Ultrasonic Nebulization for TEM Sample Preparation on Single-Layer Graphene Grids

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1. Experimental

1.1 Materials

Cetyltrimethylammonium bromide (CTAB), 99%, was purchased from Sigma. Hydrogen tetrachloroaurate (HAuCl₄ • 3H₂O), 99.9%; and tetraethyl orthosilicate (TEOS), 99.0% were purchased from Aldrich. Sodium hydroxide (NaOH), silver nitrate (AgNO₃), 99.0%; hydroquinone, 99%, and Ludox® LS, 30 wt.%, aqueous silica nanoparticle suspension were purchased from Sigma Aldrich. Sodium borohydride (NaBH₄), 99%, was purchased from Fluka. Methanol, 99.9%, was purchased from Fisher Scientific. Ethanol, 100%, was purchased from Decon Labs. All chemicals were used as received without further purification. Deionized water was purified by a Barnstead Nanopure II water purification system.

1.2 Gold Nanorod Synthesis

We adapted the methods described by Vigderman and Zubarev¹ to synthesize gold nanorods (AuNRs). First, CTAB-capped gold seeds were prepared. To 9.5 mL 0.10 M CTAB was added 500 µL 0.010 M chloroauric acid (HAuCl₄). While stirring rapidly, 460 µL of freshly prepared, ice-cold 0.10 M sodium borohydride (NaBH₄) in 0.010 M NaOH was quickly added. The seed solution changed color to dark brown immediately following the addition of NaBH₄. The seeds were aged for 1 hour before use.

A gold nanorod growth solution was prepared by adding 25 mL 0.010 M HAuCl₄ to 475 mL 0.10 M CTAB. While stirring gently, 0.75 mL 0.10 M AgNO₃ was added followed by 25 mL 0.10 M hydroquinone. The solution was stirred until it turned colorless, then 8 mL of CTAB-capped gold seeds were added. The gold nanorod solution was allowed to stand overnight.
resulting AuNRs were purified by centrifugation at 8000 rcf for 30 min. The supernatants were removed and the pellets were dispersed in water.

1.3 Silica Coating of CTAB-capped AuNRs

Silica coating was accomplished using previously published procedures. To further reduce the CTAB concentration, the CTAB-capped AuNRs were centrifuged again at 8000 rcf. After discarding the supernatant, the pellet was dispersed in water and an aliquot of 0.10 M CTAB was added to bring the CTAB concentration up to 0.8 mM. The AuNRs were allowed to stand in 0.8 mM CTAB overnight. To 10 mL of 1 nM CTAB-capped AuNRs in 0.8 mM CTAB was added 40 µL of 0.10 NaOH. The nanorods were gently shaken for 30 min. Fresh 20 vol% TEOS in methanol was prepared and 90 µL was added to the AuNRs. The silica shells were allowed to grow overnight. Silica coated AuNRs were purified by centrifugation at 8000 rcf for 30 min. After discarding the supernatant, the silica coated AuNRs were dispersed in 10 mL ethanol. This was followed by a second round of centrifugation at 7000 rcf for 30 min after which the supernatant was discarded and the pellet was dispersed in 10 mL ethanol.

1.4 MUA Functionalization of AuNRs

CTAB-capped nanorods were centrifuged a second time at 8000 rcf. The supernatant was discarded and the AuNRs were dispersed in nanopure water to a concentration of 1 nM in gold nanorods. To 40 mL of 1 nM CTAB-capped AuNRs was added 1 mL of 50 mg/mL PEG-SH (5000 M.W.) and the solution was gently shaken overnight. To purify the nanorods, they were centrifuged at 8000 rcf for 30 minutes, the supernatant was discarded, and the pellet was dispersed in 40 mL water. The AuNRs were centrifuged a second time after which the pellet was
only dispersed in 20 mL water. Then, 2 mL of 0.1 M NaOH was added to the nanorods followed by 4 mL of 20 mM MUA in ethanol. The AuNRs were then diluted to 40 mL with water and placed on a shaker overnight. Excess MUA was removed by centrifuging the AuNRs at 8000 rcf for 30 minutes. The supernatant was discarded and the pellet was dispersed in 2 mL of 0.1 M NaOH and then diluted to a total volume of 40 mL with water. This was repeated for an additional round of centrifugation.

1.5 Ultrasonic Nebulization for TEM Sample Preparation

Figure S1 shows a schematic of the nebulizer used to coat the nanoparticle solution onto a TEM grid in this paper. The ultrasonic spray setup was homebuilt, using a 1.7 MHz piezoelectric transducer, operating at a maximum of 12.5 W. The nebulization system consisted of a series of glass cells and tubes to allow for solution nebulization, solvent evaporation, and nanoparticle cluster deposition on a TEM grid within an enclosed system. The nanoparticle solution was injected into a glass cell that was placed over an ultrasonic transducer operating at 1.7 MHz. The nebulization cell is a custom-made piece of glassware with inlets for a gas stream and sample injection. The bottom of the cell opens with an o-ring flange. The bottom of the cell was sealed with a polymer membrane to facilitate the transfer of the ultrasound from the outer water bath to the inner nanoparticle solution while keeping the nanoparticle solution within the closed system, isolated from the transducer and nebulizer water bath. A flow of nitrogen gas at 0.5-1.0 L min⁻¹ carried the nebulized nanoparticle solution through the apparatus. A bump trap, horizontal drift tube, and a skimmer were employed to filter out large droplets. As shown in Figure 1, the horizontal drift tube was placed within a tube furnace so that heating could be used to increase the rate of solvent evaporation, if desired. A TEM grid was placed on a cleaned piece
of silicon on the bottom of a sealed coating cell. For convenience, we used a glass cell similar to the nebulization as our coating cell. As nebulized droplets were carried from the nebulization cell through the drift tube via the nitrogen flow, the solvent evaporates, leaving clusters of nanoparticles. While many of droplets and clusters are lost to deposition on the drift tube, some drift through the entire apparatus to gently deposit on the TEM grid. A bubbler connected to the coating cell collected any remaining airborne nanoparticles.

For a typical coating procedure, 5 mL of the nanoparticle solution were placed in the nebulizing cell. Once the cell had been sealed and nitrogen gas flow started, the ultrasonic transducer was turned on to generate a mist. The transducer was kept on for 0.5-3 minutes, then the gas was kept flowing for 2 minutes after the transducer has been turned off to make sure no airborne nebulized droplets remained in the apparatus.

1.6 Instrumentation

UV-Vis extinction spectra were collected using an Agilent Cary 5000 UV-Vis-NIR spectrophotometer. Transmission electron microscopy was carried out using JEOL 2100 Cryo and JEOL 2010 LaB$_6$ microscopes operated at 200 kV. Scanning transmission electron microscopy was carried out using both a Thermo Fisher Scientific Themis Z operated at 80 kV with a 25 mrad convergence angle and 30-pA current, and a NION100 UltraSTEM operated at 100kV with a 20 mrad convergence angle and 40-pA current. For scanning electron microscopy (SEM) both a Hitachi S-4800 and a JEOL 7000F SEM were used.

1.7 Graphene Transfer Methods

Chemical vapor deposition grown single-layer graphene on copper foil was purchased
from Grolltex Inc. Wet transfer to 200 gold mesh Quantifoil TEM grids with 2 micron sized holes and 4 micron period (purchased from Electron Microscopy Sciences) was performed adapting methods outlined in Huang et al.\textsuperscript{4} 495k molecular weight 2\% in anisole poly(methyl methacrylate) (PMMA) was purchased from MicroChem Corp. PMMA was spun at 2000 rpm for 1 minute onto the graphene on copper foil, and then etched the backside graphene (side without the PMMA coating) in oxygen plasma at 250 W power for 1.5 minutes. A 3 mm hole was cut in a polydimethylsiloxane (PDMS) square (purchased from Gel-Pak) that had dimensions of 1 cm by 1 cm by 0.2 mm, and then the PDMS was placed onto the PMMA/graphene/copper foil stack. The copper foil was then dissolved in 0.1 M ammonium persulfate in water (reagent grade 98\% purchased from Sigma). The remaining PDMS/PMMA/graphene stack was removed from the acid solution and transferred to distilled water for a series of 6 cleaning baths. Then a TEM grid was submerged, lined up with the 3 mm hole in the PDMS, and lifted forcefully to rip the PMMA from the PDMS, leaving the PMMA/graphene membrane on the TEM grid. The sample was left overnight to dry, followed by a 3 second dip in acetone and a 30 second dip in isopropyl alcohol. Then the sample was annealed at 500 °C under ~200 mTorr vacuum, ramping for 4 hours and holding temperature for 4 hours in 5\% H\textsubscript{2}/Ar, keeping the total flow below 50 sccm.

2. Variability in nanoparticle counts per cluster

In order to quantitatively measure the degree of variability in the nanoparticle counts per cluster, an aqueous solution of 20 nm colloidal gold nanosphere was prepared, concentration of 4.0 nM in particles according to UV-vis spectrophotometry.\textsuperscript{5,6} This standard solution was spray-deposited on a TEM grid and 180 clusters were individually analyzed for their
nanoparticle content (Figure S3). The geometric mean corresponded to a particle concentration of 2.7 nM.

3. Estimation of droplet size distribution

To gauge how droplet size distribution and verify the application of Lang’s equation, we nebulized a 2.5 wt% suspension of Ludox® LS nanoparticles in water and the drift tube was heated to 200 °C. We measured the diameters of the resulting silica microspheres, composed of thousands of silica nanoparticles, via scanning electron microscopy (Figure S4) and calculated the volume of each silica microparticle. The silica particles had a geometric mean (GM) volume of 0.44 µm³ with a geometric standard deviation (GSD) of 2.7. Assuming the size of the spherical clusters is proportional to the size of the initial droplet and the distribution of silica nanoparticles is uniform throughout the nebulized liquid, the droplets would be expected to have a similar GSD. The initial droplet size calculated from the size of the silica particle clusters is consistent with estimations of droplet size from Lang’s equation for an aqueous silica suspension nebulized at the frequency of the transducer in our apparatus. The GSDs for the silica coated AuNRs, 2.2-2.6, are similar to those measured for the silica microparticles as well as the MUA-capped AuNRs, suggesting that coalescence is not the major cause of error in estimating the concentration.

Similarly, a solution of 4% paraffin wax in heptane was also nebulized to study the particle size distributions. In Lang’s original paper, he nebulized molten paraffin wax to examine droplet distributions, but the apparatus we used did not allow sufficient heating of the nebulization cell to replicate Lang’s experiment. The wax particles were deposited on a piece of silicon and observed using SEM, with a typical SEM shown in Figure S4. Many of the particles
were irregularly shaped due to depositing while still wet with heptane, which limited the use of this data for droplet size distribution estimation, but the particle sizes did seem consistent with initial estimates of droplet size calculated using Lang’s equation for heptane using the transducer in our apparatus.
Figure S1. Schematic of the homebuilt nebulizer system, showing the nebulizer itself (from Mouser Electronics) with the rheostat (American Piezo); also shown is a photograph of the setup.
Figure S2: Concentration of CTAB-capped AuNRs estimated from the geometric mean of the size of the clusters that were deposited via ultrasonic spray coating are shown against the concentration as measured by UV-Vis extinction spectrophotometry. The dotted red line, added as a guide to the eye, shows where the two methods would be in agreement. Error bars depict the geometric standard deviation.
**Figure S3:** Concentrations of 4.0 nM citrate-capped 20 nm gold nanoparticles estimated from the particle per cluster density that was deposited via ultrasonic spray coating are shown against the solution concentration as measured by UV-Vis extinction spectrophotometry. The TEM grid is an ultrathin amorphous carbon one on lacey carbon, not single-layer graphene. 180 clusters were counted. The geometric mean, and the corresponding concentration estimated from this cluster size, is indicated by the orange square data point. TEM images of 193 (top), 21 (middle), and 8 (bottom) particle clusters are shown as insets with lines indicating the concentration corresponding to the particle per cluster density. The scale bars are all 100 nm.
Figure S4. Estimating the relative distribution in droplet size. A 2.5 wt% suspension of silica nanoparticles was ultrasonically nebulized, and the collected nanoparticle clusters were measured by SEM in order to study the size distribution of droplets deposited by our spray coating apparatus. In panel (a) a box-and-whisker plot (right) with individual data points on the left of the measured diameters. The overlaid curve shows the lognormal distribution of the sizes. Panel (b) shows a typical SEM image used to study the particle sizes of the silica nanoparticle clusters. The geometric mean diameter of the silica particles was 0.94 mm. A 4% paraffin wax in heptane solution was also used to evaluate the distribution of droplets produced by ultrasonic nebulization. Panel (c) shows a box-and-whisker plot (right) with individual data points on the left of the measured diameters. The overlaid curve shows the lognormal distribution of the sizes. The geometric mean diameter of the wax particles was 1.0 µm. Panel (d) shows a typical SEM image used to study the particle sizes of the wax particles. Scale bars are 20 µm.
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