Manufacturing high-quality gold sol
Understanding key engineering aspects of the production of colloidal gold can optimize the quality and stability of gold labeling components.
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As early as the first decade of the twentieth century, colloidal gold sols containing particles smaller than 10 nm were being produced by chemical methods. However, these inorganic suspensions were not applied to protein labeling until 1971, when Faulk and Taylor invented the immunogold staining procedure. Since that time, the labeling of targeting molecules, especially proteins, with gold nanoparticles has revolutionized the visualization of cellular and tissue components by electron microscopy. The silver enhancement method has extended the range of application of gold labeling to include light microscopy. The electron-dense and visually dense nature of gold labels also facilitates detection in such techniques as blotting, flow cytometry, and hybridization assays. Double- and triple-labeling systems involving immunogold methods have been used successfully to detect more than one antigen at the same time.

The first step toward manufacturing a consistent gold-protein conjugate is to make a gold sol having particles of proper size and dimension. Basically, colloidal gold sols consist of small granules of this transition metal in a stable, uniform dispersion. Most preparations of colloidal gold are made up of particles varying in diameter from about 5 to around 150 nm. For the development of diagnostic assays that make use of gold conjugates, typical particle sizes in the gold sol range between 20 and 40 nm. Since these are very small particles, the surface area of the gold in the sol is remarkably high. This means that production of colloidal gold sol involves the creation of a large surface area having a very high surface energy. Any colloidal suspension with high surface energy can lose its stability if proper operating conditions are not maintained during its production.

This article discusses some process engineering aspects of gold sol manufacture that have considerable influence over the quality and stability of the suspension. It highlights the physical, rather than chemical, factors that play important roles in gold sol production. But first, a look at process chemistry is in order.

Basic Chemistry
A variety of chemical methods can be employed to produce monodisperse colloidal gold suspensions. However, three procedures have become the most common for making
particles that fall into predictable size ranges. In all three processes, tetrachloroauric acid (HAuCl₄) in a 1% aqueous solution is reduced by an agent in order to produce spheroidal gold particles. The greater the power and concentration of the reducing agent, generally, the smaller the resultant gold particles in the suspension.

To create large-particle colloidal gold dispersions, an aqueous solution of tetrachloroauric acid is treated with trisodium citrate in aqueous solution. This results in particles sized 15-150 nm, the final range depending on the concentration of the citrate used in the reduction process. Medium-sized gold particles with diameters between 6 and 15 nm and an average size of 12 nm are formed by reducing the tetrachloroauric acid solution with an aqueous sodium ascorbate solution. The smallest particles, measuring less than 5 nm in diameter, are produced by reduction with either white or yellow phosphorus in diethyl ether.

The following discussion focuses mainly on the process-development aspects of making colloidal gold with 20- to 40-nm particles. Gold sols with particles in that range are the most suitable candidates for use in developing rapid diagnostic tests. It can be seen from the foregoing that the starting raw materials for production of these gold sols are an aqueous solution of tetrachloroauric acid and an aqueous solution of trisodium citrate, which are brought together to create a chemical reaction. As described below, such chemical reactions are broadly classified as either homogeneous or heterogeneous.

**Homogeneous Reaction.** In a homogeneous reaction, all the reactants are miscible and form a homogeneous solution. The products that are formed from the reaction are also soluble; therefore, there is no phase separation at any time during the course of reaction. The rate of such a reaction depends on the concentration of the reactants and on the operating temperature. The temperature influences the rate constant of the reaction. Usually, a 10° rise in the operating temperature enhances the rate of reaction by a factor of two.

This effect of operating temperature on the rate of reaction is exploited in order to increase reaction speed or achieve greater production throughput with a single reactor. For irreversible reactions—reactions that produce almost complete conversion of reactants to products—the upper temperature limit is established by the highest operating temperature of the material from which the reactor is constructed. The effect of temperature on reversible reactions is more complicated, because both the reaction rate and the equilibrium conversion are functions of temperature and both need to be taken into consideration. For a reversible endothermic reaction, the rate of reaction and the
equilibrium conversion increase with an increase in temperature; therefore, the highest possible temperature is suitable for large-scale production. For a reversible exothermic reaction, however, the equilibrium conversion decreases while the rate increases with an increase in temperature. These are opposing effects, and the reaction is thus conducted at varying temperatures so as to reach the optimum rate of reaction and achieve the best conversion of reactants into products. The transport factors—heat transfer, mass transfer, and so on—do not play major roles in homogeneous reaction kinetics.

**Heterogeneous Reaction.** In a heterogeneous reaction, more than one phase is present. The reaction might be gas-liquid, liquid-liquid, gas-solid, liquid-solid, gas-liquid-solid, or liquid-liquid-solid, or display some other progression of phases. Any solid involved could be a catalyst or a reactant. The relatively simple rules for controlling homogeneous reactions are not applicable to the control of heterogeneous reactions. In addition to concentrations and temperature, the physical shift of reactant from one phase to another assumes great importance in heterogeneous reactions.

The chemical reaction between an aqueous solution of tetrachloroauric acid and an aqueous solution of trisodium citrate is interesting in that the reaction begins as a homogeneous one, but then, within a minute, the reaction mixture becomes heterogeneous. This phase transition from homogeneous to heterogeneous occurs very rapidly, making the sol-manufacturing process difficult to monitor or control effectively. Moreover, the reaction is completed so quickly that operators do not have much time to take any corrective action necessary to ensure reproducible product.

**Producing Gold Colloids**

Before the addition of the reducing agent, 100% gold ions exist in solution. Immediately after the reducing agent is added, gold atoms start to form in the solution, and their concentration rises rapidly until the solution reaches supersaturation. Aggregation subsequently occurs, in a process called nucleation. Central icosahedral gold cores of 11 atoms are formed at nucleation sites. The formation of nucleation sites, in response to the supersaturation of gold atoms in solution, occurs very quickly. Once it is achieved, the remaining dissolved gold atoms continue to bind to the nucleation sites under an energy-reducing gradient until all atoms are removed from solution.

The number of nuclei formed initially determines how many particles finally grow in solution. At a fixed concentration of tetrachloroauric acid in solution, as the concentration of the reducing agent is increased the number of nuclei that form grows larger. The more nuclei, the smaller the gold particles produced. Finding the optimal concentration of the citrate in solution is therefore an important, even crucial, task. If manufacturing conditions are optimized, all nucleation sites will be formed instantaneously and simultaneously, resulting in formation of final gold particles of exactly the same size (monodisperse gold). This is indeed difficult to achieve. Most manufacturing methods fail to accommodate this ideal and generate irreproducible gold (gold inconsistent from batch to batch) that gives unstable gold conjugates in most situations.

Gold colloids are composed of an internal core of pure gold that is surrounded by a surface layer of adsorbed AuCl$_2^-$ ions. These negatively charged ions confer a negative charge to the colloidal gold and thus, through electrostatic repulsion, prevent particle aggregation. All
colloidal gold suspensions are sensitive to electrolytes. Electrolytes compress the ionic double layer and thereby reduce electrostatic repulsion. This destabilizing effect results in particle aggregation, which is accompanied by a color change and eventual sedimentation of the gold. The detrimental effect of chloride, bromide, and iodide electrolytes on the stability of the gold colloid is greatest for chlorides and least with iodides.

All gold colloids display a single absorption peak in the visible range between 510 and 550 nm. With increasing particle size, the absorption maximum shifts to a longer wavelength, while the width of the absorption spectra relates to the size range. The smallest gold colloids (2–5 nm) are yellow-orange, midrange particles (10–20 nm) are wine red, and larger particles (30–64 nm) are blue-green. Smaller gold particles are basically spherical, while particles in the range of 30–80 nm show more shape eccentricity related to the ratio of major to minor axes.

Researchers have observed several factors that affect the quality and stability of the gold colloid. An important consideration leading to the preparation of stable gold colloids is employment of thoroughly cleaned glass apparatus, 0.2-µm-filtered solutions, and, ideally, triple-glass-distilled water. The use of nanopure water is recommended. These precautions suggest the adverse effect that even trace contaminants have on the preparation of colloidal gold. Although the use of siliconized glassware is often recommended, good results have consistently been obtained without any special glassware.

The effect of the order of reagent addition—that is, adding citrate solution to the tetrachloroauric acid solution or vice versa—on the quality of the gold colloid formed has been noted by researchers. However, no clear indication of how addition order might relate to methods of manufacturing colloidal gold suspensions reproducibly has been given.

Researchers have not explicated the role of mixing in the formation of the suspension, nor have they mentioned the negative impact of the use of a stir bar (for laboratory-scale preparation) in a magnetically agitated system on the quality and stability of the gold sol. It must be kept in mind that, in a large-scale operation, it is not only the chemistry of the process that is important, but also its perhaps seemingly insignificant physical parameters. Small changes in process conditions can so adversely affect the quality of the product that its utility to end-users will be minimal.

**Batch or Continuous Process?**
Generally, manufacturing processes can be run either batchwise or in continuous mode. Certain considerations dictate the best choice. The scale of operation is an important factor.

Small-scale production calls for batch operation. The reactor used in such a case is called a batch reactor. Reactants are added to the batch reactor at a suitable temperature, the reaction proceeds, and then, at the end of the batch time, the reactor's contents are removed. The reaction products are subsequently recovered by means of a separation process. Unconverted reactants can be reused in certain cases. Whenever the unconverted reactants are discarded, they must be disposed of in accordance with pertinent environmental and safety rules. In a batch reactor, the concentrations of reactants and products change continuously with time. Thus, the reaction can be tracked
by noting carefully the fall in reactant concentration or the rise in product concentration as a function of time.

The continuous mode of production is used for high-volume manufacture. In a continuous process, the reactants are fed into the reactor steadily and the products form and come out continuously. The flow pattern of fluid in a continuous reactor will take one of three forms. A mixed flow pattern is characterized by the reactor contents being completely mixed and the exit concentration being equal to the reactor concentration. In a plug-flow reactor, the concentrations of reactants and products change progressively as the materials pass through the reactor. There is no mixing of fluid in the longitudinal direction, although the radial mixing is complete. A flow pattern between the mixed- and plug-flow configurations is possible. In such a case, the fluid in the reactor is partially rather than completely mixed. Sound experimental techniques are available for determining the specific flow pattern in an unknown vessel, and modes of describing the vessel characteristics have been established. Without proper characterization of fluid flow within a reactor, it is practically impossible to predict the behavior of the reaction taking place there.

Typical production volumes for colloidal gold are in the range of 1 to 100 L. Batch processing is appropriate for such production. Manufacturers producing gold suspension in the range of 10 to 100 L (production volume depends on the order size and the availability of suitable reactor) sell it to customers for subsequent conjugation with proteins. Typically, for a 100-L batch, the power consumption for stirring the liquid contents will be substantially large. The reactor needs an adequate piping arrangement for pumping reactants, washing solutions, etc., into the reactor. After every batch of production, the reactor must be cleaned thoroughly with a sodium bicarbonate and detergent solution, distilled water, and then with some volatile organic solvent, such as acetone. A further wash with nanopure water is recommended.

Many diagnostic companies produce gold suspension in the range of 2 to 5 L for their in-house use (captive consumption). For such small-volume production, power consumed in stirring the reactor contents can be expected to be minimal, and it is easy to clean the reactor thoroughly after every batch by dismantling the entire assembly. Complicated piping arrangements that are necessary for large-batch production or in continuous processes do not figure in small-batch processes, making cleaning simple.

The important parts of the batch reactor used for making colloidal gold are the reaction vessel, the agitation or stirring system, and a constant-temperature bath that keeps the reactor contents at a uniform and suitable operating temperature throughout the reaction. The reaction assembly is easy to set up; the only precaution that needs to be taken is that the assembly components must be cleaned scrupulously. It is best that all glassware be autoclaved before each use.

**Factors Affecting the Quality of the Final Gold Suspension**

A variety of physical parameters affect the quality of the final gold suspension that is produced by the reaction of aqueous tetrachloroauric acid with an aqueous solution of trisodium citrate. Key factors worthy of consideration are:
• The concentration of reactants.
• Mixing of the reactants.
• The order in which reactants are added.
• Operating temperature.
• Liquid head in the reactor.
• The reactor’s material of construction.

**Concentration of Reactants.** The rate of any homogeneous chemical reaction depends on the concentration of the reactants and the operating temperature. A low reactant concentration will result in low rates. A high concentration is therefore desirable for realizing high rates. Too high a concentration might yield other problems, however, particularly for competing reactions. In such cases, the desired product might not form in sufficient quantity and the reaction might produce a large amount of byproducts. Only one reaction is involved when tetrachloroauric acid and trisodium citrate are combined, but inadequate reactant concentration would result in gold particles of undesirable size and a broad distribution of particle sizes.

The procedure developed by Frens is most commonly used to produce 40-nm gold particles. In accordance with this procedure, to 50 ml of tetrachloroauric acid in 0.01% solution (weight to volume) that is at a boil, 0.5 ml of a 1% solution of trisodium citrate is added. The solution initially has a gray color which changes to lavender and then, with continued boiling, after 1 to 3 minutes develops a red hue. The resulting particle size is 41 nm. Once the colloid is formed, neither prolonged heating nor further addition of the citrate solution will produce any change in particle diameter. The use of proportionally larger reaction volumes is accompanied by an increase of some 20% in the final particle size. A 20% difference in the amount of tetrachloroauric acid or trisodium citrate used has been observed not to affect the particle size substantially. Rates of initial nuclei formation are practically uniform throughout this range of concentrations.

**Mixing of Reactants.** This is perhaps the most crucial physical process parameter. The reactants must be well mixed in order for nucleation to occur. Adequate mixing results in uniform concentration and temperature in every part of the reactor. If concentration varies from location to location, the rates of reaction in different places will be different, too.

All chemical reactions are accompanied by either heat generation or heat absorption, which gives rise to either an increase or a decrease in temperature from the desired value. Different temperatures within the reactor cause heat- and mass-transfer gradients.

With an intrinsically slow reaction, how the reactants are mixed is not going to be a cause of substantial distortion in the product. But in the gold-making process the reaction occurs in seconds, and the reactants must be brought to uniform concentration before it happens. A challenge of rapid mixing is therefore encountered here; special arrangement must be made for agitation of the liquid contents of the reactor.

In small-scale processes a small stir bar is used to agitate the liquid in the reactor. The reactor is placed on a magnetic stirrer, and, in order to promote fast mixing, the stirrer is rotated at high speed. Careful observation reveals that under such a condition a vortex is formed in the reactor (see Figure 1).