mechanisms that depend on ionic movements. This is in comparison to polar organic solvents, such as dichloromethane (DCM) which, like water, promote ionization and facilitate ionic heating mechanisms.

Bulk electrolysis was used, as we have previously, to set the oxidation state of $\text{Au}_{25}(\text{PET})_{18}$ to -1, 0 or +1 core charge states.³⁵⁻³⁷ These oxidation states correspond to diamagnetic, paramagnetic and diamagnetic compounds (shown schematically in figure 3, right column), as we and others have previously reported.³⁷⁻⁴²

We measured heating response for these cluster preparations in all three oxidation states and in polar and nonpolar solvents. Figure 3 shows heating rates of these preparations.

Essentially no thermal response is observed when the clusters are uncharged, but paramagnetic (green trace). Similarly, no thermal response is observed in the nonpolar solvent toluene, for both positively charged (purple trace), negatively charged, and uncharged clusters (data not shown). Conversely, a thermal response is observed for both positively and negatively charged clusters when dichloromethane is used as a solvent (blue trace, red trace).

The inset in figure 3 shows the theoretical fraction of observed heat attributable to the cluster (yellow bar) and the counterion (red orange bar) as calculated with equation 2. The observed heat from a cluster of unitary charge is identical, independent of whether the charge is +1 or -1. We attribute the difference in heating rates observed for the positive and negatively charged clusters entirely to the size of the counterion used in each experiment.

Previously, we studied the effect of the charge on the gold core of the $\text{Au}_{102}(\text{SR})_{44}$ cluster, which may be a proxy for paramagnetism of the cluster.¹⁹ For that cluster we observed

heating that appeared to depend on whether the cluster was paramagnetic or not. Due to the technical difficulties of working in water, we could not assign an exact oxidation or spin state to these clusters. For the organosoluble preparations studied here, where we can assign an exact spin state, we see no evidence for a magnetic heating mechanism. For $\text{Au}_{25}(\text{SR})_{18}$, heat is completely attributable to electrophoretic mechanisms.

In conclusion, heating mechanisms for inorganic nanoparticles in RF irradiation are a subject of current investigation. For the $\text{Au}_{25}(\text{SR})_{18}$ cluster, which allows mechanistic investigation through alteration of net charge, magnetic moment and solvation environment, we see only evidence for electrophoretic heating mechanisms. We modified the net charge of the particles by chemical modification of the ligand layer, changing the pH and bulk electrolysis to charge the metallic core of the particle. Overall, we conclude that in a variety of preparations, only electrophoretic mechanisms contribute to heat evolution from $\text{Au}_{25}(\text{SR})_{18}$ clusters.

Experimental Methods

Particle synthesis

$\text{Au}_{25}(\text{PET})_{18}$ particles were synthesized and purified by crystallization in the various charge states, exactly as previously reported.³⁷ The crystallized particles were thoroughly washed with toluene to remove excess salts. $\text{Au}_{25}(\text{GS})_{18}$ was synthesized as previously reported.⁴³ Fractional precipitation was used to clean up the particles prior to purification by PAGE. This allowed more material to be loaded on each gel. After gel purification the particles were precipitated with 7:1 methanol to DI H_2O and washed 3x to rinse away any excess salt. The same batch of particles was used for all heating and electrophoresis studies to ensure that any additional salt remaining after washing would be consistent for all samples allowing for an accurate comparison between different pH buffers.

Ferguson analysis

Mobility of $\text{Au}_{25}(\text{SG})_{18}$ in 1x TBE (pH is 8.3) was determined with gel electrophoresis by measuring the distance the band traveled (retention factor) as a function of gel percentage. To get a precise measurement of the distance, a gel imager was used to ensure precisely the same angle and height the picture was taken from then imageJ was used and calibrated to the size of the gel. Distances of the bands were measured from the start of the well to the darkest part (highest intensity in imageJ) of the band (corresponding to the most cluster), and band width was incorporated as error bars in the mobilities plotted. The linear fit of the cluster was then used to extrapolate the free mobility of the clusters. The mobility measured by gel is in good agreement with the mobility found using capillary electrophoresis.

Capillary electrophoresis: experiments were performed and an HP 61600AX 3DCE, using a 50 μm , 33 cm (24.5cm to the window) fused silica capillary treated with NaOH. It was run with positive polarity with an applied voltage of 15 kV and the temperature of the capillary was held constant at 25°C. Uv-vis absorption at 214, 254 and 280 nm was collected using chemstation software Rev A.08.03. Caffeine was used as a neutral electro osmotic flow indicator. Raw data from the absorbance at 280nm can be seen below bellow. Data was then

converted to the mobility domain to better visualize the effect of the pH of the background electrolytes on the electrophoretic mobility of the clusters.

Radiofrequency heating experiments

2mL samples were contained in 10mm NMR tube. They were heated in a custom constructed water cooled hollow copper solenoid powered by a 100W amplifier (ifi scx100) and a function generator (Philips PM5192) set to generate a sinusoidal current at 13.56 MHz. An impedance matching network was used and forward voltage from the amplifier was monitored and set at 50W. The temperature of the bulk solution was measure with a fiber optic temperature probe (Neoptix Nomad).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this research was provided by the NIH 5R01GM112225 We thank Dr George Collins (Colorado State University, Electrical Engineering) for help with our heating set up

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Figure 1. $\text{Au}_{25}(\text{SR})_{18}$ crystal structure with gold atoms orange, sulfur in yellow and R groups in green. Below are the ligands PET and glutathione ligand used for organic and water solubility respectively.

Figure 2.

The left absorbance at 280nm from capillary electrophoresis plotted as mobility of the particles in buffers ranging from pH 9 to pH 3. The right is the background subtracted RF heating rates measured in the same buffers with pH 3, pH 5, pH 7 and pH 9. Calculated, normalized heating rates are shown as dashed columns next to the observed heating rates. The calculated charge on the cluster is shown next to each.

Figure 3.

Heating rates as a function of charge state and solvent. Blue trace is +1 in DCM, red is -1 in DCM, green is 0 in DCM and purple is +1 in toluene. The inset shows normalized calculated heating rates of the +1 and -1 charge states next to the normalized measured heating rates in blue and red respectively. The orange color indicates heat from the counter ions, PF₆⁻ and TOA⁺ and the yellow color indicates heat from the cluster's electrophoretic movement.