Make or Buy? The Economics of Gold Nanoparticle Manufacturing for Lateral Flow Assays

Introduction

Price is an important factor in the commercial success of lateral flow diagnostics and manufacturers are under pressure to keep the cost of goods to a minimum. A key component of lateral flow assays are reporter particles which are typically gold nanoparticles or dyed latex beads. While the cost of the reporter particles is typically less than 1 cent per strip for 40 nm diameter gold nanoparticles, many companies choose to fabricate their own gold nanoparticles for use in their assays. In this paper, we look at the pros and cons of fabricating versus purchasing this critical reagent.

Figure 1:.Beaker scale fabrication of 40 nm gold nanoparticles.

The Deceptive Simplicity of Gold Nanoparticle Fabrication

In the 1950's John Turkevich described a simple method for the production of relatively monodisperse gold nanoparticles[Turkevich, 1951]. By simply heating a solution of gold chloride in the presence of sodium citrate and ascorbic acid to a boil, gold nanoparticles can be formed (Figure 1). The diameter of the particles can be adjusted between 10 and 120 nm by varying the ratios of the different reactants. Given the relative simplicity of the recipe, many companies follow a similar recipe in order to produce gold nanoparticles for their assays. In-house production reduces the reliance on outside vendors that have supply and quality risks and keeps costs to a minimum. For lateral flow assay tests where sensitivity is not a concern and where the test is qualitative ("yes/no"), batch-to-batch variance may not significantly impact the quality of the test and the cost savings is justified. However, if the lateral flow diagnostic needs to detect very low levels of an analyte or if the assay is quantitative, the nuances associated with the nanoparticle size, shape and surface become very important and it is critical that each lot is produced under stringent manufacturing controls to ensure that lot-to-lot variability is minimized.

Manufacturing Controls for Minimizing Variability

While conceptually simple, the manufacturing of gold nanoparticles is extremely sensitive to all components of the reaction. Water purity, glassware cleaning, trace element contaminants in chemical ingredients, addition rates, stir speeds, temperature control, temperature ramps and mixing dynamics all affect product quality. Even when carefully controlling all variables there is often inherent variability in the process resulting in a long investigative process to determine which variable is the culprit. Gold nanoparticle fabrication recipes that rely on "self-nucleation", where the particles experience a nucleation event which rapidly produces a large number of seed particles which then grow larger to a final size, are especially challenging to control. Alternative, "seeded growth" recipes that start with a calibrated seed concentration and then grow these calibrated seeds larger are generally more controlled and robust but are "seed" dependent in terms of reliably fabricating the same sized final product. To ensure that the nanoparticle's are made in a reproducible fashion, extensive characterization must be performed. One advantage of gold nanoparticles is that due to the particle's plasmon resonance, the particles have a distinct optical spectra that is a function of the nanoparticle size, polydispersity, and concentration (see Figure 2). Since this data can be obtained on a relatively inexpensive UV-Visible spectrophotometer, this is the main (and typically only) characterization tool used by lateral flow manufacturers to verify the quality of their nanoparticles. However, since the UV-Visible spectra is the product of many independent variables, critical differences between batches can be masked and supplementary characterization methods are highly recommended.

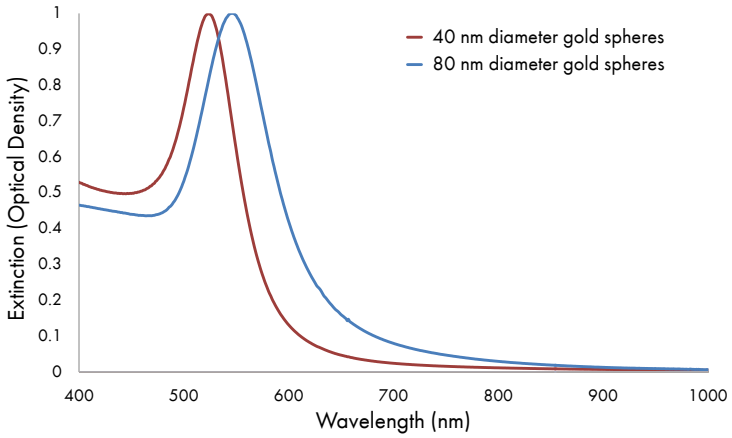

Figure 2. UV-Visible spectra of 1 OD 40 nm and 1 OD 80 nm gold nanoparticles. Differences in particle diameter result in a shift in the peak resonance and a change in the spectral shape. For a given size, peak absorption wavelength, full width at half maximum, and the ratio of a wavelength near the peak to a red-shifted wavelength (e.g. ratio of 520 nm to 700 nm) can be used to define specifications. These metrics indirectly capture particle uniformity and are an indicator of aggregation.

What's Typically Missing and Why It Matters

Unfortunately, the UV-Visible spectra of a gold nanoparticle solution does not provide all the data necessary in order to quantify the lot-to-lot variation in nanoparticle fabrication. Broadening of the spectral peak can be due to aggregation, size polydispersity or shape polydispersity all of which can impact the effective surface area of the particles and their antibody loading. Transmission electron microscopy (TEM) is the preferred method for generating size histograms and calculating the mean diameter and the coefficient of variation (CV; mean diameter divided by the standard deviation of the diameter). Using TEM size statistics, an effective surface area per unit mass can be calculated which is a critical parameter in determining optimal antibody/particle ratios during conjugation. Additionally, TEM provides information on shape. Many gold nanoparticle synthesis recipes generate a variety of semi-crystalline shapes (shape impurities). The specific crystal facet on the gold particle surface influences the adsorption of antibodies and it is important to know the number and type of non-spherical particles in solution. Agglomeration is measured with a dynamic light scattering (DLS) instrument that measures the ensemble average of the hydrodynamic size of the particles in solution. Particle charge is quantified by the Zeta Potential of the sample and is sensitive to any change to the surface of the particle. If a contaminant is absorbed to the particle surface, it can be undetectable with UV-Visible spectroscopy but still impact the ability of the particles to consistently adsorb antibodies. Since the particles are relatively dilute in solution, vanishingly small concentrations of contaminants can cause problems and Zeta Potential is one method of identifying that there is an issue.

Another important characteristic that is rarely measured is the mass concentration. When provided for lateral flow applications, gold nanoparticles are typically quoted in Optical Density (OD) units which is a measure of the amount of light that they absorb. Since UV-Visible measurements are relatively simple to make, this provides nanoparticle manufacturers a straightforward method to concentrate or dilute to a particular value. The downside to this metric is that the OD does not necessarily correlate to a particle number or to an effective surface area since different sized and shaped gold nanoparticles have different absorption cross sections. Arguably, the antibody/gold nanoparticle surface area ratio is one of the most important parameters to optimize and two gold nanoparticle solutions at the same OD can have different surface areas if the size and shape distributions are not equivalent. To accurately measure concentration, ICP-MS is used to measure the gold ion concentration and using the average particle mass calculated by TEM, a particle concentration and effective surface area can be calculated. A certificate of analysis from a typical gold nanoparticle lot that shows the critical characterization metrics is shown in Figure 3. Each lot of gold from nanoComposix is provided with a certificate of analysis that ensure minimal lot-to-lot variation.

The Surface is Everything

The self-assembly of antibodies and other proteins on the surface of a gold nanoparticle is difficult to control. Small changes in the pH or salt conditions can alter the charge, hydrophobicity, or structure of the antibody, which can affect the antibody density and orientation on the particle surface. The gold particle surface has different crystal orientations where each orientation can have a selective affinity for different portions of the antibody. For all passive adsorption processes, the starting particle should be bare, which is defined as not having any molecular ligands bound to the particle surface. Technically, it is not possible to have a stable bare nanoparticle because the charge of the colloidal double layer keeps the particles from aggregating. Therefore, to produce particles that are as bare as possible, ultra-high purity nanoparticles are fabricated and suspended in a buffer with components that only weakly associate with the surface. In the presence of an antibody or other protein, the protein will displace the weakly associated molecules and bind to the particle surface.

The most common buffer for bare nanoparticles is citrate (Figure 4). Sodium citrate is used as a reductant in many gold nanoparticle fabrication methods and provides a balance between stability during particle formation and displaceability when making particle conjugates. Each of the three carboxylic acids on the citrate molecule can weakly bind to the particle surface but are displaced in the presence of a protein. In addition to the standard citrate buffer, nanoComposix offers gold nanoparticles in a carbonate buffer (Figure 5). Carbonate is a smaller, less complex molecule and has a lower affinity to the gold nanoparticle surface than citrate. The greater displaceability of the carbonate molecules typically produces better performing conjugates, and therefore, carbonate-buffered gold particles are preferable for passive adsorption experiments. A comparison of the sensitivity of a troponin I sandwich assay that used either the citrate or carbonate stabilized BioReady Bare 40 nm gold is shown in Figure 6. Due to the superior absorption antibody absorption properties, a higher sensitivity is achieved with the carbonate surface.

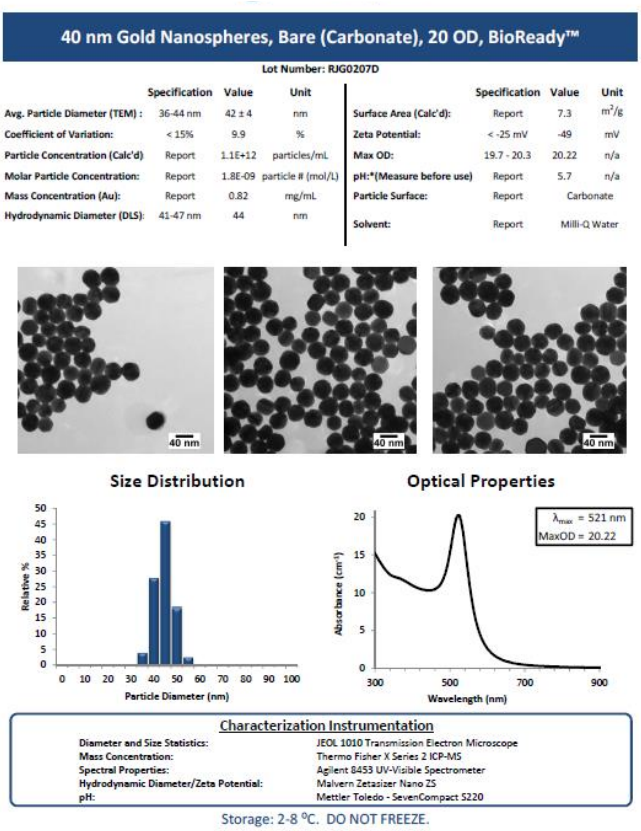

Figure 3. Example certificate of analysis provided with every lot of material from nanoComposix.

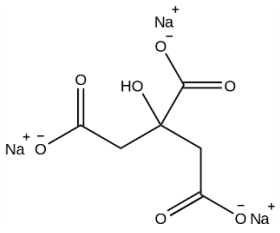

Figure 4. Trisodium citrate is used as a reductant in many gold syntheses and also acts as a stabilizing molecule and buffer component in many gold nanoparticle solutions used for physisorption of antibodies.

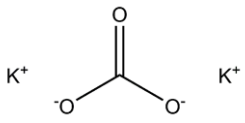

Figure 5. Potassium carbonate molecules have a weak affinity for the particle surface and is the preferred surface for generating high quality physisorbed conjugates.

BioReady™ Bare Products

The BioReady bare products are available in 40 nm and 80 nm diameter particles (Table 2). The 40 nm diameter particles are provided at a concentration of 20 OD, which is approximately 1 mg/mL of gold. The 80 nm diameter particles are provided at 5 OD. Conjugation protocols for both the 40 nm and 80 nm BioReady bare particles are provided on nanoComposix's web site.

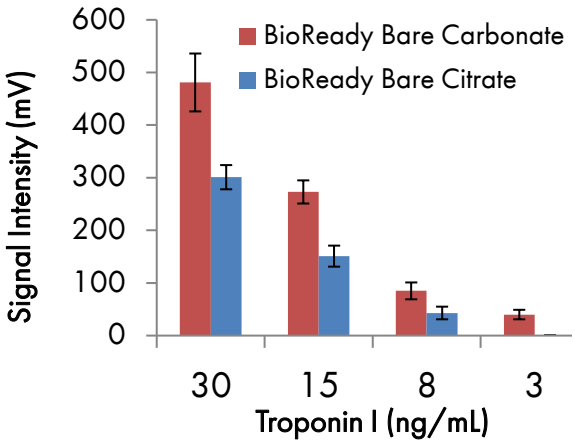

Figure 6. Signal intensity (mV) of test line intensity measured on a Qiagen benchtop reader in a lateral flow sandwich assay using anti-cTnI conjugates on either carbonate buffered BioReady Bare particles or Citrate buffered BioReady Bare particles.

Who Doesn't Love Re-optimizing?

One of the challenges for companies that fabricate their own gold is that the manufacturing scale is on the order of 1-2 liters at a concentration of 1 OD. In a typical strip, 5 uL of 20 OD gold nanoparticles might be used. This is 0.005 mL * 20 OD = 0.1 OD-mL of gold. A 2-liter reaction at 1 OD = 2000 OD-mL, which would be sufficient for about 20,000 tests. While significant, many lateral flow production runs generate hundreds of thousands or even millions of strips. If there is lot-to-lot variation between each of the gold nanoparticle fabrication batches, the assay will potentially have to re-optimized for each lot of gold requiring additional quality control checks and manufacturing delays. At nanoComposix, BioReady nanoparticles are fabricated within our ISO 13485 compliant quality system to produce high lot-to-lot consistency of the particle properties. Lot sizes as large as 400,000 OD-mLs (equivalent to 400 L of 1 OD gold) are consistently produced which is sufficient to generate up to 4,000,000 lateral flow assay strips. Specific lots can be reserved under a supply contract that allows for purchasing from the same lot for up to a year.

Product	Concentration (OD)	Concentration (mg/mL Au)	Buffer
BioReady™ Bare 40 nm Citrate	20	0.8 – 0.95	0.02 mM citrate
BioReady™ Bare 40 nm Carbonate	20	0.8 – 0.95	DI water
BioReady™ Bare 80 nm Carbonate	5	0.18 – 0.21	2 mM potassium carbonate

Table 2: . BioReady bare gold particles with citrate and carbonate surfaces that are optimal for passive adsorption of antibodies.

Time is Money

The processes of performing optimizations for each lot of gold manufactured not only adds cost by delaying manufacture, it is also expensive to pay a team of scientist to perform these optimizations regularly, and even more costly when troubleshooting is required. While purchasing gold nanoparticles may slightly increase the cost of raw materials, the increase in cost is more than recovered in the time saved during development and manufacture.

Advantages of Purchasing Gold from NanoComposix

- Large lot sizes
- Minimal lot-to-lot variation
- High optical density = minimized volumes for processing
- Guaranteed quality and shape purity

- Long-term stability (> 1 year)
- Multiple highly displaceable stabilizing ions to choose from
- Technical support from a team with experts in nanoparticle fabrication and bio-functionalization

So How Much Are You Really Saving?

As one of the largest gold nanoparticle fabricators in the world, nanoComposix has leveraged its manufacturing volume to provide precisely engineered and highly characterized gold nanoparticle solutions optimized for lateral flow at an extremely competitive cost. To compare prices between vendors, it is important to convert the volume and concentration to a price per OD-mL value. Table 2 below shows a comparison between nanoComposix's pricing to various nanoparticle vendors. Further discounts (as low as $0.45 / OD-mL) are available at larger scales.

	nanoComposix	Vendor B	Vendor BW	Vendor NH	Vendor IB
Price ($)	$1250	$4820	$2487	$2750	$2990
Volume (mL)	100	100	100	100	100
Optical Density (OD)	20	15	15	25	20
Price/OD-mL	**$0.62**	**$3.21**	**$1.66**	**$1.10**	**$1.50**

Table 2: . Commercial 40 nm diameter gold nanosphere pricing comparison.

Conclusion

NanoComposix's BioReady Bare gold nanospheres maximize sensitivity for passive conjugation and consistently produce reproducible high quality conjugates. While some manufacturers fabricate gold nanoparticles in-house, the characterization that is necessary to ensure that each batch meets specification and the time lost in re-optimizing when using small volume lots adds significant costs. Citrate stabilized BioReady Bare particles are a drop-in replacement for existing gold nanoparticles currently being provided by other vendors. Carbonate stabilized BioReady Bare particles have shown increased performance compared to citrate stabilized gold nanoparticles due to the highly displaceable stabilizing molecule. NanoComposix offers a three-lot evaluation kit for those interested in evaluating gold nanoparticles for their assays.

References

Turkevich J. Stevenson, P.C., Hillier, J. "A study of the nucleation and growth processes in the synthesis of colloidal gold" Discuss. Faraday Soc. 1951, 11, 55-75.

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