Biosynthesis of gold nanowires using sugar beet pulp

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A B S T R A C T

Sugar beet pulp was used as reductor and capping agent for the synthesis of gold nanowires. Reduction of tetrachloroaurate with sugar beet pulp is a simple, room temperature and environmentally friendly method. Polysaccharides and proteins are involved in the bioreduction and synthesis of nanoparticles. Different pH and molar concentration ratios of HAuCl4 were studied for the synthesis of gold nanowires. The formation of nanowires was induced by both basic mediums, due to the competence between biomolecules and hydroxide ions, and high concentrations of gold ions, because of the lack of capping agent to stabilize the preliminary nanoparticles formed that stick together producing wire-like nanostructures instead of nanospheres. This method allowed the synthesis of crystalline gold nanowires in the absence of a surfactant or polymer to direct nanoparticle growth, and without externally added seed crystallites. The synthesis of other metallic nanostructures such as silver and platinum could be achieved following a similar procedure.

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1. Introduction

The synthesis of nanoparticles with a controlled shape and size is one of the most promising research areas. The excellent properties of some materials strongly depend on crystallographic and morphological characteristics. One-dimensional nanostructures have unusual anisotropic properties. For example, if metal nanoparticles are smaller than approximately tens of nanometers, that is, the mean free path of a conduction-band electron in a metal, conductivity will decrease because electrons are scattered by the surface and, consequently, the effective optical constants decrease. In such case, electrons should be confined along the diameter of long metallic nanowires. Metallic nanowires are useful to connect different components in nanodevices. Metal nanorods and nanowires are able to absorb and scatter light along the long and the short axis because they have two plasmon bands [1]. These structures have been used as components in flexible electronics [2] and biological [3] or gas sensing applications [4].

Nanowires of noble metals have been fabricated using various methods such as wet chemical synthesis based on a seed mediated growth mechanism [5], solutions phase methods based on capping agents [6], reverse micellar or polyol process [7] and template-based methods using nanoporous silica [8], polycarbonate membranes [9] or carbon nanotubes [10]. Self assembly of nanowires to integrate these structures directly into various devices has been developed [11]. Furthermore, top-down methods based on nanolithography and laser ablation have been studied [12]. Nanowires have also been obtained by physical methods like photochemistry [13], radiolysis [14] and sonochemistry [15].

At present, biological methods have an increasing interest because of the necessity to develop new clean, cost-effective and efficient synthesis techniques. Lately, many biological systems such as microbes [16,17], fungi [16,18] and several plant extracts [16,19,20] have been investigated due to their ability to reduce metal ions and form nanoparticles. However, the production of biologically inspired nanostructures of a desirable size and morphology is critical to their commercial applicability. The synthesis of gold nanowires using biological resources has hardly ever been reported. Recently, Chen et al. synthesized networked nanowires with an extract of Rhodopseudomonas capsulata modulating the concentration of HAuCl4 [21].

In this work, biosynthesis of gold nanowires has been investigated using aqueous chloroaurate ions and sugar beet pulp as a clean technology to recover gold from dilute solutions. To our knowledge, biosynthesis of gold nanowires using industrial wastes as reducing agent has not been reported yet. Sugar-beet (Beta vulgaris) pulp is a residue obtained by heating sugar-beet cossettes in water during the extraction of the juice used for the crystallization of sugar. The morphology of the nanoparticles produced could be controlled varying the initial pH value and the concentration of gold ions in the reaction medium.

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2. Experimental

2.1. Materials

All chemical reagents including chloroauric acid (HAuCl₄), sodium hydroxide flakes and hydrochloric acid (37%) were obtained from Panreac Spain and used as received.

2.2. Sugar beet pulp preparation

The sugar-beet pulp was provided by Azucarera Ebro Agrícola plant in Toro (Zamora, Spain). The pulp was collected directly from the final drying line to ensure freshness and easy-handling compared to the pelletized form. The sugar-beet pulp was washed repeatedly with tap water and filtered through a cheese-cloth to remove the molasses. The pulp was then dried in a stove at 60°C and ground with an agate mortar. In batch experiments, a pulp particle size lower than 0.5 mm was used.

2.3. Synthesis of gold nanoparticles

The experimental method consists of mixing 50 mL of aqueous solution of tetrachloroaurate with sugar beet pulp (5 g/L) and stirring the mixture at room temperature. The influence of solution pH (9, 10, 11) was investigated in 100 mg/L gold aqueous solutions. The influence of gold ions concentration (50, 100, 200 mg/L of HAuCl₄) was studied at pH 10. The biomass was removed from the reaction mixture to analyze the samples at several times and the biomass after the process. The mixture was filtered using nylon membrane filters 0.2 μm from Whatman. After 5 h of reaction, the pulp was separated by centrifugation (4500 rpm) for 10 min and washed four times with deionized water.

2.4. UV–vis absorbance spectroscopy studies

The UV–vis spectra of samples at different pH values and gold ions concentration were analyzed using a Libra S11 single beam spectrophotometer operated at a resolution of 5 nm with quartz cells. Blanks of each sample set were prepared with deionized water.

2.5. TEM measurements

The shape of the gold nanoparticles was observed by transmission electron microscopy. TEM samples of the gold nanoparticles synthesized were prepared by placing drops of the product solution onto carbon-coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on a JEOL model JEM-2000FX instrument operated at an accelerating voltage of 200 kV.

2.6. FTIR measurements

Previous to FTIR analysis, the biomass before and after the reduction was dried, and gold nanoparticles biosynthesized were centrifuged at 5000 rpm for 20 min and then dried. These samples were pelletized mixing 0.5–3 mg of sample (sugar beet pulp plus gold nanoparticles) with approximately 250 mg of KBr. The spectra were recorded with a Nicolet Magna 750 in the region of 500–4000 cm⁻¹ at a resolution of 4 cm⁻¹.

2.7. pH and redox potential measurements

pH and redox potential of the gold solution (2 mL) were measured at the end of process using a pH meter Crison Basic 20.

3. Results and discussion

The present work was focused on the development of a biosynthetic method for the production of gold nanowires using green chemistry. Authors proposed an efficient exploitation of industrial wastes from sugar production. Sugar beet pulp contains organic acids, amino acids and proteins as well as the important presence of saccharides, which provides reduction power for the gold nanostructures preparation. In order to improve the nanowires formation, two variables in the reaction between aqueous chloroaureate and the components in sugar beet pulp were studied: the initial solution pH and the concentration of HAuCl₄.

3.1. Influence of pH

The first variable studied in this process was the initial pH value of the tetrachloroaurate solutions in different alkaline mediums.

The effect of hydroxyl ions in the shape and size of the nanoparticles synthesized using sugar beet pulp has been previously reported by our group [22]. In this study, the morphology of the nanoparticles obtained at different initial pH values was examined using UV–vis spectroscopy and TEM observations. We found the possibility of controlling the nanoparticles shape through the dependence on solutions pH producing polygonal nanoparticles at acidic mediums and nanowires in basic conditions. The especial properties of gold nanowires as well as their great number of applications suggested the optimization of the process in order to improve the nanowires production.

It is well known that the differences in UV–vis absorption spectra and color of solution could be dependent on different surrounding mediums and size, shape and structure of the gold nanoparticles. The color changed from pale yellow to pink or blue depending on the pH tested, indicating a change in metal oxidation and the formation of gold nanoparticles. As shown in the inset in Fig. 1, the color of the colloidal gold solutions is a function of pH. Fig. 1 shows UV–vis absorption spectra of the nanoparticles solutions obtained at different pH values. At pH 9 and 10, the spectra exhibit a unique resonance wavelength at approximately 530 nm associated to the formation of the nanospheres since all their electronic oscillations are equivalent. Unlikely, at pH 11, the absorbance curve is practically flat in the range of 500–825 nm and corresponds to the light blue sample. Generally, one dimensional nanostructures exhibit two plasmon absorption peaks with energies characteristic of the longitudinal and the transversal axes of these particles. In this case, the two peaks cannot be observed due to the overlap of the longitudinal absorption of the nanowires with different aspect ratios at relevant wavelengths [21,23].

The corresponding TEM images for the samples at different pH values are shown in Fig. 2. At pH 9, the nanoparticles are spherical and small, approximately 10 nm of diameter (Fig. 2a). The biomolecules acted as capping agent and kept them stable in solution. Increasing alkalinity at pH 10, the particle size increased up to 25 nm because of the aggregation of gold nuclei to form nanorods (Fig. 2b). Finally, very thin nanowires were obtained at pH 11 (15 nm of diameter) (Fig. 2c). HRTEM was employed to observe the structure of the resulting nanowires. The coexistence of nanowires and multi-shaped nanoparticles is shown in Fig. 3a. In consequence, the production of gold nanowires is not optimum controlling the pH but this variable allows to improve the yield of wires obtained. Fig. 3c shows the fine nanostructure of one section of the gold nanoparticles presented in Fig. 3a. Moreover, elemental gold peaks were found in the EDS study to confirm the nature of the metallic wires (Fig. 4).

These results show that nanowires were produced increasing solution pH. The explanation for this probably involves Ostwald ripening and the capping action of the biomolecules. In spite that...
molecules of the biomass were available, there may be competition from the hydroxide ion for the gold ion. In fact, hydroxide is a strong complexing agent of gold ion ($\log K_{3} = 38.6$) and could interfere on the capping ability of molecules present in the sugar beet pulp [24, 25].

3.2. Influence of gold concentration

The effect of the solution pH in the production of gold nanowires led us to investigate Ostwald ripening effect at high pH value increasing the concentration of HAuCl$_4$. In Ostwald ripening, smaller particles dissolve preferentially with subsequent crystallization onto larger particles, which involves nucleation and growth processes of larger particles from smaller ones.

The solution color changed from pale yellow to pink or blue depending on the tetrachloroaurate concentration, as shown in the inset of Fig. 5. It could be concluded that the concentration of gold ions in solution affects the shape and size of the nanoparticles obtained using sugar beet pulp. UV–vis absorption spectra of solutions at 50 and 100 mg/L show only one peak indicating the presence of nanospheres (Fig. 5). The red-shifted of the surface resonance peak at higher concentration is an indication that the size of nanoparticles increased. Furthermore, the flat spectrum recorded with the gold concentration of 200 mg/L is related to the formation of nanowires with different aspect ratios.

TEM images of the samples at different gold concentrations showed: spherical nanoparticles with a size about 15 nm at 50 mg/L (Fig. 6a), aggregated with a size about 25 nm at 100 mg/L (Fig. 6b) and nanowires of less than 50 nm of diameter at 200 mg/L (Fig. 6c). At the lowest initial gold concentration, the amount of capping agent was enough to stabilize the first particles formed in solution. Increasing concentration, gold nanoparticles size increased since they were not thermodynamically stable and stuck together. At the highest concentration, gold nanowires were formed by aggregation of tiny gold particles from large spherical aggregates. The TEM image in Fig. 6d is the SAED pattern of the corresponding gold nanowire. The electron diffraction spots could be attributed to the diffraction of (1 1 0) zone, indicating that the synthesized nanowires were monocrystalline. The formation of these nanowires can be related to the Ostwald ripening due to the collision and join between small nanoparticles (Fig. 7) [23].

3.3. Functional groups involved in the biosynthesis of gold nanowires

The nature of the biomolecules involved in the reduction and formation of gold nanoparticles was studied by FTIR analysis of the biomass before and after reduction of the nanoparticles (Fig. 8).

The FTIR spectrum of the unreacted sugar beet pulp shows bands at 1742 and 1636 cm$^{-1}$ (Fig. 8a). The first band is characteristic of stretching vibrations of the carbonyl functional group in ketones, aldehydes and carboxylic acids. The second absorption at 1636 cm$^{-1}$ corresponds to the amide I band. The intense broad absorbance at 3412 cm$^{-1}$ is attributed to the O–H stretching vibration modes of hydroxyl functional group in alcohols and N–H stretching vibrations in amides and amines. Moreover, the 1059 cm$^{-1}$ band can be assigned to C–O stretching vibrations. The absorption peak at 2930 cm$^{-1}$ corresponds to C–H stretching vibrations of the hydrocarbon chains.

The spectrum of the biomass after gold reduction shows the same bands that in the untreated biomass (Fig. 8b). The main difference between both spectra is that the treated biomass exhibits peaks of less intensity for the amide I band. This suggests that proteins are responsible for Au (III) reduction and gold nanoparticles stabilization. In addition, polysaccharides in the sugar beet pulp would be involved in the reduction process and their hydroxyl groups oxidized to carbonyl groups since the corresponding band at 1742 cm$^{-1}$ increased its relative intensity.

The particles would have been stabilized in solution by the capping agent provided by proteins of the sugar beet pulp. FTIR analysis of gold nanoparticles shows the presence of three bands at 1635, 1541 and 1457 cm$^{-1}$ (Fig. 8c). The two first absorptions are...
characteristic of amide I and II bands, respectively. The other band at 1457 cm\(^{-1}\) is assigned to the methylene scissoring vibrations of proteins. Another band at 1043 cm\(^{-1}\) can be related to S=O stretching vibrations. Sugar beet pulp may contain proteins which can act as capping and stabilizing agents of gold nanoparticles. The surface-bound is established through free amine groups or cysteine residues in the proteins [26,27].

Biosynthesis using beet pulp suggested the action of sugar as reducing agent and the role of proteins as stabilizing and/or capping agent. The coordination between amino groups and gold ions would be responsible for the stabilization of nanoparticles. These protein-conjugated nanoparticles have potential use as labels for living cells and tissues since they are not capped by toxic products and could be used in medicine and the pharmaceutical industry.

3.4. Mechanism of gold nanowires formation

Gold nanorods and nanowires have usually been synthesized by electrochemical reduction with cetyltrimethyl ammonium bromide (CTAB) and by seed-mediated growth method using a surfactant template. Reduction with sugar beet pulp is a method to produce nanowires in the absence of surfactant or polymer to control nanoparticles growth and without externally added seed crystallites.

As it is known, gold is a fcc metal and its surface energies associated with different crystallographic planes are usually different and the free energies decrease in the order \(\gamma_{\{111\}} > \gamma_{\{100\}} > \gamma_{\{110\}}\), because of the package and the distinct surface atoms density. Surfactants and capping agents usually can cap planes with \{100\} growth, which might lead to anisotropic growth of gold nanoparticles through \{111\} planes [28,29].
Aminoacids in the proteins, $\text{RCH(NH}_2\text{)}\text{COOH}$, are oxidized by a variety of agents and $\text{–NH}_2$ and $\text{–COOH}$ groups, in general, undergo chemical transformations. Furthermore, polysaccharides contain a great deal of hydroxyl and aldehyde groups that could be oxidized easily to form the structure of carboxyl releasing the electrons for the reduction of gold atoms. In our experiments, proteins in the sugar beet pulp might selectively bind to the $\{100\}$ facets of gold nanoparticles.

**Fig. 6.** Effect of gold ions concentration. TEM images of gold nanoparticles obtained at (a) 50, (b) 100, and (c) 200 mg/L. (d) SAED pattern of the gold nanowires.

**Fig. 7.** HRTEM images of the gold nanoparticles obtained at 200 mg/L HAuCl₄. (a) Representative image of nanoparticles sample and (b) detail of individual nanowire.
The experiment performed in a basic medium showed that hydroxide ions played a key role in the production of nanowires. In spite that the capping agent was available, competition between the biomolecules of the biomass and hydroxide ions for gold ions favored the aggregation of nanoparticles due to the lower ability of biomolecules to stabilize the nuclei formed. In turn, nanoparticles aggregation led to the formation of nanowires. This process could be favored not only in the presence of hydroxide ions but also with increasing gold ions concentration.

FTIR analyses indicate that proteins were the biomolecules that capped and stabilized gold nanoparticles in solution. Nevertheless, sugar beet pulp contains about 7–10% of proteins [30] and this low concentration of capping agent led to gold nanowires formation. A scheme of the mechanism of gold nanowires formation is shown in Fig. 9. The proteins of the biomass were insufficient to stabilize completely gold nuclei. The first gold nanoparticles formed were thermodynamically unstable due to their small size and the insufficient capping agent. The uncovered face of gold nanoparticles could orientate against each other through dipole–dipole interaction of biomolecules and the intermolecular hydrogen bonding interaction of biomolecules and hydroxide ions could act as possible driving forces for the formation of 1D assembly. In addition, the surface energy of larger particles is lower than that of smaller ones [29]. These small nanoparticles were suitable to dissolve in the solution and grow onto larger ones. The nanoparticles grew and joined together because of their Brownian motion in the solution, forming wire-like structures. The end of these nanorods may be bounded by {111} facets whose interaction with OH− and with biomolecules is very weak and thus reactive to the new formed atoms. This was followed by deposition of newly reduced gold atoms onto the concave region of the connected particles.

4. Conclusions

Reduction of tetrachloroaurate with sugar beet pulp is a simple, room temperature, efficient and clean method to synthesize gold nanostructures from dilute hydrometallurgical solutions. Advantages over other biological methods include the reuse of industry wastes, easy handling and scale-up.

Nanowires production can be controlled adjusting initial pH and chloroaurate concentration. Biomass proteins perform multiple tasks (reducing agent of gold ions and capping agent for nanoparticles).

The key points of this process are: competition between hydroxide ion and biomolecules for gold ions, the insufficient quantity of biomolecules to cap the nanoparticles and the preferential adsorption of AuCl4− on the gold nanoparticles surface.

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