Synthesis of Gold Nanoparticles and Association of DNA-Gold Nanoparticles
Weon Bae Ko1*, Young Min Lee1, Sung Kyu Hong1, Sung Sook Choi2, Sang Jin Lee3
Department of Chemistry1, College of Pharmacy2, Division of Animal Science3, Sahmyook University, Seoul, 139-749 Korea

Abstract
This study examined the synthesis of gold nanoparticles using a non-ionic surfactant, polysorbate 80, and KAuCl4 in water. The gold nanoparticles, which were well dispersed in water, were analyzed by UV-vis spectroscopy and transmission electron microscopy (TEM). In addition, the SRY(sex-determining region Y) gene of the Bos taurus specific primer was designed, and this primer solution was mixed with the aqueous gold nanoparticles solution. The binding ability of DNA and gold nanoparticles was identified by polyacrylamide gel electrophoresis. The products of DNA linked with gold nanoparticles were also characterized by UV-vis spectroscopy and TEM.

Introduction
Among the various metal nanoparticles reported thus far, gold nanoparticles have attracted remarkable interest over the last few years on account of their high stability to oxidation and their optical and well-defined size-related electronic properties (e.g. quantized charging) [1, 2]. The synthesis of monolayer-protected gold nanoparticles [3] is based on the reactive head groups, which allow the self-assembly of organic monolayers onto the nanoparticles surface. Gold nanoparticles are protected with polysorbate 80 in an aqueous solution. The Polysorbate 80 is a non-ionic surfactant that is used in the preparation of gold and silver nanoparticles in aqueous solutions at room temperature without reducing agents [16-17]. This study examined whether polysorbate 80 could act not only as a stabilizing agent but also as a reducing agent for the synthesis of gold nanoparticles in an aqueous solution at room temperature. The products were characterized by UV-vis spectra and transmission electron microscopy (TEM). The surface of the gold nanoparticles was functionalized with thiolated oligonucleotides in solution, which typically exhibits a red color due to the optical absorption peak at approximately 525 nm caused by surface plasmon resonance [4-5]. Upon aggregation of the nanoprobes induced by the high salt concent-

*corresponding author. E-mail: kowb@syu.ac.kr

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pe. The TEM specimens were prepared by placing a few drops of the sample solution onto a carbon-coated copper grid. The 3 wt% polyacrylamide gel in electrophoresis was used at 100 volts.

**Synthesis**

**Synthesis of gold nanoparticles by polysorbate 80**

In a typical experiment, 0.4 mM KAuCl$_4$ was added to 10ml of 1 wt% polysorbate 80 of an aqueous solution and kept at room temperature. The solution turned from yellow to colorless, then to a pink color, and finally to red after 5h, indicating the formation of gold nanoparticles.

**Preparation of DNA-gold nanoparticles**

The SRY (sex-determining region Y) gene of Bos taurus specific primer and HPLC purified 50 bases 3’ thiolated primer were designed. This primer was dissolved in 1M DTT (dithiothreitol) at a concentration of 100 μM and left to stand at room temperature for 10 min. The resulting primer was then stored at -20°C. Immediately before use, the primer solution was thawed, and extracted three times with ethylacetate. The aqueous phase was used for the gold nanoparticles binding experiment. 100 μl of an aqueous phase solution containing DNA was added to 250 μl of an aqueous gold nanoparticles solution. The resulting mixture was stirred for 30 min and 60 min. The products of DNA linked to the gold nanoparticles were characterized by UV-vis spectroscopy, electrophoresis and TEM. With electrophoresis, the product was loaded onto 3 wt% polyacrylamide gel (0.5x Tris-Borate-EDTA as a running buffer), run at 7V/cm for 1 h and stained with ethidium bromide.

**Results and Discussion**

No reducing agent was used, only polysorbate 80 and KAuCl$_4$ were employed to obtain the reduced gold nanoparticles Au(0). The technique for synthesizing the Au(0) nanoparticles was simple compared to that reported previously [14-16]. A set of typical color changes for the preparation of Au(0), were observed during the course of the reaction at room temperature. Initially, the solution turned from yellow to colorless. However, after 3h, the solution turned pink and finally red after 5h. The gold surface plasmon band, which is indicative of Au(0), was observed after 5h at room temperature. The characteristic surface plasmon band in the UV-vis spectra of the gold nanoparticles in the range of $\lambda_{max}$=520~540 nm was observed (figure 1 $\lambda_{max} = 525$ nm). This is related to the characteristic surface plasmon band of gold nanoparticles [6], corroborating the formation of Au(0).

Fig. 1. UV-vis spectrum of gold nanoparticles synthesized using polysorbate 80 in water.
Figure 2 shows a TEM image of the Au(0) nanoparticles synthesized in an aqueous 1 wt% polysorbate 80 solution. The gold nanoparticles had a spherical and hexagonal shape with a particle size ranging from 5 nm to 20 nm, as shown in figure 2.

A non-ionic surfactant-induced reduction mechanism of a gold salt is proposed that may involve the repeat unit(-CH$_2$-CH$_2$-O-) (scheme 1) of polysorbate 80 in the reaction with gold nanoparticles. Polysorbate 80 has an oxyethylene group(-CH$_2$-CH$_2$-O-) and is an efficient reducing agent as a non-ionic surfactant.

The gold nanoparticles and thiolated DNA made the product, which DNA linked with gold nanoparticles by sulfonation. The peak of gold nanoparticles-DNA was shifted to a longer wavelength at 534 nm (figure 3 b and c) in water (from at 525 nm (figure 3 a)) compared to gold nanoparticles.

Scheme 1. Chemical structure of polysorbate 80.

Fig. 3. UV-vis spectra of the gold nanoparticles (a), gold nanoparticles reacted with DNA by stirring for 30min (b), gold nanoparticles reacted with DNA by stirring for 60 min (c).
Figure 4 shows a TEM image of the gold nanoparticles-DNA aqueous solution. The gold nanoparticles-DNA aggregated in an aqueous solution after stirring for 90 min in figure 4. The shape of the DNA linked with gold nanoparticles is shown in figure 4 as a thread line connected to small spots on the outside of the gold nanoparticles. This means that the DNA is combined with gold nanoparticles. The size of the DNA linked with gold nanoparticles ranged from 5 nm to 20 nm in diameter, as shown in figure 4.

![TEM image](image_url)

**Fig. 4.** TEM image of the DNA linked with gold nanoparticles.

The binding ability of Au nanoparticles was checked using a DNA oligomer. The DNA linked with gold nanoparticle samples were loaded on 3 wt% polyacrylamide gel (0.5x Tris-Borate-EDTA as a running buffer), run at 7V/cm for 1 h and stained with ethidium bromide. As shown in Figure 5, the gold nanoparticle-unbound DNA molecules (2) migrate faster than the gold nanoparticle-bound DNA molecules (1).

**Conclusions**

Polysorbate 80 can act as both a reducing agent and stabilizing agent to produce gold nanoparticles in an aqueous solution at room temperature. UV-vis spectroscopy and TEM confirmed that the products were reduced gold nanoparticles. DNA linked with gold nanoparticles was prepared using the gold nanoparticles and thiolated DNA. UV-vis spectroscopy, TEM, and electrophoresis confirmed that the gold nanoparticles were linked to the 3'-thiolated DNA oligomer by sulfonation.

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