Journal of Nanoparticle Research **6:** 377–382, 2004.

Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology

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Received 13 April 2004; accepted in revised form 10 May 2004

Key words: gold, nanoparticles, oat biomass, pH, nanobiotechnology

Abstract

Oat (*Avena sativa*) biomass was studied as an alternative to recover Au(III) ions from aqueous solutions and for its capacity to reduce Au(III) to Au(0) forming Au nanoparticles. To study the binding trend of Au(III) to oat and the possible formation of Au nanoparticles, the biomass and a solution of Au(III) were reacted for a period of 1 h at pH values ranging from 2 to 6. The results demonstrated that Au(III) ions were bound to oat biomass in a pH-dependent manner, with the highest adsorption (about 80%) at pH 3. HRTEM studies showed that oat biomass reacted with Au(III) ions formed Au nanoparticles of fcc tetrahedral, decahedral, hexagonal, icosahedral multitwinned, irregular, and rod shape. To our knowledge, this is the second report about the production of nanorods as a product of the reaction of a Au(III) solution with a biological material. These studies also showed that the pH of the reaction influenced the nanoparticle size. The smaller nanoparticles and the higher occurrence of these were observed at pH values of 3 and 4, whereas the larger nanoparticles were observed at pH 2.

Introduction

Gold nanoparticles have been used for more than 400 years for the treatment of certain illnesses, and the staining of glass and enamels (Tanaka, 1999). Nowadays, the preparation of nanoscaled gold materials has become very important due to their unique properties, which are different from those of the bulk materials. The special properties of nanomaterials allow their potential utilization in a broad branch of new developing technologies such as catalysis, chemical industry, biotechnology, electronics and electro-optical devices (Brust et al., 1998; Martin & Mitchell, 1998; Tanaka, 1999; McConnell et al., 2000; Kohler et al., 2001; Troiani et al., 2003). These new technologies demand for better control over nanoparticle size and shape, characteristics that are associated to the chemical method used for the production of determined nanoparticles. It has been observed that Au colloids exhibit colors ranging from red, violet, or blue, which are associated with the particle size and shape (Turkevich, 1985a,b). It has also observed that the final shape and size of Au nanoparticles depend on the chemical method used for their production. The most common method to prepare these colloids is the chemical reduction of salts. However, other techniques such as ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction of Au, have also been widely used and investigated (Tolles, 1996; Magnusson et al., 1999;

Okitsu et al., 2001; Sau et al., 2001; Mafune et al., 2002). While these methods may successfully produce pure and well-defined Au nanoparticles, they remain expensive and sometimes involve the use of hazardous chemicals. In order to reduce the cost of the production of Au nanoparticles and the use of toxic materials, intensive efforts are being made in the development of cost effective and environmentally safe methods to reduce Au(III) and form Au nanoparticles. Bioreduction seems to possess these advantages. Successful gold(III) bioreduction and Au nanoparticle formation has been produced by using dead and live tissues of alfalfa (*Medicago sativa*) (Gardea-Torresdey et al., 1999, 2002a,b, 2003), dead biomass of hops (*Humulus lupulus*) (Lopez et al., 2004), fungus such as *Verticillium* sp. and *Fusarium oxysporum* (Mukherjee et al., 2001, 2002), and algae (Greene et al., 1986; Hosea et al., 1986; Kuyucak & Volesky, 1989). Previous studies with alfalfa biomass have indicated that pH is an important factor in the bioformation of colloidal gold (Gardea-Torresdey et al., 1999). The adsorption of Au ions to alfalfa biomass has shown to be independent of the initial solution pH. However, the resulting nanoparticle size greatly varied with pH. In this study, stalk dead-tissues of oat were investigated for the adsorption of Au(III) ions from aqueous solutions and the possible formation of Au nanoparticles. Batch experiments for pH studies were performed in order to have a better understanding of the mechanisms involved in metal binding and Au nanoparticle formation. In addition, high resolution transmission electron microscopy (HRTEM) was used in order to characterize the nanoparticles produced by oat biomass reacted with Au(III) at different pH values.

Experimental

pH profile experiments were performed following the procedure previously published by Gardea-Torresdey et al. (1999). A sample of 0.150 g of oat ground stems were weighed and washed twice with 0.1 M HCl and three times with deionized (DI) water in order to remove any material that could interfere with the binding of Au(III) ions to the biomass or the formation of the nanoparticles. The biomass was resuspended in 30 ml of DI water in order to have a final concentration of 5 mg of biomass per ml of solution. Using diluted concentrations of HCl and NaOH, a portion of the biomass suspension was adjusted to pH 2. Subsequently, aliquots of 2.0 ml each were taken and placed into three test tubes. These test tubes were centrifuged for 5 min at 3000 rpm in a Marathon Centrifuge (Fisher Scientific 6K) and the supernatants were discarded. Batch of three test tubes were adjusted to pH 3, 4, 5 and 6 using the same method described above.

In a separate beaker, a 0.1 mM Au(III) solution was prepared using $KAuCl_4$, reagent grade. A portion of this solution was adjusted to pH 2 using diluted HCl and NaOH and three aliquots of 2 ml each were transferred to the test tubes containing the oat biomass previously adjusted to pH 2. A separate aliquot was transferred to a clean test tube in order to use it as a control. The same procedure was followed for the different pH values. All the test tubes were then agitated for 1 h, followed by centrifugation. The supernatants were separated from the biomass and used for further gold analysis using flame atomic absorption spectroscopy (FAAS) (Perkin–Elmer model 3110). For the nanoparticle analysis, a drop of the supernatant was placed on 100 mesh carbon grid and was allowed to air dry. The samples were analyzed using a JEOL 4000EX high resolution electron microscope equipped with an EDS system.

Results and discussion

The percentage of Au(III) (from aqueous solution) bound to oat biomass is shown in Figure 1. As observed in this figure, the binding of Au(III) was pH dependent, in contrast to previous studies with alfalfa biomass (Gardea-Torresdey et al., 1999). The maximum Au(III) adsorption (81%) was achieved at pH 3, which indicates the potential use of oat biomass as an alternative biosorbent for the recovery of Au(III) ions from aqueous solutions. These results are slightly similar to previous studies performed with hops biomass where the binding of Au(III) was also higher at low pH values (Lopez et al., 2004). Figure 1 also shows that the percentage of Au(III) bound to the biomass decreased as the pH of the reaction increased reaching a minimum of about 15% at pH 6. This binding behavior suggests that the Au-biomass interaction is through ionic binding, since Au(III)

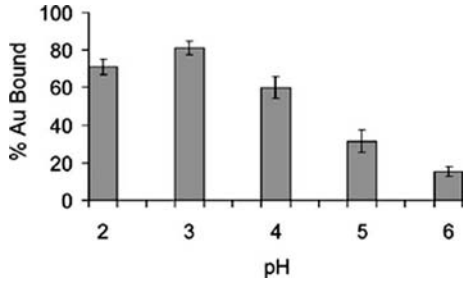

Figure 1. Percentage of Au(III) bound to oat biomass at different pH values. A gold solution of 0.1 mM was reacted with oat biomass for 1 h. The error bars indicate a 95% confidence interval.

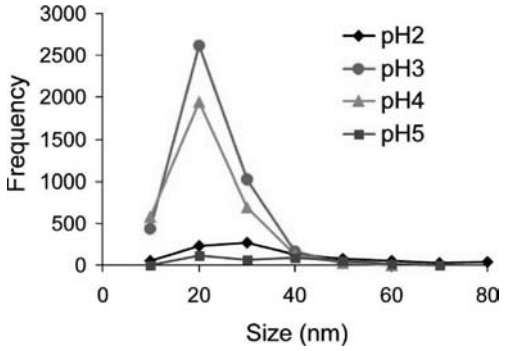

Figure 2. Size distribution of Au nanoparticles produced by the reaction of 0.1 mM Au(III) solution with oat biomass.

is present as an anion in aqueous solutions, and at low pH values, the biomass might carry more positive functional groups which allows the Au(III) ions to get closer to the binding sites. Previous research suggested that Au(III), which is a soft metal, binds to the biomass mainly through amino and sulfhydryl groups which are considered soft ligands and carry a more positive charge at low pH values, which makes them available for the binding and reduction of Au(III) to Au(0) (Gardea-Torresdey et al., 2002b). Also carboxylic groups, which are abundant in the biomass, are protonated at low pHs and could also contribute to the binding of Au(III) ions even though this group is considered a hard ligand (Gardea-Torresdey et al., 2002b).

HRTEM was used to determine the possible formation of Au nanoparticles when the oat biomass was exposed to the Au(III) solution at various pH values. The size distribution of nanoparticles formed by oat biomass at various pH values is shown in Figure 2. This figure shows that the reaction of Au(III) with oat biomass at pH values of 3 and 4 yielded nanoparticles with a narrower distribution in diameters. At pH 4, about 2500 nanoparticles were found with diameters ranging from 5 to 20 nm, and at pH 3 almost 2000 nanoparticles were found with similar diameter size. On the other hand, at pH 2 larger nanoparticles were observed with diameters ranging from 25 to approximately 85 nm; these nanoparticles however were present in small quantities compared to those observed at pH values of 3 and 4. The nanoparticles observed at pH 5 had a small diameter. However, these particles have the disadvantage of being in small quantities since only approximately 330 particles were observed. A similar variation in the size of the nanoparticles at different pH values have been previously reported by Goia and Matijevic (1999), who observed larger nanoparticles at pH values of 3 or lower, by reducing Au(III) with iso-ascorbic acid in aqueous solutions.

Different shapes of Au nanoparticles were produced by the exposure of Au(III) to oat biomass. Some of these shapes were fcc tetrahedral, decahedral, hexagonal, icosahedral multitwinned, irregular shape, and rod shape nanoparticles, which resembles the particles observed by the reaction of Au(III) ions with alfalfa biomass (Gardea-Torresdey et al., 1999). It is important to point out that we found the presence of rod shape nanoparticles at every pH value. Rod shape gold nanoparticles have been reported to be produced mainly by electrodeposition and to our knowledge, this is the second report about the production of nanorods as a product of the reaction of a Au(III) solution with a biological material (Armendariz et al., 2004).

Figures 3–6 are HRTEM micrographs of the nanoparticles formed by the reaction of Au(III) with oat biomass. Figure 3 corresponds to the nanoparticles produced by oat biomass at pH 2. As it can be observed from the nanoparticle size distribution showed in Figure 2, the nanoparticles formed at this pH are larger than those formed at other pH values. The diameter of these nanoparticles ranged from 25 to about 85 nm, and nanoparticles with more than 100 nm in diameter were also observed. It is well known that small nanoparticles tend to aggregate and form larger nanoparticles, especially at low pH values, which may explain the presence of larger nanoparticles

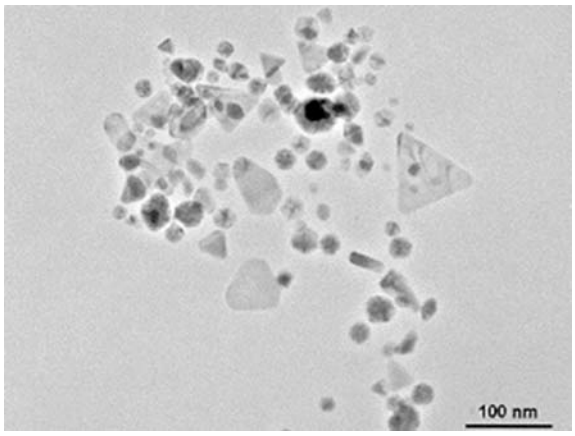

Figure 3. HRTEM micrograph of the gold nanoparticles produced by the reaction of 0.1 mM Au(III) solution with oat biomass at pH 2.

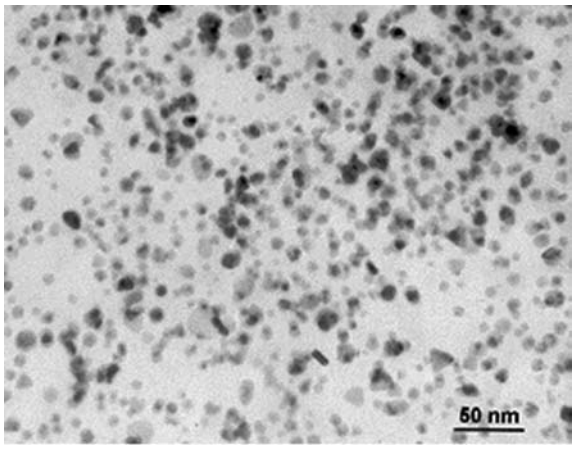

Figure 4. HRTEM micrograph of the gold nanoparticles produced by the reaction of 0.1 mM Au(III) solution with oat biomass at pH 3.

observed in this micrograph (Gardea-Torresdey et al., 1999, 2002b). Most of the nanoparticles formed at this pH had irregular shape, which could be the result of aggregation of nanoparticles. In addition, fcc tetrahedral nanoparticles were also observed.

Figure 4 is a micrograph of the nanoparticles obtained from the reaction of the Au(III) solution with oat biomass at pH 3. This figure shows that these nanoparticles are smaller than those produced by the oat biomass–Au(III) reaction adjusted at pH 2, which is confirmed by the size distribution graph shown on Figure 2.

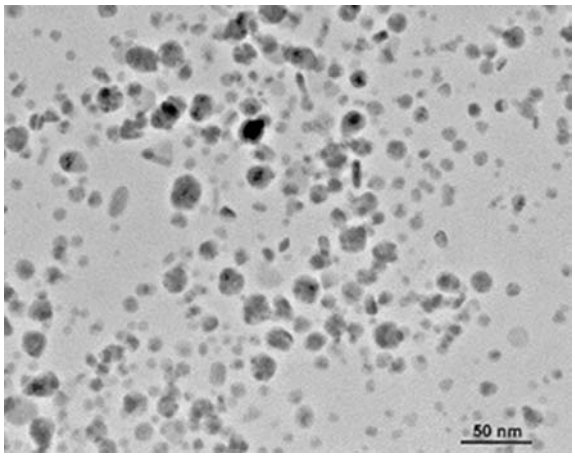

Figure 5. HRTEM micrograph of the gold nanoparticles produced by the reaction of 0.1 mM Au(III) solution with oat biomass at pH 4.

The majority of the nanoparticles formed at pH 3 had a diameter ranging between 10 and 20 nm; this indicates that there was a reduction in size at this pH, and an increase in the uniformity of the nanoparticles produced. In addition, at this pH, the number of rod shape nanoparticles increased compared to those observed at pH 2, where only one nanorod was observed.

Figure 5 shows the Au nanoparticles produced by oat biomass at pH 4. At this pH, the number of nanoparticles formed increased. In addition, the nanoparticles were smaller in size. This figure shows that most of these nanoparticles had an average size of 10 nm in diameter (see also Figure 2). The nanoparticle shapes observed at this pH are similar to those observed at previous pH values, which indicates that the variation in the pH of the reaction has a major impact on the size of the nanoparticles rather than on their shape. The regularity of the nanoparticles formed at this pH shows that oat biomass might provide an alternative for the controlled production of Au nanoparticles, which is essential for their use in nanotechnology.

Figure 6 shows a HRTEM micrograph of Au nanoparticles produced by the reaction of Au(III) solution with oat biomass at pH 5. The shape of the nanoparticles observed was similar to those obtained at previous pH values. In addition, it can be observed in this micrograph that there were fewer nanoparticles, but they have a slightly

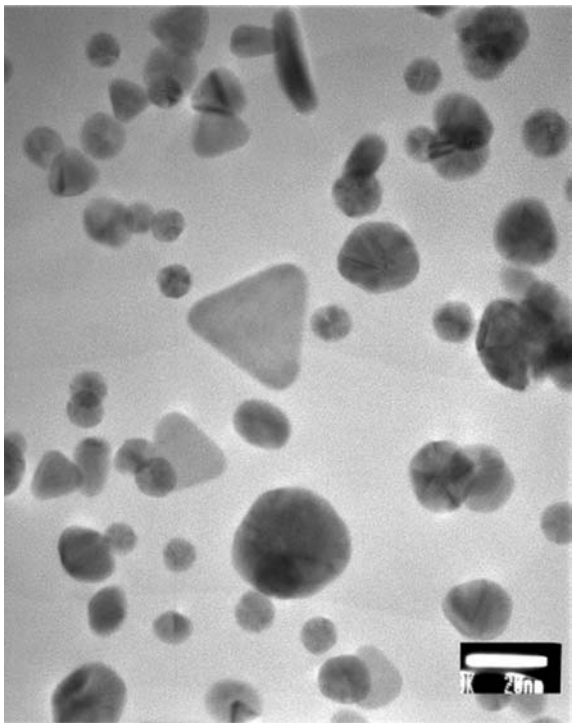

Figure 6. HRTEM micrograph of the gold nanoparticles produced by the reaction of 0.1 mM Au(III) solution with oat biomass at pH 5.

larger diameter as compared to those obtained at pH 3 and 4.

Overall, the size variation on the nanoparticles formed by oat biomass depended on the pH of the reaction. Large nanoparticles were found at pH 2, the size of the nanoparticles decreased at pH 3 and 4, and there was a slight increase in the size of the nanoparticle at pH 5 and 6, forming small irregular shape nanoparticles (the micrograph corresponding to pH 6 is not shown). This behavior indicates that at pH 2 the process of aggregation of Au particles to form large nanoparticles is favored over nucleation to form new nanoparticles. At pH values of 3 and 4, more functional groups could be available for gold binding, thus a higher number of Au(III) complexes can bind to the biomass at the same time, which allows the subsequent formation of larger amounts of nanoparticles with smaller diameters. However, at pH 5 the biomass carries an overall negative charge due to functional groups present in the biomass such as carboxyl groups (Gardea-Torresdey et al., 2002b). Therefore the negatively charged Au(III) ions do not easily approach the binding sites which prevent the binding of Au(III) and its reduction to Au(0) leading to fewer nanoparticles formed. Additionally, the same electrostatic repulsion could also prevent the growth and aggregation of small nanoparticles.

Conclusions

The results of this study show that oat biomass possesses the capacity to recover Au(III) ions from aqueous solutions forming Au nanoparticles of different shapes and sizes. Presumable, functional groups present in the cell walls of the inactivated tissues of the plant such as carboxyl, amino and sulfhydryl, may contribute to the reduction of Au(III) to Au(0) forming Au nanoparticles. Shapes such as fcc tetrahedral, decahedral, hexagonal, icosahedral multitwinned, irregular shape, and rod shape nanoparticles are formed when Au(III) is reacted with oat biomass at different pH values. On the other hand, a high number of nanoparticles of 20 nm in diameter were obtained at pH values of 3 and 4. Thus, the size of the nanoparticles can be controlled by changing the pH of the reaction. Finally, dead plant tissues, in this case oat biomass, could be used as an environmentally friendly method to produce Au nanoparticles.

Acknowledgements

The authors would like to acknowledge the National Institutes of Health (Grant S06 GM8012-33) and the University of Texas at El Paso's Center for Environmental Resource Management (CERM) through funding from the Office of Exploratory Research of the US Environmental protection Agency (cooperative agreement CR-819849-01). We also thank the financial support from the Southwest Center for Environmental Research and Policy (SCERP) program, and the HBCU/MI Environmental Technology Consortium that is funded by the Department of Energy. In addition, we acknowledge the financial support of the National Institute of Health (NIH) through the Minority Access for Research Careers Program

MARC and the support of the Model Institutes for Excellence Program MIE at the University of Texas at El Paso. Dr. Gardea-Torresdey also acknowledges the funding from the National Institute of Environmental Health Sciences (Grant R01ES11367-01).

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