

THE GREEN SYNTHESIS OF GOLD NANOPARTICLES USING THE ETHANOL EXTRACT OF BLACK TEA AND ITS TANNIN FREE FRACTION

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Abstracts: In this research the ethanol extract of black tea and its tannin free fraction used for green synthesis of gold nanoparticles. All the extracts were used separately for the synthesis of gold nanoparticles through the reduction of aqueous $AuCl_4^-$. Transmission electron microscopy and visible absorption spectroscopy confirmed the reduction of gold ions to gold nanoparticles. The ethanol extract of black tea and its tannin free ethanol extract produced gold nanoparticles in the size ranges of 2.5-27.5 nm and 1.25-17.5 nm with an average size of 10 nm and 3 nm, respectively. The prepared colloid gold nanoparticles, using the ethanol extract of black tea, did not show the appropriate stability during storage time (24 hours) at 4 °C. In contrast, gold colloids, which were synthesized by a tannin free fraction showed no particle aggregation during short and long storage times at the same conditions. To the best of our knowledge, this is the first report on the rapid synthesis of gold nanoparticles using ethanol extract of black tea and its tannin free fraction.

Keywords: *Camellia sinensis*, Gold nanoparticles, Synthesis, Tannin, Black tea, Chloroauric acid

1. INTRODUCTION

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology [1,2]. Today, nano metal particles, especially gold, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of chemistry, electronics, medicine and biotechnology at the nanoscale [2-4]. Gold nanoparticles could also have many new applications in biology; for example, they are used for the development of biosensors and DNA labeling [5, 6]. In medicine, gold nanoparticles are used for different proposes. For example, after cellular uptake, they can act as tiny, precise and powerful heaters (thermal scalpels) to kill cancer [7-8], and they are capable of inducing apoptosis in B-chronic lymphocytic leukemia [9].

Many reports have been published in the literature on the biogenesis of gold nanoparticles using several plant extracts, particularly Neem

leaf broth (*Azadirachta indica*), alfa alfa (*Medicago sativa*), *Eucalyptus camaldulensis*, *Pelargonium roseum* and [10-14]. Furthermore, very recently, a method for gold nanoparticles synthesis using a boiling extract of a conventional green tea bag (*Camellia sinensis*) has been published. Although the synthesis of gold nanoparticles, using a green tea sample, has been reported, the capacity of black tea leaves and its tannin free extract to form gold nanoparticles has not yet been understood. Black tea leaves, as a fully fermented product of *Camellia sinensis*, is the most popular tea in the world [15]. Through a limited screening process involving a number of black tea leaves used in Iran, we observed that the ethanol extract of black tea provided from Lahijan, the first town in Iran to have a tea plantation located in the Gilan province, was a potential candidate for the rapid synthesis of gold nanoparticles. *Camellia sinensis* leaves have been reported to contain considerable amounts of tannin products [16]. We removed the tannin from the ethanol extract of Lahijan black

tea and used it for the synthesis of gold nanoparticles. These plant extracts (with and without tannin) were used for the synthesis of gold nanoparticles by the reduction of aqueous AuCl_4^- . The characterizations of the prepared gold nanoparticles using the ethanol extract and a tannin free fraction of the plant sample (Lahijan black tea), was investigated before and after a storage period at 4°C and reported in this paper.

2. MATERIALS AND METHODS

2.1. Plants Materials and Extraction

Lahijan black tea leaves (*Camellia sinensis*) were supplied from the Lahijan Tea Research Center, Lahijan, Iran. The plants were pulverized 50g and the ethanol extract was prepared by macerating the powder for 48 h with three changes of the solvent at room temperature. Tannins absorbed by the Sephadex LH 20 beads [17] and all compounds, except the tannins, were removed by washing gently with 95% ethanol and the filtrate collected. The combined solvent extracts, with or without tannins, were separately evaporated to yield brownish, viscous residues. Different stock solutions (10 mg ml^{-1}) were prepared in ethanol for further tests. All experiments were performed on the basis of dry mass of the concentrated extracts.

2.2. Synthesis and Characterization of Gold Nanoparticles

An aqueous chloroauric acid solution (10^{-3} M) was added separately to the reaction vessels containing the ethanol extract of black tea and its tannin free fraction (10% v/v), and the resulting mixture was allowed to stand for 15 min at room temperature. Chloroauric acid was purchased from Merck, Darmstadt, Germany. The ethanol solution (10% v/v) was used as a negative control. The reduction of the Au^{+3} ions by these ethanol extracts in the solutions was monitored by sampling the aqueous component (2 ml) and measuring the UV–visible spectrum of the solutions. All samples were diluted three times with distilled water and the UV–visible spectra of these samples were measured on a Labomed

Model UVD-2950 UV-VIS Double Beam PC Scanning spectrophotometer, operated at a resolution of 2 nm. Furthermore, gold nanoparticles were characterized by transmission electron microscopy (model EM 208 Philips).

3. RESULTS AND DISCUSSION

3.1. Screening and synthesis of gold nanoparticles

The chemical reduction of the aqueous solution of chloroauric acid (HAuCl_4) is one of the most widely used methods for the synthesis of gold colloids [10-14]. In this study, the capacity of the ethanol extract of black tea provided from Lahijan, Gilan Province (Iran) to form gold nanoparticles was investigated. In a series of parallel experiments, the formation of gold nanoparticles by a tannin free ethanol fraction of

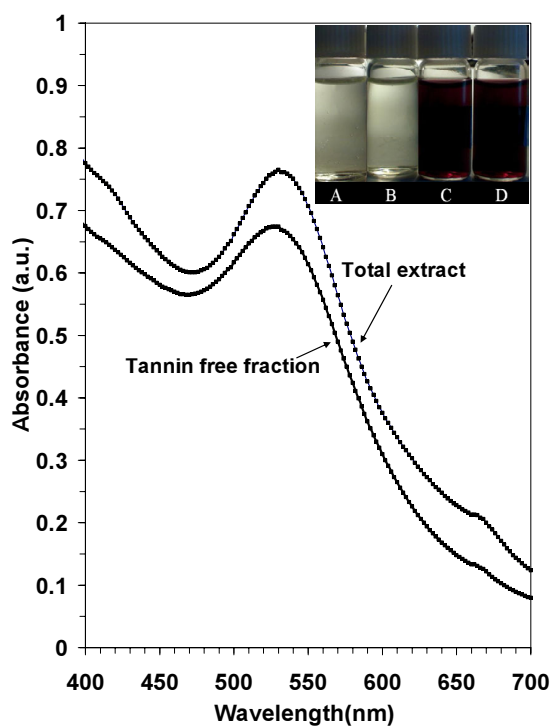


Fig. 1. UV-visible spectra of gold colloids: Spectra recorded after adding the ethanol extract of black tea and its tannin free fractions (10ml) to 90 ml of a chloroauric acid solution (1 mM). The curves are recorded after a period of 15 min. The inset shows a photograph of the solutions of chloroauric acid (1 mM) before (A, B) and after exposure to the ethanol extract of black tea (C) and its tannin fraction (D)

black tea was also investigated. The appearance of a purple color in the reaction vessels suggested the formation of gold nanoparticles with a size < 20 nm [18]. The inset to Fig.1 shows the tubes containing the black tea ethanol extract and its tannin free fraction before (tubes A and B) and after the reaction with Au³⁺ for 15 min (tubes C and D). The gold-containing solutions (tubes A

and B), that were a transparent yellow at first, turned into purple on completion of the reaction by the black tea ethanol extract (tube C) and its tannin free fraction (Tube D).

These reaction mixtures were further characterized by UV–visible spectroscopy. The technique outlined above proved to be very useful for the analysis of nanoparticles [15-17].



Fig. 2. Transmission electron micrographs recorded from a small region of a drop-coated film of chloroauric acid solution treated with the ethanol extract of black tea (upper left picture) and its tannin fraction (lower left picture) for 15 min (scale bars correspond to 50 nm). The related particle size distribution histograms (right side pictures) obtained after counting 350 individual particles for each sample.

As illustrated in Fig 1, a strong, surface plasmon resonance maxima of the ethanol extract of black tea and its tannin free fraction were centered at ca. 530 nm and 527 nm, respectively. This peak is assigned to a surface plasmon phenomenon that is well-documented for various metal nanoparticles with sizes ranging from 2 nm to 100 nm [19-21].

3.2. Particle Size Analysis

Figure 2 shows representative TEM images recorded from the drop-coated film of the as-prepared gold nanoparticles, synthesized by

treating the chloroauric acid solution with a total extract of black tea (upper left picture) and its tannin free fraction (lower left picture) after 15 min. The particle size histogram of gold particles, produced with the ethanol extract of black tea (upper right illustration in Fig. 2), shows that the particles range in size from 2.5 nm to 27.5 nm, and possess an average size of 10 nm. Furthermore, the tannin free fraction of black tea reduced Au^{3+} to gold nanoparticles (particle size from 1.25 nm to 17.5 nm) with an average size of 3 nm (lower right illustration in Fig.2). Fisher's Exact Test shows that the difference between the frequencies of the particle size of the gold

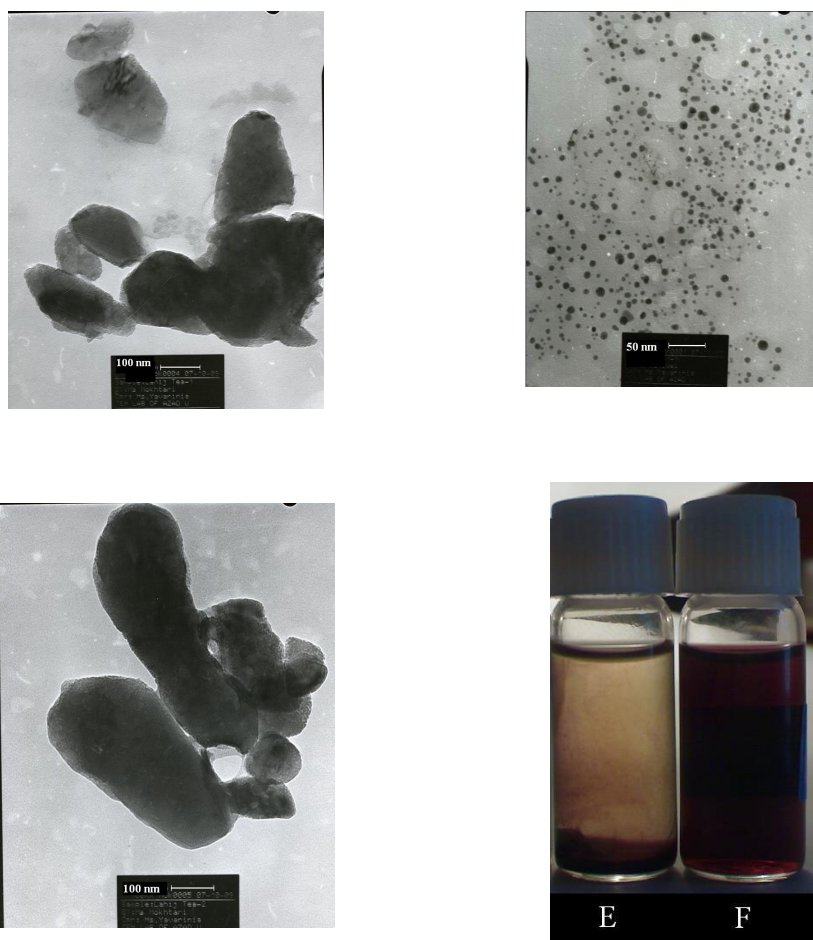


Fig. 3. Transmission electron micrographs of the stored gold nanoparticles at 40C for 24 hrs. The pictures on the left (scale bars correspond to 100 nm) show aggregated nanoparticles in the samples prepared with the ethanol extract of black tea. The upper right photograph (scale bars correspond to 50 nm) show TEM images of gold nanoparticles prepared by a tannin free fraction of black tea after a short storage time of 24 hrs at 40C. The lower right picture is a photograph of tubes containing gold nanoparticles synthesized by the ethanol extract of black tea (tube E) and its tannin free fraction (tube F) which was preserved for 24 hrs in a refrigerator (40C).

nanoparticles, prepared with the ethanol extract of black tea and its tannin free fraction, is statistically significant ($\chi^2(1) = 7.254$, $p < 0.05$).

The lower right picture in Fig. 3 shows a photograph of tubes containing gold nanoparticles prepared by the above method and reserved in 4°C for 24 hrs. We observed a considerable aggregation in the test tube containing the gold nanoparticles prepared by the total extract of black tea (tube E). In contrast, the gold colloids which were prepared using the tannin free fraction of black tea showed appropriate stability after 24 hrs at 4°C and we did not observed any aggregation in the sample during the short (tube F) or long storage times (6 months, picture not shown).

Representative TEM images of these samples, after 24 hrs of incubation in the refrigerator (4°C), are also shown in Fig. 3. The upper right photograph in this figure show gold nanoparticles synthesized by a tannin free fraction of black tea and kept in 4°C for 24 hrs. Gold nanoparticles have been aggregated, during the storage period (24 hrs) at 4°C, in the sample which was synthesized by a total extract of black tea gold. The photographs on the left in Fig. 3 demonstrated that these aggregated particles cause a visible sedimentation in the test tube (tube E, lower right picture in Fig.3). The total extract of black tea containing considerable tannin [16] and this compound may be involved in the aggregation of the nanoparticles during the storage period at 4°C. We observed no aggregation in the sample prepared by the tannin free fraction after 24 hrs at 4°C (Right picture in Fig.3) or that sample which kept for a longer storage time (6 months) at the same conditions (Figure not shown).

4. CONCLUSION

In conclusion, the potential ability of the ethanol extract of a black tea sample provided from Lahijan, Gilan Province, Iran and its tannin free fraction for the reduction of Au^{+3} to gold nanoparticles was investigated. Characterization by UV-visible, TEM, confirmed the reduction of gold ions to gold nanoparticles. To the best of our knowledge, and based on thorough literature

surveys, this is the first report on the synthesis of gold nanoparticles using the total extract of black tea as a fermented product of the *Camellia sinensis* leaves. When using this extract, the prepared colloid gold nanoparticles did not show the appropriate stability during the storage time (24 hrs) at 4°C. In contrast, the gold nanoparticles which were synthesized by the tannin free fraction showed a good stability for further application. Therefore, the tannin free fraction of black tea can be used successfully for the green synthesis of gold nanoparticles.

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