Fabrication of Size-tunable Gold Nanoparticles Using Plasmid DNA as a Biomolecular Reactor

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Gold nanoparticles (AuNPs) have been synthesized largely because of their wide range of potential applications, including drug delivery systems, catalysts, optical sensors and antimicrobial agents [1-5]. However, the harsh conditions employed in several synthetic approaches have forced researchers to investigate milder routes [6]. Biological macromolecules such as proteins [7], viruses [8], and plasmid DNA [9] have been shown to be successful candidates to ensure a milder pathway in the formation of AuNPs. Many of the aforementioned methodologies employing biological precursors present other drawbacks such as lack of size tunability, broad dispersity, and poor shape control partially due to the tendency of cationic gold to disproportionate in aqueous solutions [10], as well as the difficulties in stabilizing metallic NPs. Plasmid DNA has proven to be a successful template because it is readily available, inexpensive, and malleable i.e. it can exist in different topologies [9]. Using different plasmid DNA species as sacrificial molds (i.e. in their toroidal condensation states [9]) and employing a photo-oxidative synthetic approach, we previously have been able to stabilize and control the size of AuNPs.

Here we present a novel synthetic method combining plasmid DNA and organic gold salts in an aqueous medium. Implementing a kinetically based, rather than a photo-oxidative approach, we have been able to stabilize and control the size of AuNPs. The plasmid DNA utilized (pcDNA 3.1(+)/GFP, approx. 6kbps) acts as a reactor to initiate and control the formation of AuNPs. This approach provides a facile procedure that uses less energy, time, and fewer chemicals than most previously reported methods. The size of the AuNPs can be kinetically controlled by varying the incubation times (Table 1).

A DNA suspension (35ng/μl in TE buffer, pH 8) was incubated with gold metal salts dissolved in acetone (0.5 wt. % chloro-trimethyl phosphine-gold (I), referred as gold phosphine solution) in the dark at 70^{o}C. The size tunability of the AuNPs was later verified by TEM measurements to display correlation between UV-Vis data and corresponding incubation times (Table 1, Fig. 1 & 2). The metallic nature of the AuNPs was confirmed by superimposing the ED pattern obtained from the experimental samples with that of the gold standard (Fig. 3). The DNA fragments, formed upon template degradation, act as capping agents and ensure NP stability over time (Fig. 4).

The results demonstrate an easy method to synthesize size tunable spherical AuNPs, usually very challenging to obtain when biological macromolecules are employed. The high reproducibility of the results is also due to the minimization of unstable parameters, e.g. the varying rates of reduction of the metal salts initiated by UV light (oxidation of DNA [9]) or chemical reductants due to inherent concentration changes and diffusion.

Since linearized plasmid DNA does not yield the same results, circular plasmid DNA is essential for maintaining a narrow dispersed NP outcome (Fig. 5). Finally, the employment of mild synthetic conditions makes this method environmentally friendly while the simplicity of its procedural steps as well as the variety of plasmid utilized ensures general feasibility.

[1] S. Yugang, X. Younan, *Science Magazine* 298 (2002) 2176
[2] J. Wagner, J.M. kohler, *ACS nano letters* 5 (2005) 685
[3] N. Jana, L. Gearheart, C. Murphy, *Langmuir* 17 (2001) 6782
[4] S. Shanmugam et al., *Materials Chemistry and Physics* 95 (2006) 51
[5] H. Hiramatsu, F. Osterloh, *Chemistry of Materials* 16 (2004) 2509
[6] J. Campelo et al., *Green Chemistry* 10 (2008) 853
[7] P. Ravindra, *Jmseb* 163 (2009) 93
[8] J. Slocik et al., *J. Mater. Chem.* 15 (2004) 749
[9] J. Samson et al., *ACS Nano* 3 (2009) 339
[10] J. Kimling et al., *J. Phys. Chem. B* 110 (2006) 15700

INCUBATION TIME (h)	SIZE NPs (nm)	
	PLASMID IN TE BUFFER	NO PLASMID (CONTROL)
1	6+/-2 narrowly dispersed NPs	6+/-2; 10+/-3 aggregated NPs
2	8+/-2 narrowly dispersed NPs	8+/-3, 14+/-3 aggregated NPs
4	9+/-2 narrowly dispersed NPs	10+/-5 aggregated NPs
7	11+/-2 narrowly dispersed NPs	very aggregated NPs
12	18+/-2 narrowly dispersed NPs	N/A
15	21+/-2 narrowly dispersed NPs	N/A

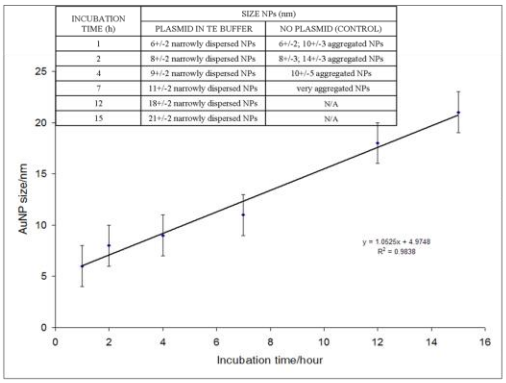

TABLE 1 Summary of the AuNP sizes yielded at different incubation times at 70°C in the dark in the presence and absence of plasmid DNA. The NP size using the plasmid DNA reactor is linearly correlated with time. In absence of plasmid, a correlation cannot be determined since all AuNPs are aggregated.

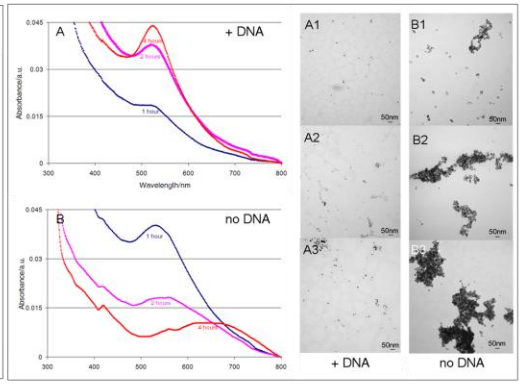

FIG. 1 A. UV-Vis spectra and TEM images of TE buffered plasmid-containing samples incubated with gold phosphine solution at 70°C in the dark for 1 (A1), 2 (A2) and 4 hours (A3), respectively. B. UV-Vis spectra and corresponding TEM images of TE buffer controls incubated with gold phosphine solution at 70°C in the dark for 1 (B1), 2 (B2), and 4 hours (B3), respectively.

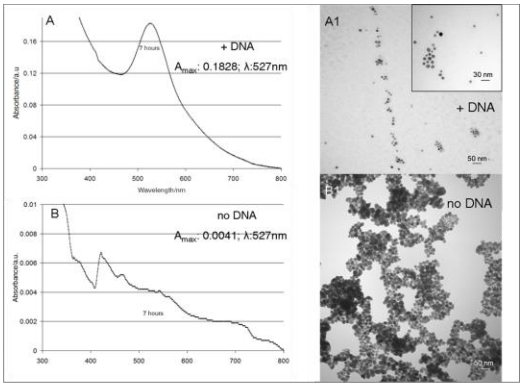

FIG. 2 A. UV-Vis spectrum and corresponding TEM image (A1) of TE buffered plasmid-containing samples incubated with gold phosphine solution for 7 hours at 70°C in the dark; Inset panel: higher magnification of sample in A1. B. UV-Vis spectrum and corresponding TEM image (B1) of TE buffer control incubated with gold phosphine solution under the same conditions.

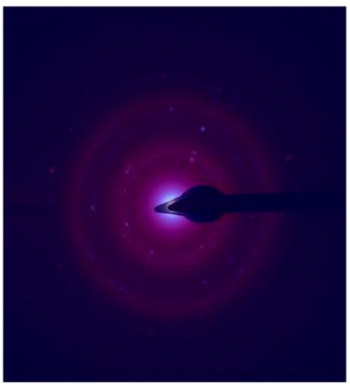

FIG. 3 ED pattern of gold standard solution (blue) superimposed on the experimental ED pattern obtained from AuNPs analysis (red).

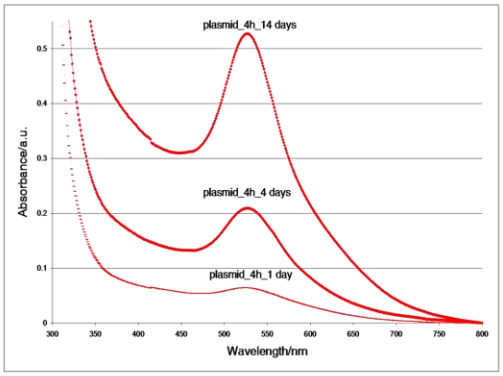

FIG. 4: UV-Vis spectra showing the peaks corresponding to the gold absorption of AuNPs. These NPs were prepared in the presence of the circular plasmid, incubated for 4 hours, and left undisturbed in the dark in TE buffer for up to two weeks indicating that the AuNPs are highly stable. The absence of a red shift confirms that the particles in solution neither re-aggregate nor collapse through time.

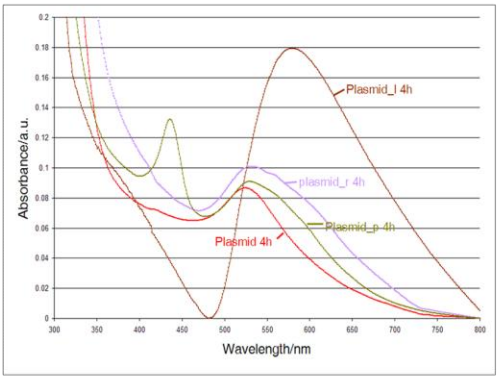

FIG. 5 UV-Vis spectra of circular plasmid (red) and control experiments (plasmid degraded by porphyrin: lime; plasmid with rearranged initial topologies: lavender; plasmid linearized by ECO RI enzyme: brown) incubated for 4 hours at 70°C. The circular DNA yields AuNPs with the highest degree of monodispersity