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TEM characterization of silver and gold nanoparticles synthesized by a 'green' method using water extract of *Rosa Damascena* petals waste and beer yeast

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In this study we present "green" synthesis of Ag and Au nanoparticles by reduction of $AgNO_3$ and $HAuCl_4$ using water extracts of solid waste from *Rosa Damascena* essential oil industry, as well as pasteurized and live beer yeasts and combinations thereof. Morphology, microstructure and phase composition of the Ag and Au NPs obtained by ten different recipes are investigated by High Resolution Transmission Electron Microscopy (HRTEM) and Selected Area Electron Diffraction (SAED). Histograms of nanoparticles distributed by their diameters are constructed using the data acquired with Image J computer program. The histograms demonstrate that the predominant population of Ag NPs has diameter between 0 and 5 nm, regardless some of Ag NPs reach larger sizes, up to 50 nm. In the same time, the main part of Au NPs possesses diameters in the range 0–15 nm. The indexing of SAED patterns revealed the presence of cubic and hexagonal metal Ag and cubic metal Au phases. The relation between synthesis conditions, phase composition and size distribution of Ag and Au nanoparticles is followed and discussed.

Keywords: Ag and Au nanoparticles, green synthesis, *Rosa Damascena* water extract, beer yeast, TEM.

INTRODUCTION

Metal nanoparticles (NPs) with a variety of shapes and sizes are of the most studied nanomaterials due to their great potential for application in medicine, pharmacy and electrochemistry, as biosensors and catalysts for detection and elimination of biotoxins, etc. [1]. Silver (Ag) and gold (Au) NPs are of high importance, because of their specific properties – high thermal and electrical conductivity, chemical inertness, non-cytotoxicity, biocompatibility, antioxidative stress, antitumor and anticancer activity, possibility to provide tunable optical properties of glasses, etc. [2–4]. Despite the huge number of chemical and physical methods for synthesis of NPs (chemical, electrochemical and photochemical reduction, gas-phase synthesis and deposition, laser ablation, sol-gel techniques, etc.[5]), recently the efforts of the scientists have been focused on the development of new, perspective, cheap and environmentally friendly methods. Among them are the so-called "green" methods for synthesizing metal NPs[6], which are based on natural products and wastes from agricultural and food industries [7, 8]. One of the "green" methods is the plant extract-based method [9] using water extracts of leaves [10–14], barks [15], fruits [16, 17] and petals [18]. Another example of NPs "green" synthesis is the biosynthesis realized by microorganisms such as beer yeast [19, 20]. The rose oil industry is one of the most popular manufactures in Bulgaria. More than 5500 tons of rose petals were processed in 2015 [21] and almost as much is the solid waste. In the same time, the rose petals and the water extracts of rose petals are rich of polyphenols and flavonoids – compounds which are the main reducing agents in the metal NPs "green" synthesis. The mechanism of the oxidation-reduction reaction between Ag and Au precursors and polyphenols and flavonoids is described by S. M. Ghoreishi et al. [22]. According to our current research, the water extract of *Rosa*

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Damascena waste is also rich in polyphenols and flavonoids as Neochlorogenic acid, Caffeic acid, Quercetin, Myricetin, etc. and it is able to reduce the Ag^+ and Au^{3+}. The spent live and pasteurized beer yeasts are found to contain fewer amounts of phenolic compounds than *Rosa Damascena* waste water extract. That's why the beer yeasts are also able to reduce Ag^+ and Au^{3+}, but they start the synthesis of Ag and Au NPs relatively slowly. Indication for the presence of Ag or Au NPs is the color change of the solution from colorless to brown or black. Using *Rosa Damascena* water extract as reducing agent, the color is changed in few minutes, while for live beer yeast the color change is observed after 35 min and for pasteurized one – after 24 hours. It could be expected that the combinations of pasteurized and live beer yeasts with water extract of *Rosa Damascena* waste are able also to produce Ag and Au NPs relatively quickly. This is the way to intensify the process of synthesis of Ag and Au NPs using beer yeasts.

This study presents a detailed research of the microstructure and phase composition of the "green" synthesized Ag and Au NPs by means of water extracts of *Rosa Damascena* waste, spent live and pasteurized beer yeasts and combinations thereof. Data on the morphology, size distribution and phase composition of Ag and Au NPs, synthesized by the aforementioned method, contribute to clarifying the relation between preparation conditions and potential applications.

EXPERIMENTAL

Materials and methods

The *Rosa Damascena* wastes were obtained from ECOMAAT distillery (Mirkovo, Sofia region, Bulgaria, 2017 harvest). The beer yeasts both pasteurized and live were obtained from ABM Production Ltd. (Plovdiv, Bulgaria). Prior to the synthesis of Ag and Au NPs, the water extracts of *Rosa Damascena* and of both pasteurized and live beer yeasts were prepared according to procedure described as follows: 75 g dry rose waste was mixed with one liter distilled water for 60 min at 60 °C then left for 24 h at room temperature (22 °C) at constant stirring. The mass was filtered and the solid residue was treated with 250 mL water at the same conditions; the pasteurized beer yeast was dissolved in distilled water at 1% w/v at constant stirring for 24 h and then filtered; the live beer yeast was diluted with distilled water (1:13) to 1% dry content. Silver nitrate ($AgNO_3$) of Merck, Germany, and Chloroauric acid ($HAuCl_4$) of Sigma-Aldrich, USA, were used as initial substances for synthesis reactions. The "green" synthesis of Ag NPs was performed by mixing in five test tubes each 0.3 ml 0.01 M $AgNO_3$ with 0.1 ml distilled water and then the following extracts were added: 1) for sample 0B – 0.1 ml extract of *Rosa Damascena*; 2) for sample 1B – 0.1 ml pasteurized beer yeast; 3) for sample 2B – 0.1 ml live beer yeast; 4) for sample 3B – 0.1 ml extract of *Rosa Damascena* and 0.1 ml pasteurized beer yeast; and 5) for sample 4B – 0.1 ml extract of *Rosa Damascena* and 0.1 ml live beer yeast. The "green" synthesis of AuNPs was performed by mixing in five test tubes each 0.4 ml 0.001M $HAuCl_4$ and the following extracts: 1) for sample 0G – 0.1 ml extract of *Rosa Damascena*; 2) for sample 1G – 0.1 ml pasteurized beer yeast; 3) for sample 2G – 0.1 ml live beer yeast; 4) for sample 3G – 0.1 ml extract of *Rosa Damascena* and 0.1 ml pasteurized beer yeast; and 5) for sample 4G – 0.1 ml extract of *Rosa Damascena* and 0.1 ml live beer yeast.

The study of morphology, microstructure and phase composition of Ag and Au NPs was performed by TEM. High Resolution Transmission Electron Microscope JEOL JEM 2100 (JEOL Ltd., Japan) was used for acquisition of bright field images and SAED patterns of the samples at accelerating voltage of 200 kV. For TEM investigation, the samples were sonicated and then microquantities of their suspensions were dropped on standard Cu grids coated with amorphous carbon and finally dried in a clean atmosphere under ambient conditions.

Using ImageJ software [23] the particles' diameters were measured and their size distribution was presented as histograms. The phase composition of Ag and Au NPs was determined on the basis of SAED patterns by means of PCPDFWIN program and PDF-2 data base of the International Centre for Diffraction Data (ICDD).

RESULTS AND DISCUSSION

TEM micrographs of Ag and Au NPs synthesized by the receipt described in the Experimental section are presented in Figs. 1 and 2, respectively. The shown TEM images and size distribution histograms in the two figures correspond to reduction of Ag^+ or Au^{3+} by: a) water extract of *Rosa Damascena*, b) pasteurized beer yeast, c) live beer yeast, d) combination of water extract of *Rosa Damascena* and pasteurized beer yeast, and e) combination of water extract of *Rosa Damascena* and live beer yeasts. Representative SAED patterns and HRTEM micrographs are also included it the figures as Fig. 1f and g and Fig. 2f and g.

It is seen that the shape of AgNPs is spherical in all samples and the particles are relatively separated from each other. Organic components existing in

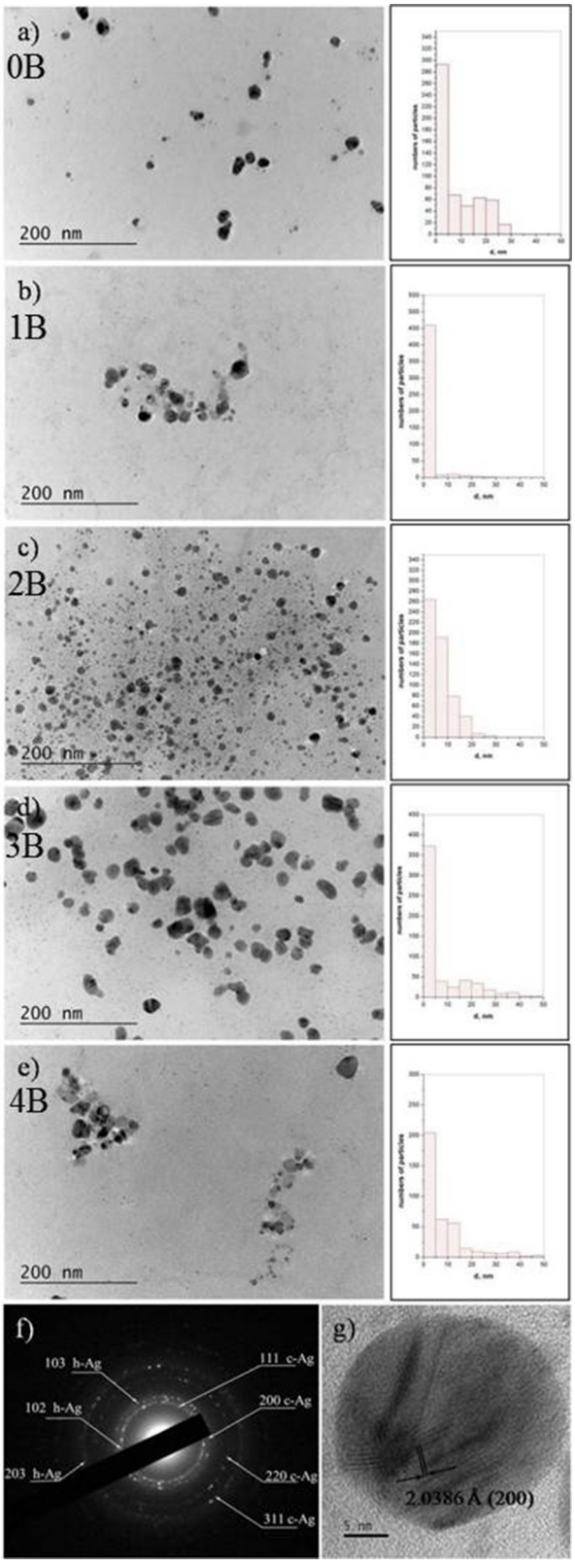

Fig. 1. TEM images and corresponding size distributions of Ag nanoparticles synthesized using reduction agents: water extract of *Rosa Damascena,* sample 0B (*a*); pasteurized beer yeast, sample 1B (*b*); live beer yeast, sample 2B (*c*); both water extract of *Rosa Damascena* and pasteurized beer yeast, sample 3B (*d*); both water extract of *Rosa Damascena* and live beer yeast, sample 4B (*e*); and representative SAED pattern (*f*); and HRTEM image (*g*).

the *Rosa Damascena* water extracts or beer yeasts solutions are also visualized as envelopes attached to NPs with less contrast than that of the metal NPs. They are well seen in some cases, especially in Fig. 1b and e. There are some procedures as centrifugation and dialysis, described in the literature [24], which allow the separation of NPs from the organic components in suspensions. These procedures were not applied in the present study because the organic components in the samples were amorphous and did not hinder the use of TEM or diffraction methods. The measured Ag NPs' diameters are in the nanometric scale, below 50 nm, with the maximal frequency in the interval 0–5 nm. Broad size distribution of Ag NPs is established for samples, reduced by *Rosa Damascena* water extract (Fig. 1a) and live beer yeast (Fig. 1c), while a narrow size distribution is observed for samples, reduced by pasteurized beer yeast (Fig. 1b). The reason is that pasteurized bear yeast contains the lowest quantity of polyphenols (1.53 μmol/100 g), compared to live beer yeast (8.4 μmol/100 g) and *Rosa Damascena* water extract (133.70 μmol/100 g). The low content of polyphenols, known as the main reducing agent of Ag^+, ensures limited number of Ag^0 in the solution and respectively the Ag NPs grow slowly and remain predominantly with small sizes, below 5 nm. Conversely, the polyphenol-rich water extract of *Rosa Damascena* predisposes to the formation of larger amounts of Ag^0 and larger size of Ag NPs. Therefore the size distribution is narrow for the sample with pasteurized beer yeast and broad in the case of *Rosa Damascena* water extracts. The NPs size distributions obtained for the mixtures of *Rosa Damascena* and the two beer yeasts (Fig. 1d and e) are also broad because of the effect of the rose extract. In addition, SAED pattern and HRTEM image recorded for the sample produced by the mixture of *Rosa Damascena* and pasteurized beer yeast water extracts are presented in Fig. 1f and g. Experimental SAED pattern consists of 7 diffraction rings, 4 of them corresponding to 4 interplanar distances of cubic Ag (S.G. Fm-3m, PDF 87-0720) and 3 of them – to hexagonal Ag (S.G. $P6_3/mmc$, PDF 87-0598). This result is in accordance with the investigations of E. Rodriges-Leon et al., who have also identified cubic and hexagonal silver in their samples, produced by "green" method [25]. In our study the two silver phases are found in all samples, except this one, reduced by pasteurized beer yeast, where the

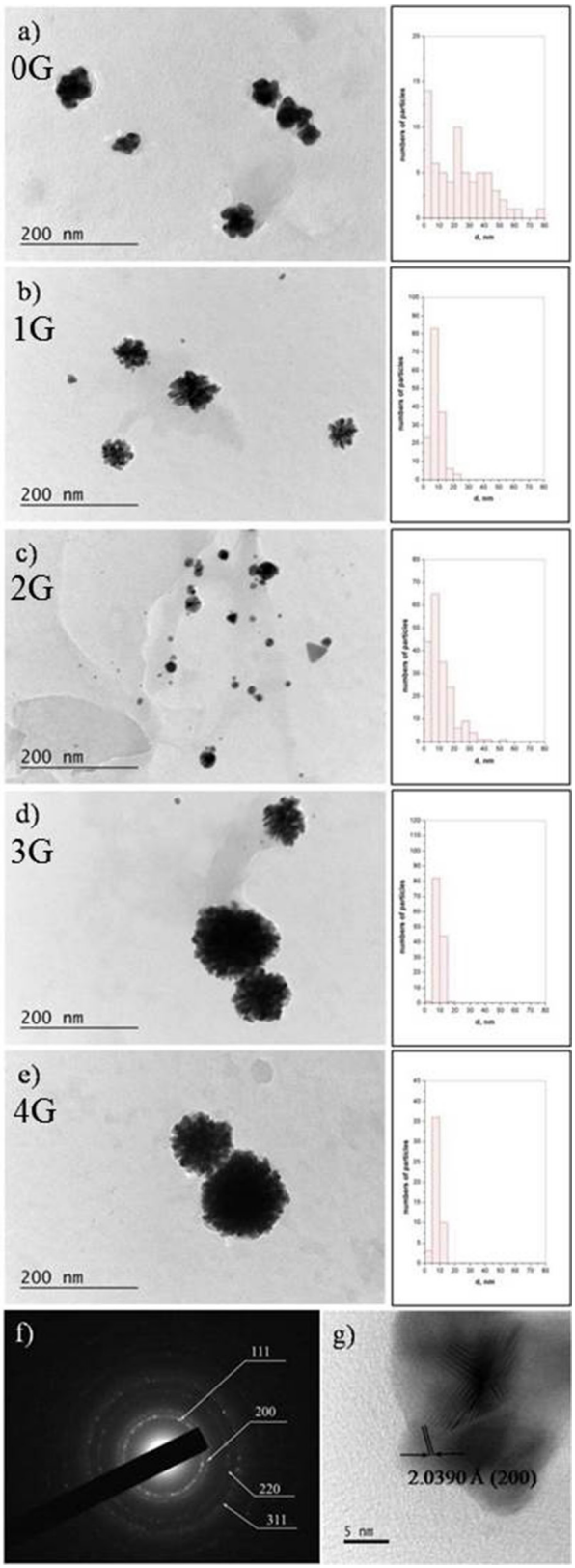

Fig. 2. TEM images and corresponding size distributions of Au nanoparticles synthesized using reduction agents: water extract of *Rosa Damascena*, sample 0G (*a*); pasteurized beer yeast, sample 1G (*b*); live beer yeast, sample 2G (*c*); both water extract of *Rosa Damascena* and pasteurized beer yeast, sample 3G (*d*); both water extract of *Rosa Damascena* and live beer yeast, sample 4G (*e*); and representative SAED pattern (*f*); and HRTEM image (*g*).

only phase is the cubic silver. In this sample, which is poor in polyphenols, the quantity of atomic silver is insufficient for growing of large Ag NPs and as a result the small Ag NPs with a cubic crystal structure dominate [25]. Hexagonal AgNPs appear in the solution when already formed small cubic AgNPs become seeds for following rapid growth of hexagonal Ag in Ag^0-rich medium [25]. Some of the diffraction rings in the pattern are diffusive, especially those that are close to the central beam. These rings are embedded by the amorphous hallo in the center of the pattern, which is due to the amorphous component in the solution. In addition, some of the inner rings, corresponding to two phases – cubic and hexagonal, are very close to each other and look as single, but vague ring. The presence of small Ag NPs, especially these with diameters less than 5 nm also influence the rings and make them diffusive.

Typical Ag NPs with lattice fringes at measured distance equal to 2.03 Å is presented in the HRTEM image in Figure 1g. This value correspond well with the interplanar distance d = 2.0386 Å of (200) planes in Ag face centered cubic crystal lattice. Similar particles are found in all samples. Although, the diffraction signal from h-Ag was registered by SAED, nanoparticles of this phase were not visualized in HRTEM mode.

From TEM images in Figure 2a–e it is seen that the shape of Au NPs is also spherical, but large aggregates are generally visualized for Au samples. However, in the peripheral areas of these aggregates the individual Au NPs are still distinguished so their shape and size could be defined. Gold NPs grow larger than silver ones and their diameters are within interval 0–80 nm as the prevailing part is below 15 nm. This is not concerned for the case of Au NPs, produced by *Rosa Damascena* water extract (Fig. 2a), where a broad size distribution of NPs is observed, similarly to the corresponding Ag NPs (Fig. 1a). SAED pattern and HRTEM image of a Au NP produced by mixture of *Rosa Damascena* water extract and pasteurized beer yeast are presented in Figure 2f and g. The indexing of SAED patterns gives evidence for the presence of face centered cubic Au (S.G. Fm–3m, PDF 04-0784) in all samples. These data are confirmed by HRTEM image in Figure 2g, which visualizes the lattice fringes corresponding to interplanar distance d_{200} = 2.0390 Å of the face centered cubic Au.

The synthesized and characterized in the present study AgNPs will be applied as a component of graphite electrode – catalyst for reduction of H_2O_2 and will be tested for amperometric quantitative determination of H_2O_2 and other analytes. As AuNPs, synthesized by *Rosa Damascena* water extract and beer yeasts tend to aggregate, it is needed to be stabilized and separated using surfactants [24] before finding an application.

CONCLUSIONS

The microstructure, phase composition and size distribution of Ag and Au NPs, synthesized for the first time by "green" method, using *Rosa Damascena* water extract and pasteurized and live beer yeasts are studied. The presence and co-existence of two Ag phases – face centered cubic and hexagonal and face centered cubic for Au NPs are proved by SAED and HRTEM. The shape of both Ag and Au NPs is spherical and their diameters are in the interval 0–50 nm for Ag NPs and 0–80 nm for Au NPs. The prevailing parts of Ag NPs are below 5 nm and of Au NPs - below 15 nm, which could be used in electrochemical, medical and other applications of these nanoparticles.

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