Layer-by-Layer Modification of High Surface Curvature Nanoparticles with Weak Polyelectrolytes using a Multiphase Solvent Precipitation Process

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Abstract

The layer-by-layer modification of ≈ 5 nm mercaptocarboxylic acid stabilized gold nanoparticles was studied in an effort to illustrate effective means to overcome practical issues in handling and performing surface modification of such extremely small materials. To accomplish this, each layer deposition cycle was separated into a multi-step process wherein solution pH was controlled in two distinct phases of polyelectrolyte adsorption and centrifugation. Additionally, a solvent precipitation step was introduced to make processing more amenable by concentrating the sample and exchanging solution pH before ultracentrifugation. The pH-dependent assembly on gold nanoparticles was assessed after each layer deposition cycle by monitoring the plasmon peak absorbance location, surface charge, and the percentage of nanoparticles recovered. The selection of solution pH during the adsorption phase was found to be a critical parameter to enhance particle recovery and maximize surface charge when coating with weak polyelectrolytes. One bilayer was deposited with a high yield and the modified particles exhibited enhanced colloidal stability across a broad pH range and increased ionic strength. These findings support the adoption of this multi-step processing approach as an effective and generalizable approach to improve stability of high surface curvature particles.

Introduction

Nanomaterials exhibit unique size, shape, and surface dependent properties that can be tailored for the development of nanotherapeutic and diagnostic devices.¹⁻⁵ A subset of such nanomaterials are inorganic in nature; these nanoparticle (NP) colloids are typically synthesized in organic solvents as a variety of core and core/shell nanomaterials that require further surface modification for stabilization, functionalization, and transfer to aqueous conditions.⁶ Facilitating nanomaterial interaction with biological media requires passivation of the inorganic core with an organic shell to prevent degradation and aggregation that would otherwise compromise the nanomaterial physiochemical properties, leading to undesirable nanotoxicological effects.⁷ Numerous surface modification techniques currently exist with varying tradeoffs between method complexity, resulting colloidal stability, chemical reactivity, and coating thickness.⁸ Modification with charged polymers is of particular interest due to the combination of a steric barrier and electrostatic repulsion that provides a superior electrosteric stabilization.⁹ Layer-by-layer (LbL) is recognized as a low-cost and versatile nanofabrication technique to modify a broad range of templates with a diverse array of materials to produce tunable, responsive, and multifunctional devices.¹⁰⁻¹³

The application of LbL has been thoroughly investigated on planar substrates for the hierarchical assembly of multifunctional films for an assortment of applications¹⁴⁻¹⁶ and was easily adapted for microparticle templates by exchanging the rinsing steps with multiple centrifugation/dispersion cycles.¹⁷ However, translating this process to NP templates is significantly more challenging due to the added restrictions to prevent aggregation and becomes especially difficult for NPs smaller than 30 nm.¹⁸⁻³⁰ For very small NPs, the high surface curvature resists wrapping by rigid polyelectrolytes (PEs), with both theoretical and empirical work demonstrating that polyelectrolyte chain length should be approximately equal to NP

circumference to promote stable wrapping.^{18-19, 31-32} Schneider and Decher contributed significantly to this area by finding the optimal conditions to prevent flocculation by mainly controlling stoichiometry between a dilute gold NP (AuNP) concentration and a large polyelectrolyte excess; demonstrating a correlation between the aggregation state of AuNPs viewed by TEM and the plasmon peak location of the UV-Vis absorbance.²⁰⁻²¹ We adapted this technique for coating dihydrolipoic acid modified quantum dots, but found that controlling stoichiometry alone was not sufficient to prevent aggregation and successful coating required careful control of solution pH throughout the process.³³ Solution pH is recognized as a critical parameter for controlling deposition thickness and morphology of the weak PEs poly (allylamine hydrochloride) (PAH) and poly (acrylic acid) (PAA) on planar,³⁴⁻⁴⁰ microparticle,⁴¹⁻⁴⁵ and NP⁴⁶⁻⁴⁸ templates. However, there has been no systematic investigation of the effect of solution pH on the interaction of weak PEs and high surface curvature NPs less than 10 nm.³⁰

Mayya and coworkers studied the coating of 7 nm AuNPs with strong PEs, poly (diallyldimethylammonium chloride) (PDADMAC) and poly (sodium-4-styrenesulfonate) (PSS), maintaining solution at pH 9 throughout the LbL process to ensure that the initial AuNP surface ligand remained fully ionized.²³ The effect of solution pH was not investigated and only strong PEs were used. Dorris and coworkers studied how pH affected the interaction of 5 nm cationic AuNPs with PDADMAC or PAH and PSS in terms of retention of the initial stabilizing ligand, but the study was limited to a single bilayer due to limited NP recovery.¹⁹ Both of these publications considered the importance of choosing the correct solution pH in relation to the NP charge density, but the influence of polyelectrolyte charge density was not investigated. Furthermore, they did not address the resulting colloidal stability of the modified NPs.

Strong PEs maintain a high charge density over a broad pH range that enhances electrostatic stability and limits film reorganization. However, coating of high surface curvature NPs with strong PEs requires an increase in ionic strength to reduce chain rigidity,³¹⁻³² which simultaneously reduces NP surface potential, leading to flocculation if the concentration is not kept fairly low. In contrast, the advantage of PEs containing weak acid/base moieties arises from the ability to manipulate their linear charge density and conformation through solution pH. NPs coated with weak PEs exhibit pH dependent properties that can be exploited as stimuli responsive triggers but this also renders them susceptible to aggregation under pH extremes. Poly (4-styrenesulfonic acid-*co*-maleic acid) (PSS-*co*-MA) is an inexpensive, low molecular weight, commercially available, and high charge density weak-strong random copolymer of styrene sulfonate (SS) and maleic acid (MA). The deposition of this weak-strong copolymer can be tuned by controlling the MA ionization but shows more resistance to post exposure film reorganization because of the highly charged SS groups.⁴⁹⁻⁵³ Using this copolymer for NP templates is expected to impart better colloidal stability for these same reasons, but to our knowledge this has not been reported on high surface curvature NPs. In this work, we explore the process for coating high surface curvature AuNPs with weak and weak-strong PEs with an emphasis on the effect of solution pH; assessing the polyelectrolyte modified AuNPs for aggregation, surface charge and colloidal stability.

Materials and Methods

Poly (allylamine hydrochloride) (PAH) ($M_w = 15000 \text{ g} \cdot \text{mol}^{-1}$), poly (acrylic acid) (PAA) ($M_w = 15000 \text{ g} \cdot \text{mol}^{-1}$), poly (4-styrenesulfonic acid-*co*-maleic acid) sodium salt (PSS-*co*-MA) ($M_w = 20000 \text{ g} \cdot \text{mol}^{-1}$), 11-mercaptoundecanoic acid (MUA) (98% or 95%), 3-mercaptopropionic acid (MPA) (99%) , gold (III) chloride trihydrate (99.9%), tetraoctylammonium bromide (98%) and sodium borohydride (99%) were all obtained from Sigma Aldrich.

AuNP synthesis: The Brust-Schiffrin method was utilized to synthesize monodisperse gold nanoparticles (AuNPs).^{23, 54-55} 3 mL of aqueous gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 25.4 mM) was transferred into 8 mL of toluene by adding 25 mM of tetraoctylammonium bromide followed by cleaning three times of deionized (DI) H_2O . The cleaned gold salt in toluene phase was reduced by adding 1 mL of sodium borohydride aqueous solution (793 mM) under vigorous stirring. The reduced gold salt phase was washed with 0.1 M HCl, 0.1 M NaOH and DI- H_2O . 1 mL of MUA in toluene (2.29 M) was prepared at 60°C and then added slowly into the preheated AuNP solution (at 60°C) causing precipitation. The black precipitated MUA modified AuNPs were gathered and cleaned with toluene to eliminate excessive MUA. The final precipitates were suspended in 50 mM TRIS buffer. The same procedure was used for producing MPA modified AuNPs. AuNP concentration was determined spectrally⁵⁶ and reported in nM calculated as number of nanoparticles per liter. Approximate size was confirmed by TEM for both AuNP_{MUA} (Figure S1) and AuNP_{MPA} (Figure S2).

PAH/PAA LbL: PAH was dissolved in 50 mM TRIS buffer at pH 7.2 or pH 9 and PAA was dissolved in 50 mM sodium acetate buffer at pH 4.5 or 50 mM TRIS buffer at pH 7.2; some additional titration was required with NaOH/HCl. The polyelectrolyte solutions were then sonicated for 1 h. A Branson 2510 bath ultrasonic cleaner was used for all sonication steps. A 5

mL solution of 12 nM AuNP_{MUA} in TRIS buffer was rapidly added to 5 mL of PAH in a 50 mL conical tube under sonication for 10 min and incubated with mild shaking for at least 1 h. The solution was precipitated by the addition of isopropanol (2:1 V/V) and centrifuged at 10 000 RCF at room temperature for 10 min. For PAH at pH 9, TRIS HCl was added at 60 mg/mL before the addition of isopropanol. The supernatant was decanted, leaving a gelatinous pellet that was resuspended in 1 mL of pH 7.2 50 mM TRIS buffer. Excess PAH was removed by three rounds of ultracentrifugation at 180 000 RCF (2 h, 1 h, and 30 min respectively). A 5 mL, 12 nM solution of PAH coated AuNPs was added to the PAA solution under sonication for 10 min and incubated with mild shaking for 1 h. The solution was precipitated by the addition of isopropanol (2:1 V/V) and centrifuged at 10 000 RCF at room temperature for 10 min. Excess PAA is removed by three rounds of ultracentrifugation at 180 000 RCF (2 h, 1 h, and 30 min respectively).

PAH/PSS-co-MA LbL: PSS-co-MA was dissolved in DI-H₂O, sonicated for 1 h, and was titrated to pH 2 with HCl. A 5 mL solution of 12 nM PAH coated AuNPs (produced using the buffer method mentioned previously) in DI-H₂O was rapidly added to 5 mL of PSS-co-MA in a 50 mL conical tube under sonication for 10 min and incubated with mild shaking for 1 h. The solution was titrated with NaOH to pH 9 and precipitated by the addition of isopropanol (2:1 V/V) and centrifuged at 10,000 RCF at room temperature for 30 min. The supernatant was decanted leaving a gelatinous pellet that was resuspended in 1 mL of pH 9, 50 mM TRIS buffer. Excess PSS-co-MA is removed by three rounds of ultracentrifugation at 180,000 RCF (2 h, 1 h, and 30 min respectively).

Microelectrophoresis: The zeta potential of AuNPs were determined with a ZetaSizer Nano Series ZEN 3600 spectrometer (Malvern). AuNPs (6-10 nM) were dispersed in 1 mM TRIS in a

DT1070 cuvette and measured 3 times. For pH dependent measurements the MPT-2 autotitrator accessory (Malvern) and pH probe (Malvern) were used. A 10 mL, 10 nM AuNP solution in DI-H₂O was titrated with either 0.1 M NaOH or 0.1 M HCl at 0.5 pH increments. The 10 mL AuNP solution was sampled 4 times for measurement at each pH increment. The electrophoretic mobility was converted to zeta potential using the Smoluchowski relation and reported as raw data.

UV-Vis: Absorbance spectra were obtained on a Cary 300 UV-Vis spectrophotometer (Agilent) with a 6x6 multi-cell Peltier block (Agilent). Measurements were acquired at 300 nm/min, 0.5 nm resolution, and 0.1 s integration time. Samples were measured in a semi-micro PMMA cuvette and baseline corrected with a cuvette filled with buffer. Raw spectra were filtered with a 25 wide median filter to remove noise.

TEM Analysis: 10 μ L of the AuNP stock solution for analysis was dropped onto a commercially-available carbon film coated-300 square mesh copper grid (CF-300 Cu, Electron Microscopy Sciences, Hatfield, PA) followed by 24 h of atmosphere drying. The AuNPs were examined by TEM (HF3300, Hitachi Scientific Instruments, Tokyo, Japan) operated at 300 kV with a cold field emission gun (FEG).

Results & Discussion

Scheme 1: (A) Mercaptocarboxylic acid stabilized AuNPs after the addition of a (B) cationic polyelectrolyte followed by an (C) anionic polyelectrolyte. (D) Multilayer coated AuNP after repeating steps B and C several times.

Figure 1: The LbL experimental process shown visually: (A) AuNP_{MUA} are mixed with excess polyelectrolyte, (B) solvent precipitation by the addition of isopropanol, (C) sedimentation of polyelectrolyte and AuNPs by centrifugation, (D) resuspension in buffer, (E) ultracentrifugation, (F) decanting of supernatant and resuspension in buffer.

The basic process for LbL modifying AuNPs is depicted in Scheme 1. AuNPs are synthesized with a stabilizing ligand, which is commonly a mercaptocarboxylic acid (Scheme 1A), followed by coating with a cationic polyelectrolyte (Scheme 1B) and then an anionic polyelectrolyte (Scheme 1C) with multiple centrifugation/redispersion cycles between each step. This process can theoretically be repeated indefinitely to yield a multilayer coated particle with a desired thickness and terminal coating (Scheme 1D). The procedure we developed for NP LbL splits each layer deposition cycle into a multistep process. Initially, the MUA stabilized AuNP template is mixed with the oppositely charged polyelectrolyte (Figure 1A) at the adsorption pH.

The adsorption pH is selected to maximize the surface charge density of template AuNP but can be varied around the polyelectrolyte pKa to tune the polyelectrolyte charge density. Using a modification of a controlled precipitation technique,⁵⁷ the mixture was precipitated by the addition of isopropanol (Figure 1B) and centrifuged to completely sediment both coated AuNPs and excess polyelectrolyte into a gelatinous pellet (Figure 1C). The solvent precipitation (SP) step serves to make sample processing more amenable by concentrating the sample volume ($\approx 10:1$) and changing suspension media from the adsorption pH to the centrifugation pH before ultracentrifugation. The sediment is resuspended in a small volume of buffer at the centrifugation pH (Figure 1D) and ultracentrifuged to sediment the coated AuNPs (Figure 1E). Since the high speed of ultracentrifugation can often lead to complete aggregation of NPs that have a low surface charge, it is imperative that the centrifugation pH be selected to maximize the charge density of the terminal polyelectrolyte. The supernatant containing the excess polyelectrolyte is decanted and the AuNP sediment is resuspended in buffer (Figure 1F). The ultracentrifugation and supernatant decanting is repeated twice more to ensure removal of excess polyelectrolyte. NP stability can be visually assessed after centrifugation steps (Figure 1C and Figure 1E) as we found that stable NPs always form a sediment at the bottom of the tube that can be easily redispersed without sonication. The sample can then be analyzed or used for deposition of the next layer following the same process, while exchanging the adsorption pH and centrifugation pH to correspond to the next polyelectrolyte layer. For brevity, the adsorption pH and centrifugation pH for each polyelectrolyte layer is denoted by a superscript and subscript respectively (polyelectrolyte^{Adsorption pH}_{Centrifugation pH}).

Figure 2: (A) Plasmon peak absorbance location, (B) plasmon peak absorbance increase relative to the absorbance at 450 nm and (C) microelectrophoresis measurements after each layer deposition cycle for AuNP_{MUA}-(PAH_{7.2}^{7.2}/PAA_{7.2}^{7.2}) multilayers (blue □) and AuNP_{MUA}-(PAH_{7.2}⁹/PAA_{7.2}^{4.5}) multilayers (red ○). (D) Percent recovery of AuNPs per layer (blue bars) and cumulative (blue --□--) for AuNP_{MUA}-(PAH_{7.2}^{7.2}/PAA_{7.2}^{7.2}) and per layer (red bars) and cumulative (red --○--) for AuNP_{MUA}-(PAH_{7.2}⁹/PAA_{7.2}^{4.5}). For AuNP_{MUA} the percent recovery represents three rounds of ultracentrifugation only and no solvent precipitation. Error bars represent standard deviations of three different samples (n = 3).

Scheme 2: Two different scenarios for pH dependent LbL: (A) polyelectrolyte is fully ionized and rigid resulting in thin films of linear polyelectrolyte, (B) polyelectrolyte is partially/weakly ionized and randomly coiled leading to thicker films of coiled polyelectrolyte.

The AuNP_{MUA} was synthesized using procedures described in the literature.^{23, 54-55} The resulting average diameter was determined spectrally⁵⁶ to be approximately 5 nm and was confirmed by TEM (Figure S1). Adsorption of the first cationic polyelectrolyte layer onto the anionic AuNP must occur above the surface group's pK_a (≈ 4.5) and near or below the pK_a of the cationic polyelectrolyte. Two cases for polyelectrolyte ionization shown in Scheme 2 were tested to understand how the choice of pH affects polyelectrolyte adsorption. Deposition at a pH where the polyelectrolyte is expected to be more strongly ionized and rigid, leading to thinner film growth (Scheme 2A), and at a pH where the polyelectrolyte is weakly ionized and coiled, resulting in thicker film growth (Scheme 2B).³⁹ Experimentally, the weakly ionized case involved deposition of PAH (pK_a $\approx 8 - 9$)³⁶ at pH 9 and PAA (pK_a $\approx 4.5 - 6.5$)^{36, 38} at pH 4.5, while the strongly ionized case used pH 7.2 for both PAH and PAA; in both the weakly and strongly ionized cases the centrifugation was at pH 7.2.

AuNPs are used as a model template because their aggregation state can be determined by monitoring changes in the plasmon peak location, which correlates to the aggregation state observed by TEM.²⁰⁻²¹ When AuNPs aggregate, interparticle coupling produces larger redshifts in the plasmon peak location, while polymer adsorption without aggregation produces dielectric changes manifested as smaller redshifts.²⁰⁻²¹ The magnitude of these redshifts can vary depending on AuNP properties (size, concentration, stabilizing ligand, *etc.*) limiting direct comparison of our results to those of others. However, by measuring the relative change in plasmon peak absorption location after each layer deposition cycle, the conditions for preventing aggregation during this pH dependent LbL can be explored. For the weakly ionized case an

approximate 4 nm redshift occurs in the plasmon peak absorbance location after the deposition of the first PAH layer, followed by redshifts of less than 1 nm for subsequent layers (Figure 2A). For the strongly ionized polyelectrolyte case, slightly smaller redshifts are observed for the first bilayer but deposition of the third layer causes a larger redshift in plasmon peak absorbance location followed by a sharp blueshift (Figure 2A). For the weakly ionized conditions plasmon peak absorbance intensity follows the same trend as plasmon peak absorbance location but for the strongly ionized case a sharp decrease in plasmon peak intensity after deposition of the 4th layer (Figure 2B). The blueshift at the 4th layer indicates some change in the surface dielectric constant, which could be either removal of the previous PAH layer or some rearrangement of the multilayer structure. From the raw UV-Vis spectra (Figure S3) there is an increase in scattering that is contributing to this blue shift. The scattering is not likely significant aggregation because there are no secondary peaks or broadening in the raw spectra to indicate strong interparticle coupling (Figure S3). It is also not clear why the plasmon peak absorbance intensity decreases at the 4th deposition stage, but we suspect it is due to the pH effects causing a change in the multilayer structure. The microelectrophoresis measurements for the weakly ionized case show a large magnitude of charge reversal greater than 40 mV after the addition of both PAH and PAA, but a decrease in magnitude of 5 mV between the first and last layer of PAH (Figure 2C). For the strongly ionized charge reversal is 4 – 12 mV less than the weakly ionized case for PAH and PAA layers (Figure 2C). The high charge reversal in all cases is indicative of high colloidal stability.

AuNP concentration and percent recovery was determined after each layer deposition cycle by the absorbance at 450 nm.⁵⁶ The cumulative percent recovery represents the accumulating loss of the multilayer process. Overall, the percent recovery for each layer and the

cumulative percent recovery are lower for the strongly ionized case compared to the weakly ionized case (Figure 2D). After the deposition of 4 layers only 13.5% of AuNPs remained for the strongly ionized case compared to 35% recovery for the weakly ionized case (Figure 2D). We did not continue past layer 4 for the strongly ionized case due to this low recovery. For the weakly-ionized case, an average recovery of 78% per layer is achieved, which is lower than the reported 94%²⁰ recovery for 13.5 nm AuNPs; however, it is much higher than the 30%¹⁹ reported for similarly-sized 5 nm AuNPs.

The loss of AuNPs can be attributed to the combination of several factors: (1) transfer loss between the adsorption, solvent precipitation and ultracentrifugation steps; (2) accidental discarding during supernatant removal after each ultracentrifugation cycle; (3) unstable particles that cannot be recovered after ultracentrifugation. The first two factors can be mitigated by careful handling and represent only a small percentage of the lost AuNPs ($\approx 2.2\%$ for AuNP_{MUA}) (Figure 2D). The third factor represents AuNPs that become unstable because of inadequate polyelectrolyte coating or are ultracentrifuged under improper pH conditions. These unstable AuNPs become strongly immobilized on the tube wall or form a tightly packed pellet that is not redispersible by vortexing or sonication. In contrast, when properly coated AuNPs are ultracentrifuged they form sediment on the bottom of the tube (Figure 1E) that is easily redispersed by pipette aspiration. For these reasons ultracentrifugation can be used to qualitatively assess AuNP stability, while also serving to purify the sample of poorly coated AuNPs. The much higher loss in the strongly ionized case indicates that the pH conditions used are leading to a higher percentage of unstable AuNPs that are being excluded during ultracentrifugation. The weakly ionized case is able to provide very high stability even after only a single layer for two possible reasons: (1) charge overcompensation by a more weakly ionized

polyelectrolyte on the AuNP template requires more polyelectrolyte chains to adsorb, leading to a more homogenous coating of the particle surface⁴⁷ and/or (2) the coiled conformation of the weak polyelectrolyte provides a more effective steric barrier.²⁸

Figure 3: Zeta potential measured as a function of pH for (A) AuNP_{MUA} (red ○), AuNP_{MUA}-(PAH_{7.2}/PAA_{4.5})₁ (black ◇) and (B) AuNP_{MUA}-(PAH_{7.2}) (grey □), AuNP_{MUA}-(PAH_{7.2}/PAA_{4.5})_{1.5} (blue △). Error bars represent standard deviations (n = 4).

To understand the pH dependent surface potential and electrostatic stability the zeta potential as a function of pH was measured starting from pH 9 (where surface charge and particle stability is expected to be highest) to pH 3 (Figure 3A). The AuNP_{MUA} and AuNP_{MUA}-(PAH_{7.2}/PAA_{4.5})₁ show an expected profile of a carboxylic acid terminal coating with surface charge decreasing as pH decreases near the carboxylic acid pKa. From pH 7 – 9 both MUA and PAA terminated AuNPs maintain a strong negative charge between -45 mV and -55 mV, suggesting that the difference in pH adsorption from pH 7.2 to pH 9 was only affecting the PAH ionization and not the anionic AuNP surface potential. The pH dependent microelectrophoresis for AuNP_{MUA}-(PAH_{7.2}) and AuNP_{MUA}-(PAH_{7.2}/PAA_{4.5})_{1.5} reveals an interesting apparent dynamic instability when the sample is titrated from pH 4-5 to pH 9 (Figure 3B). The zeta potential for all PAH terminated particles at pH 7.2 was initially measured greater than 40 mV, however after pH was decreased to pH 4-5 before titration and then increased again, the particle charge became neutral when returning to pH 7.2, leading to aggregation. We suspect that

exposure to a lower pH is causing multilayer rearrangement or multilayer interpenetration similar to what is observed for planar films.³⁸ This dynamic instability is an interesting observation considering our previous work found that PAH coated QDs stored at pH 8 were stable for several months,³³ while under these dynamic conditions the AuNPs rapidly aggregate. The AuNP_{MUA}-(PAH_{7.2}⁹/PAA_{7.2}^{4.5})_{1.5} profile also shows an apparent shift in the surface pK_a towards basic pH, which is consistent with reports of PAH/PAA multilayers on larger NPs.⁴⁶

Figure 4: (A) Plasmon peak absorbance location and (B) microelectrophoresis measurements after each layer deposition cycle for AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1) (black □) and AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 3:1) (red ○) multilayers. (C) Percent recovery of AuNPs per layer (black) and cumulative (black --□--) for AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1) and per layer (red) and cumulative (red --○--) for AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 3:1). For AuNP_{MUA} the percent recovery is after three rounds of ultracentrifugation only and no solvent precipitation. Error bars represent standard deviations of three different samples (n = 3).

PSS-*co*-MA is commercially available as two copolymer ratios of 1:1 and 3:1 (SS:MA), each having two pK_a values of 2.9/8.8 and 2.7/8.3 respectively.⁵¹ Multilayers of AuNP_{MUA}-

(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1) and AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 3:1) were constructed in TRIS for PAH and in titrated DI-H₂O for both PSS-*co*-MA copolymer ratios. Assembly of both copolymers at pH 2 represents two different cases of polyelectrolyte rigidity where the 1:1 copolymer is expected to be less charged and more coiled than the 3:1, leading to thicker and thinner coatings respectively. The plasmon peak absorbance location extracted from UV-Vis absorbance measurements shows that after the addition of one layer of PSS-*co*-MA 1:1 the plasmon peak redshifts 2 nm, but no further shift is observed with additional coating (Figure 4A). The first layer of the PSS-*co*-MA 3:1 formed an apparently stable coating and resulting in a 1 nm redshift in the plasmon peak absorbance location, but the addition of another PAH layer causes complete particle flocculation that cannot be recovered after ultracentrifugation for further analyses (Figure 4A). We postulate that the higher ratio of SS:MA of PSS-*co*-MA 3:1 makes the polyelectrolyte too rigid to completely wrap the AuNP as effectively as the 1:1 copolymer. The subsequent addition of the third PAH layer removes some PSS-*co*-MA 3:1 chains resulting in charge neutralization and complete particle flocculation. Microelectrophoresis shows high charge reversal after coating with both 3:1 and 1:1 PSS-*co*-MA for all layers, but for PAH terminated layers the zeta potential decreases from about +49 mV to +35 mV from the first to third PAH layer respectively (Figure 4B). The percent recovery after the first PSS-*co*-MA 1:1 layer was very high (97%) but subsequent layers show a decreased recovery (70 – 80%) with a cumulative recovery of only 25% after 6 layers (Figure 4C).

Figure 5: Zeta potential measured as a function of pH for (A) AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)₁ (green Δ), AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 3:1)₁ (purple $\square$) and (B) AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)_{1.5} (orange $\diamond$). Arrows indicate direction of titration. Error bars represent standard deviations (n = 4).

The surface potential profiles of both 1:1 and 3:1 copolymer ratios of AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)₁ show a strong negative charge of -55 mV at pH 9 and maintain a strong net negative charge of -40 mV even when pH is lowered to pH 3.5. This shows the ability of PSS-*co*-MA to enhance the electrostatic stability of AuNPs, especially below pH 5. Interestingly, the PSS-*co*-MA 1:1 terminated AuNPs could even be recovered at the end of the titration cycle by centrifugation; however the PSS-*co*-MA 3:1 aggregated upon centrifugation after the end of titration (at pH 3.5). Analysis of AuNP_{MUA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)_{1.5} shows the full hysteresis that occurs when the AuNPs are titrated from pH 7.2 to pH 4 and back to pH 7.2 again (Figure 5B); the profile is nearly identical to AuNP_{MUA}-(PAH_{7.2}⁹). The PSS-*co*-MA/PAH multilayer system is expected to be more stable when exposed to low pH compared to PAA/PAH due to SS groups. However, the similar instability when PAH is deposited on either PAA or PSS-*co*-MA suggests that the source of the dynamic instability may be the increasing charge of the terminal PAH, leading to surface rearrangement and possibly unwrapping of the AuNP, rather than the loss in charge of the underlying anionic layer. Unfortunately, that cannot be

proven from the current data, but it serves as our current working hypothesis to be tested in future studies.

Figure 6: (A) Plasmon peak absorbance location and (B) Microelectrophoresis measurements for AuNPMPA, AuNPMPA-(PAH_{7.2}⁹), and AuNPMPA-(PAH_{7.2}⁹/PSS-co-MA₉² 1:1)₁ at polyelectrolyte:AuNP ratios of 60,000:1 (black), 30,000:1 (red ○) and 15,000:1 (green Δ). Error bars present standard deviations of three different samples (n = 3). (C) Representative TEM micrograph of AuNPMPA-(PAH_{7.2}⁹/PSS-co-MA₉² 1:1)₁ produced at the 30,000:1 ratio. Scale bar is 20 nm.

The smaller capping ligand mercaptopropionic acid (MPA) was used to stabilize AuNPs (AuNP_{MPA}) using the same procedure as AuNP_{MUA}. MPA stabilized NPs are expected to have a much smaller hydrodynamic size, about 5 nm less than MUA stabilized NPs.⁵⁸ This allows us to test the effect of ligand type and template hydrodynamic size on this LbL process. Additionally, several different polyelectrolyte to AuNP ratios of 60,000:1, 30,000:1 and 15,000:1 were tested by increasing AuNP concentration, while keeping polyelectrolyte concentration constant. Under weakly ionized conditions the coiled polyelectrolyte's decreased linear size should reduce the probability of interparticle bridging, which could potentially allow increasing the scale of production without compromising the AuNP aggregation state. The PAH/PSS-co-MA 1:1 polyelectrolyte combination was used because previous data indicated this to be a stable combination with the highest percent recovery for the first bilayer. The plasmon peak absorbance location for AuNP_{MPA}-(PAH_{7.2}⁹) is about 524 nm for all polyelectrolyte to AuNP ratios tested (Figure 6A). The added layer of PSS-co-MA 1:1 has a much larger shift to 529.3 nm for the 60,000:1 case and to 531.5 nm and 531.7 nm for the 30,000:1 and 15,000:1

respectively (Figure 6A). There are no observed secondary peaks in the spectra associated with high levels of interparticle bridging or aggregation for all ratios tested (Figure S4). The zeta potential of the initial ligand capped AuNP_{MPA} (-14 mV) is much lower than AuNP_{MUA} (-39.7 mV) and after PAH coating only a modest charge reversal (+20 mV) is achieved for all polyelectrolyte to AuNP ratios (Figure 6B). However, the addition of PSS-*co*-MA 1:1 resulted in very high charge reversal (-55 mV) for every case, showing that with only a single bilayer electrostatic colloidal stability can be greatly enhanced. To further assess the aggregation state after LbL modification, TEM images were acquired for the AuNP_{MPA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)₁ produced at the 30,000:1 ratio (Figure 6C). The image clearly shows that particle monodispersity has been maintained after coating with one bilayer. The polyelectrolyte films cannot be resolved in these images; consequently, for statistical analysis all contacting AuNPs were considered aggregates. Analysis of ~1400 NPs revealed that 95.4% of the AuNP_{MPA}-(PAH_{7.2}⁹/PSS-*co*-MA₉² 1:1)₁ clearly exist as single particles and less than 1% exists as aggregates of 4 or more (Figure S5, Table S1).

Figure 7: (A) AuNP_{MPA} in DI-H₂O (black –) and 0.1 M phosphate buffer pH 7.2 with 0 mM (red – –), 50 mM (green •) and 100 mM (blue •) NaCl. (B) AuNP_{MPA}-(PAH/PSS-co-MA 1:1)₁ in 0.1 M phosphate buffer with 0 mM (black –), 50 mM (orange •), 100 mM (green •), 500 mM (blue •), 1 M (red •), or 5 M (purple •) NaCl immediately after mixing, and (C) after 48 h and (D) 7 days at room temperature.

The electrosteric stability of the AuNPs was assessed by exposure to varying concentrations of NaCl in 0.1 phosphate buffer at pH 7.2. The AuNP_{MPA} are stable in DI-H₂O but immediately aggregate when exposed to phosphate buffer with and without NaCl, as noted by the substantial redshift and presence of secondary peaks in the absorbance spectra (Figure 7A). In contrast, the AuNP_{MPA}-(PAH_{7.2}⁹/PSS-co-MA₉² 1:1)₁ remain stable immediately after mixing with 0.1 M phosphate buffer with up to 1 M NaCl (Figure 7B) and only a small shift in the plasmon peak is observed even at 5 M NaCl (Figure 7B). After a 48 h incubation at room temperature AuNP_{MPA}-(PAH_{7.2}⁹/PSS-co-MA₉² 1:1)₁ settling is observed in 500 mM and 1 M NaCl samples, but no decrease in absorbance is observed for 0 – 100 mM NaCl (Figure 7C). The 5 M

NaCl sample completely precipitated after 48 h and had to be redispersed for measurement and contained substantial aggregation, as noted by the peak shift and peak broadening. After 7 days of storage undisturbed at room temperature, a more notable decrease in absorbance intensity occurs due to AuNPs settling out of solution. However, there is no substantial shift in plasmon peak absorbance location from 0 – 500 mM NaCl. This clearly shows how just one bilayer of LbL coating may exert such a powerful influence to enhance the AuNP electrosteric stability and to preserve the AuNP optical properties.

Conclusions

This work has shown the capability for LbL modification of high surface curvature AuNPs with weak and weak-strong PEs using a solvent precipitation assisted multistep layer deposition process. The choice of solution pH during polyelectrolyte adsorption plays an important role to enhance AuNP surface coverage to increase surface charge and improve recovery throughout the LbL process. The usage of solvent precipitation was a critical innovation to allow for controlled pH-dependent deposition under conditions that would normally lead to aggregation. An average recovery of 78% was achieved when depositing PAH/PAA multilayers under weakly-ionizing conditions; this is a significant improvement over the 30% recovery per layer achieved by others for similarly-sized AuNPs.¹⁹ Even with this higher recovery the compounding loss still results in less than 25% of AuNPs remaining after 6 layer deposition cycles, placing limitations on the application of LbL on high surface curvature NPs as a multilayer tool. However, this technique yielded a surprising electrosteric stabilizing effect, even with a single bilayer of PAH and PSS-*co*-MA 1:1 deposited under weakly-ionized conditions. The PAH/PSS-*co*-MA 1:1 coated AuNPs could be produced with a very high percent recovery and displayed enhanced stability over a broad pH range and in high concentrations of NaCl. Thus, the major outcome of this work is an approach that overcomes the major shortcoming of the time-intensive nature of NP LbL by using a solvent precipitation method to deposit a single bilayer that greatly enhances electrosteric stability. Future work will focus on applying this technique to other nanomaterials. Furthermore, the PAH/PSS-*co*-MA formulation will be developed towards biofunctionalization through covalent attachment, which will be particularly useful in realizing multifunctional components in biological applications that necessitate high colloidal stability.

TOC Graphic

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ABBREVIATIONS

PAH, poly(allylamine hydrochloride); PDADMAC, poly(diallyldimethylammonium chloride); PSS, poly(sodium-4-styrenesulfonate); PAA, poly(acrylic acid); QD, quantum dot; LbL, layer-by-layer; MPA, mercaptopropionic acid; MUA, 11-mercaptopundecanoic acid; AuNP, gold nanoparticle; PSS-*co*-MA, poly (4-styrenesulfonic acid-*co*-maleic acid); SS, styrene sulfonate; MA, maleic acid; DI, deionized; nanoparticle, NP;

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