Hassan Korbekandi*, Rasoul Mardani Jouneghani, Soudabeh Mohseni, Meraj Pourhossein and Siavash Iravani

Synthesis of silver nanoparticles using biotransformations by *Saccharomyces boulardii*

Abstract: Green synthesis of nanoparticles (NPs) using microorganisms is ecofriendly and gives researchers greater opportunities in sizes and shapes of the synthesized NPs. The aims of this research were the biosynthesis of silver NPs by biotransformations using *Saccharomyces boulardii* and analysis of the sizes and shapes of the produced NPs. Dried (commercially available) and freshly cultured *S. boulardii* were used as the biocatalyst. Dried yeast synthesized few NPs, but freshly cultured yeast produced a large amount of NPs. It was seen from transmission electron microscopy (TEM) micrographs that individual silver NPs as well as a number of aggregates with almost spherical shapes and sizes of about 8–26 nm were synthesized. It was shown that the NPs were mainly synthesized intracellularly, which might be due to the reductases inside the cells. Therefore, the biomass should be used in the synthesis, instead of discarding it. Previous researchers used only the supernatant and reported that the synthesis is extracellular.

Keywords: biosynthesis; biotransformation; green synthesis; *Saccharomyces boulardii*; silver nanoparticles.

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1 Introduction

Recently, silver nanoparticles (NPs) have been of interest because of their unique properties which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, electronic components and cancer treatments [1]. Several physical and chemical methods have been used to synthesize silver NPs, but these methods suffer from drawbacks such as polydispersity, low yield and use of harsh, toxic and expensive materials. Therefore, there is a need to develop clean, nontoxic and ecofriendly synthetic approaches for synthesizing NPs [2–4].

Recently, microorganisms and biological systems have been used for green and ecofriendly synthesis of metal and metal oxide NPs [2–6]. Green biosynthesis of NPs using organisms is mainly advantageous in terms of environmental friendliness. However, major drawbacks associated with this method are longer reaction times, tedious purification steps, greater sizes of all NPs and poor understanding of the mechanism. Furthermore, optimization of the process is critical to fast and clean synthesis of NPs with desired sizes and shapes. Among the organisms, yeasts have received attention in the biosynthesis of NPs. For instance, Dameron et al. [7] reported that yeasts such as *Schizosaccharomyces pombe* and *Candida glabrata* produced intracellular CdS NPs when challenged with cadmium salt in solution. Yan et al. [8] showed the synthesis of zinc phosphate [$Zn_3(PO_4)_2$] NPs by chemical precipitation using yeast cells. The yeast *Torulopsis* sp. was capable of intracellular synthesis of semiconductor PbS nanocrystallites when exposed to aqueous Pb^{2+} ions [9]. They also showed that the CdS nanocrystals in the size range of 1–1.5 nm exhibiting ideal diode characteristics were synthesized intracellularly in *S. pombe* yeast cells. It was also illustrated that when MKY3 (silver-tolerant yeast strain) was challenged with 1 mM soluble silver in the log phase of growth, silver NPs (2–5 nm in size) were synthesized extracellularly [10]. Jha et al. [11] demonstrated that baker's yeast (*Saccharomyces cerevisiae*) was able to produce spherical antimony trioxide (Sb_2O_3) NPs. Sb_2O_3 could be used as an opacifier in porcelain, fining agents, a degasser and a catalyst for production of polyethylene terephthalate (PET) plastic [11]. Agnihotri et al. [12] reported biosynthesis of gold NPs by the tropical marine yeast *Yarrowia lipolytica* NCIM 3589.

***Corresponding author: Hassan Korbekandi,** Genetics and Molecular Biology Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, 81744-176, Iran, e-mail: korbekandi@pharm.mui.ac.ir
Rasoul Mardani Jouneghani and Soudabeh Mohseni: Science and Research Branch (Kurdistan), Islamic Azad University, Kurdistan, Iran
Meraj Pourhossein and Siavash Iravani: Genetics and Molecular Biology Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, 81744-176, Iran

S. boulardii is a nonpathogenic yeast, which is able to protect the intestine against *Clostridium difficile* and cholera toxins and is clinically effective in preventing antibiotic-induced diarrhea, diarrhea associated with tube-feeding in critically ill patients and recurrence of *C. difficile* infection [13]. *S. boulardii* grows fast and its industrial use is simple. It seems that *S. boulardii* is one of the best candidates for NP synthesis, and this green approach can also be suitably scaled up for large-scale synthesis of metal NPs. Therefore, the aims of this study were the biosynthesis of silver NPs by biotransformations using this yeast and analysis of the sizes and shapes of the produced NPs.

2 Materials and methods

2.1 Culture and media

2.1.1 Freshly cultured *S. boulardii*

Silver nitrate ($AgNO_3$, 99.99%) was purchased from Sigma-Aldrich (Steinheim, Germany). *S. boulardii* was purchased from Himedia (India). Seed culture was developed by inoculating a single colony of *S. boulardii* into growth medium (10 ml) containing peptone (3 g/l), yeast extract (3 g/l), and dextrose (10 g/l). The culture was incubated at 37°C for 24 h at 200 rpm after adjusting the pH to 7. Inoculum (2% v/v) was transferred to the production medium of the same composition and the culture was grown for 48 h at 37°C and 200 rpm. After sufficient growth, the cell mass was harvested by centrifugation (3080 *g*, 20 min, Gallenkamp centrifuge 200).

The reaction mixture contained the following ingredients (final concentrations): $AgNO_3$ (1 mM) as the biotransformation substrate, freshly cultured *S. boulardii* as the biocatalyst, glucose (56 mM) as the electron donor and phosphate buffer (pH=7, 100 mM). The aforementioned ingredients were added in appropriate volumes into Duran bottles (100 ml) and incubated (80 rpm, 25°C). Samples (1.5 ml×3) were taken from the reaction mixtures at different times and the absorbance (430 nm) of the colloidal suspensions of silver NPs (hydrosols) was read freshly (without freezing).

2.1.2 Dried *S. boulardii*

In another experiment, we used commercially available dried *S. boulardii* (0.1 g/ml) as the biocatalyst with the aforementioned reaction mixture containing phosphate buffer (pH=7, 100 mM), glucose (56 mM) and $AgNO_3$ (1 mM). These ingredients were added in appropriate volumes into Duran bottles (100 ml) and incubated (80 rpm, 25°C).

2.2 Characterization and analysis

2.2.1 UV-visible analysis

Absorption spectra were measured on a Shimadzu (UVmini-1240) spectrophotometer.

2.2.2 Transmission electron microscopy analysis

Transmission electron microscopy (TEM) was performed on selected samples to investigate the process of formation of silver NPs and to study the size and shape of them. Samples for TEM were prepared by drop-coating the Ag NP solutions onto carbon-coated copper grids. Micrographs were obtained using an EM 10C ZEISS (Germany) transmission electron microscope.

2.2.3 Dynamic light scattering analysis

In dynamic light scattering (DLS) analysis, particle size distribution of NPs was analyzed using Nano-Zeta Sizer (Nano ZS, ZEN 3600, Malvern Nano, UK).

3 Results and discussion

3.1 Visual inspection and UV-visible spectral analysis

When *S. boulardii* biomass was exposed to silver ions ($AgNO_3$, 1 mM), the color of the reaction mixture, turned to yellowish brown and then dark brown, which was in agreement with previous studies and was considered as the production of colloidal suspension (hydrosol) of silver NPs. The appearance of dark brown seems to be due to excitation of surface plasmon resonance in the NPs [14].

Most researchers have used the optical absorption of colloidal silver as an indicator of production of silver NPs [15–20]. We examined the UV-visible absorption spectrum of colloidal silver to verify this. As a result, the formation of silver NPs in the solution was confirmed by UV-visible spectral analysis. Figure 1 shows the UV-visible spectrum

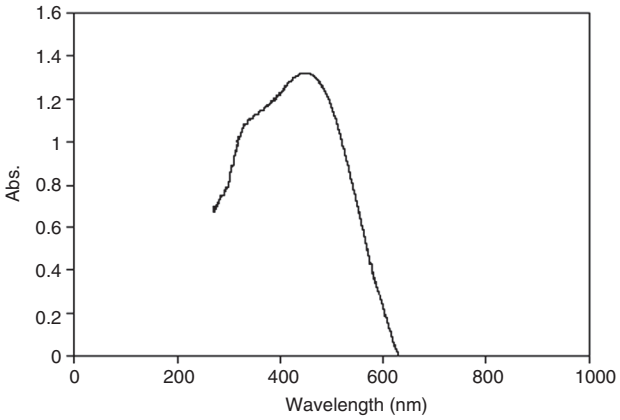

Figure 1 UV-visible absorption spectrum of the produced colloidal silver. The spectrum was obtained at 72 h after the start of $AgNO_3$ (1 mM) reduction using *S. boulardii*.

of the reaction mixture containing *S. boulardii* biomass and silver nitrate (1 mM) incubated for 72 h. The λ_{max} was about 430 nm. Strong absorption at 430 nm confirmed the formation of silver NPs in solution. Parallel control experiments containing only *S. boulardii* biomass (without silver nitrate) and silver nitrate solution (without *S. boulardii* biomass) did not show any absorption at about 430 nm.

3.2 TEM and DLS analysis

Dried yeast synthesized few NPs (Figure 2), but freshly cultured yeast produced a large amount of NPs (Figure 3). TEM demonstrated the localization of the NPs inside and outside the cells, the mean particle size and the size distribution. From TEM micrographs, it was shown that individual silver NPs as well as a number of aggregates with almost spherical shapes and sizes of about 8–26 nm were produced (Figure 4). Corona could be seen around the NPs (Figure 4C). NPs were seen inside the cells, within the cell membrane, attached to the cell membrane during the exocytosis and outside the cells (Figure 5).

It might be interpreted that silver ions firstly were trapped by *S. boulardii* cells, and then were reduced by enzymes present within the cytoplasm. It was shown that the NPs are mainly synthesized intracellularly, which might be due to reductases inside the cells. Therefore, the biomass should be used in the synthesis, instead of discarding it. It is recommended that both the cells and supernatant are used for future studies using these biocatalysts or the other yeasts. Previous researchers using Fourier transform infrared (FTIR) analysis mentioned involvement of proteins in the capping process of NPs

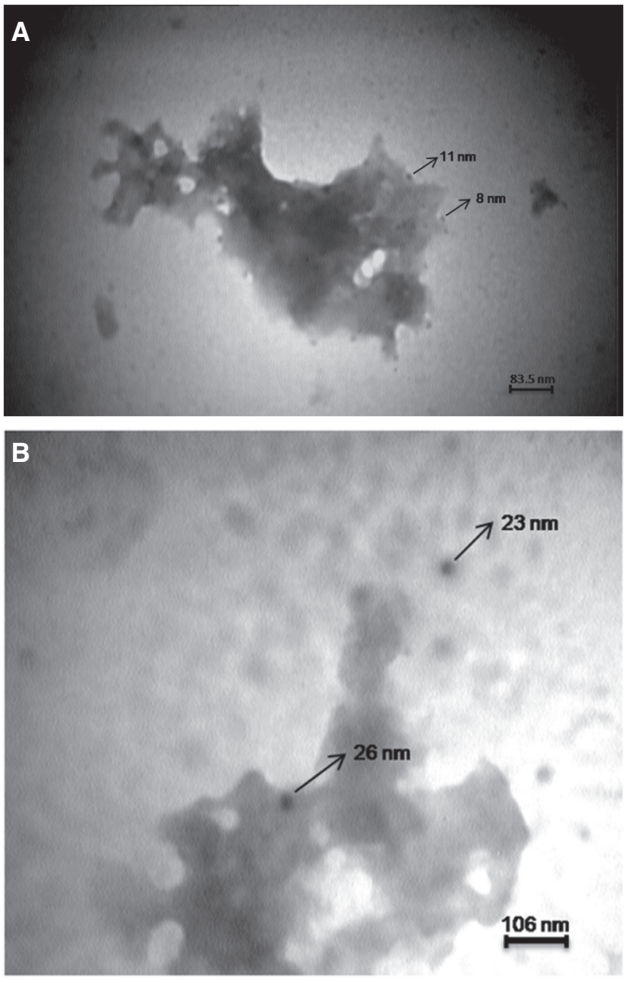

Figure 2 Transmission electron microscopy (TEM) micrographs recorded from a drop-coated film of an aqueous solution of Ag^+ ions incubated with dried *S. boulardii* (after 5 h biotransformation). Magnifications are: (A) 25,000 and (B) 20,000.

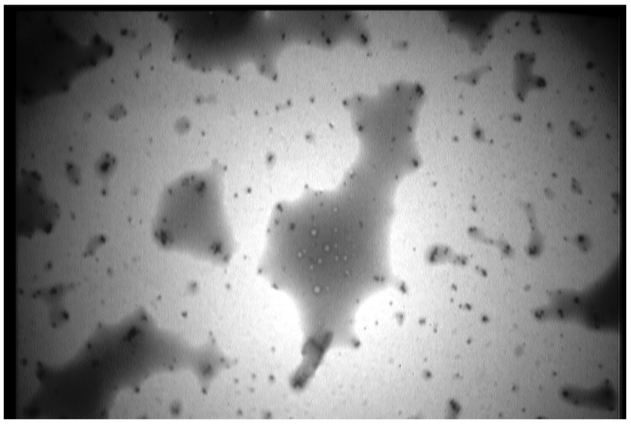

Figure 3 Transmission electron microscopy (TEM) micrograph recorded from a drop-coated film of an aqueous solution of Ag^+ ions incubated with freshly cultured *S. boulardii* (after 5 h biotransformation). Magnification is: 5000.

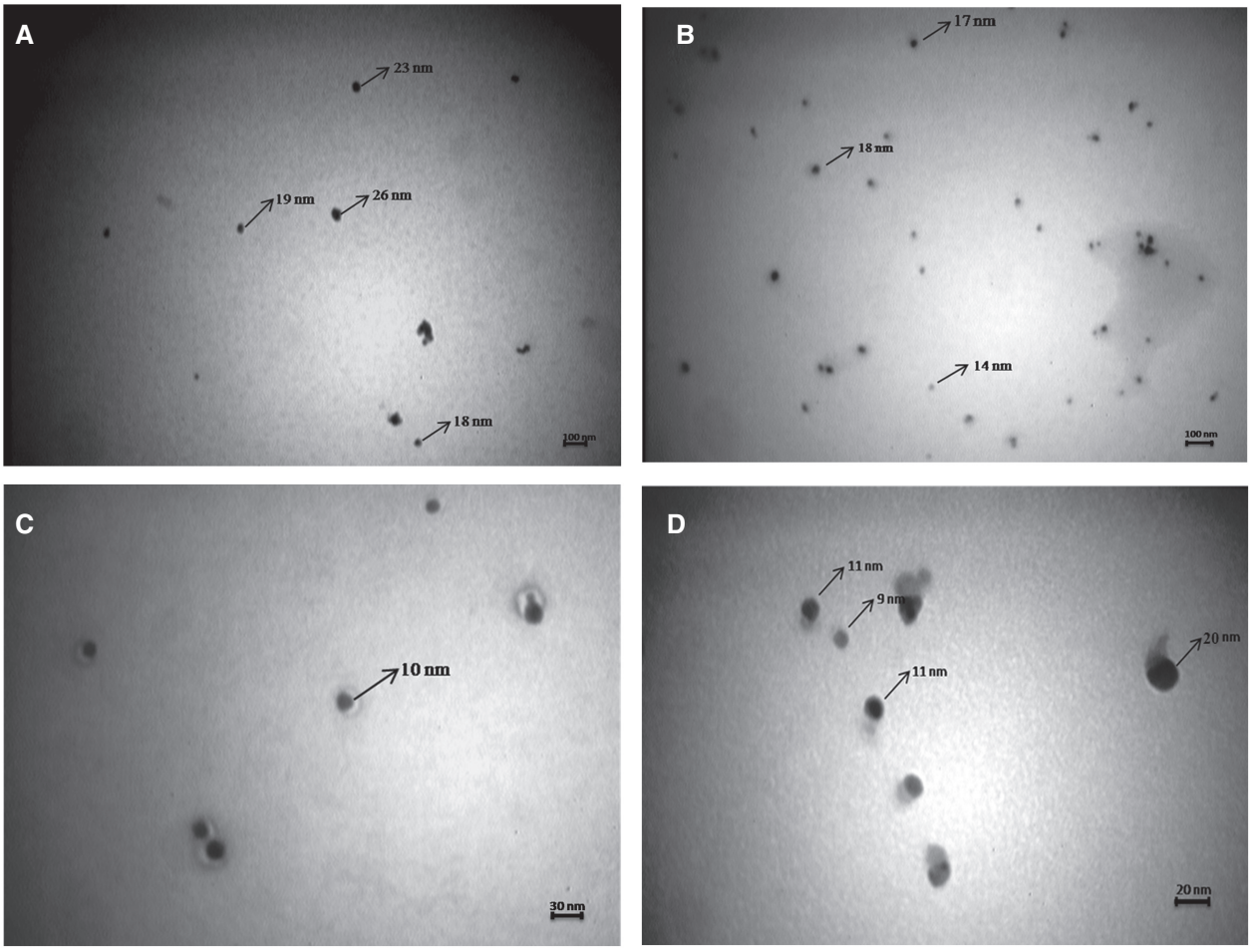

Figure 4 Transmission electron microscopy (TEM) micrographs recorded from a drop-coated film of an aqueous solution of Ag^+ ions incubated with freshly cultured *S. boulardii* (after 5 h biotransformation). Magnifications are: (A) 16,000, (B) 20,000, (C) 63,000 and (D) 100,000.

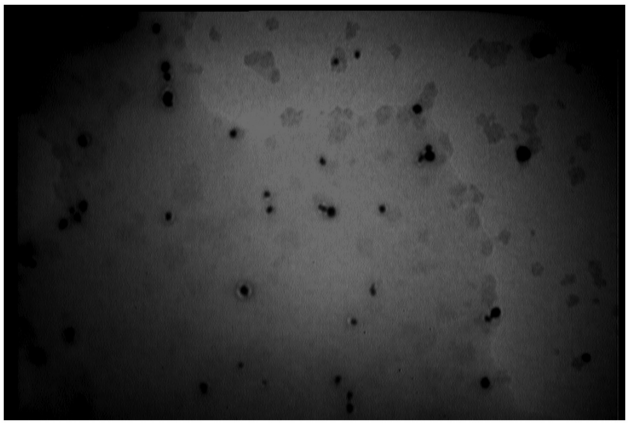

Figure 5 Transmission electron microscopy (TEM) micrograph recorded from a drop-coated film of an aqueous solution of Ag^+ ions incubated with freshly cultured *S. boulardii* (after 5 h biotransformation).

synthesized by yeasts, and existence of a protective coat on the NP surface produced by fungi [21, 22]. Some authors believed that biosynthesis of the NPs might be the result of tautomerization of membrane-bound (as well as cytosolic) quinones or the pH sensitive oxidoreductases (e.g., monooxygenases or dioxygenases and mixed function oxidases/oxygenases) [11].

One peak was seen in DLS analysis of the reaction mixture after 24 h (Figure 6). The polydispersity index (PDI) was 0.323. Single NPs with diameter of 166.1 nm were the most frequent ones (100%) and the mean size was 208 nm (Z-Average). DLS analysis of the colloidal solution further confirmed monodispersity of the synthesized particles. Moreover, two peaks were seen in DLS analysis of the reaction mixture after 48 h (Figure 7). The PDI was 0.270. Single NPs with diameter of 320.1 nm were the most frequent ones (97.3%) and the mean size was 284.9 nm (Z-Average). The aggregates with diameters of 5170 nm were 2.7% of the NPs. Results from TEM (Figures 2–5) after 5 h and DLS (Figures 6 and 7) after 24 and 48 h analysis demonstrated that by increasing the time of reaction, the NPs were aggregated further.

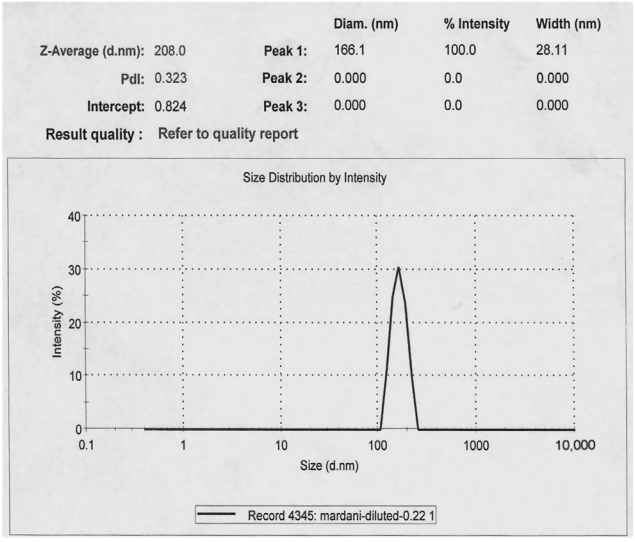

Figure 6 Particle size analysis of nanoparticles (NPs) produced after 24 h biotransformation.

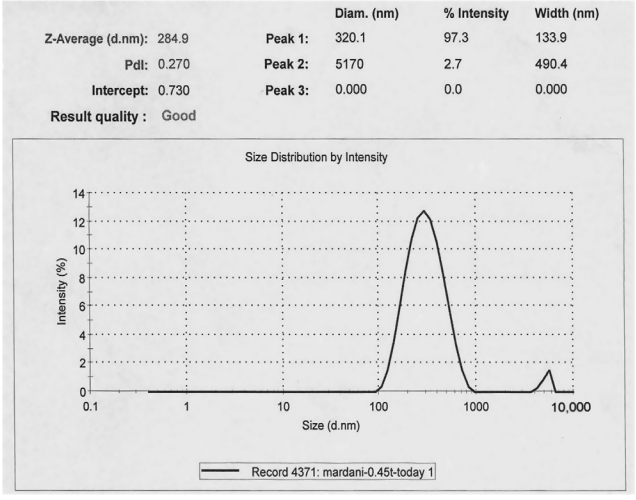

Figure 7 Particle size analysis of nanoparticles (NPs) produced after 48 h biotransformation.

4 Conclusion

Biosynthetic methods of NPs provide a new possibility of conveniently synthesizing NPs using natural reducing and stabilizing agents. As possible ecofriendly alternatives to chemical and physical approaches, biosynthesis of metal and semiconductor NPs using yeasts has been suggested. Monodispersity and particle size and shape are very important parameters in the evaluation of NPs synthesis. Therefore, efficient control of the morphology and monodispersity must be explored.

The present study demonstrated the bioreductive synthesis of silver NPs using *S. boulardii* at room temperature. Silver NPs were successfully synthesized by using this yeast. Dried yeast synthesized few NPs, but freshly cultured yeast produced a large amount of NPs. The NPs were monodispersed and some of them were aggregated outside the cell. This is the first time that it has been shown that the NPs are mainly synthesized inside the *S. boulardii* intracellularly, which is in agreement with our previous works about the bacteria and the fungi. Therefore, the biomass should be used in the synthesis, instead of discarding it. Previous researchers used only the supernatant and reported that the synthesis is extracellular. It seems that discarding the cells is a waste of reduction potential of the biocatalyst. It is recommended that both the cells and supernatant are used for future studies using these biocatalysts or the other yeasts. Previous researchers using FTIR analysis mentioned involvement of proteins in the capping process of NPs synthesized by yeasts, and existence of a protective coat on the NP surface produced by fungi.

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Declaration of interest: The authors report no conflicts of interest.

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Hassan Korbekandi was born in Iran (PhD in Biotechnology, UMIST, Manchester). He has worked on several projects at the Isfahan University of Medical Sciences and University of Manchester Institute of Science and Technology, including environmentally friendly and ecofriendly synthesis of nanoparticles, biotransformations using *Peptostreptococcus productus*, directed mutagenesis to improve human Interferon- beta (hIFN-B) activity and stability, etc. Moreover, he has worked in the areas of nanomaterials and green nanotechnology, nanobiotechnology, biotransformations, bioinformatics and food biotechnology. He has published over 20 papers in reviewed journals. Currently, he is an assistant professor at Isfahan University of Medical Sciences.

Rasoul Mardani Jouneghani was born in Iran. He obtained his Master's degree, and he has worked in the areas of nanoparticle synthesis and biological methods for synthesizing metal and metal oxide nanoparticles.

Soudabeh Mohseni was born in Iran. She obtained her Master's degree, and she has worked in the areas of nanoparticle synthesis and biological methods for synthesizing metal and metal oxide nanoparticles.

Meraj Pourhossein was born in Iran (PhD in Molecular Biology, UMIST, Manchester). He is interested in molecular biology related techniques. He has experience in the following techniques: *in silico* analysis, homology studies, electron microscopy (SEM and TEM), atomic force microscopy (AFM), DNA purification of animal and bacterial cells, preparation of plasmid DNA, polymerase chain reaction (PCR) based techniques, using restriction endonucleases for partial and multiple digestions of DNA, ligation and cloning techniques, transformation of plasmid DNA into cells, screening of recombinant DNA, DNA sequencing, Western blotting, Coomassie blue and silver staining, complementation assays, protein purification, production of polyclonal antibodies, large scale protein production for crystallographic analysis, site-directed mutagenesis, hybridization studies, phage sensitivity analysis, radioactive incorporation and autoradiography. He has published over 20 scientific papers in reviewed journals.

Siavash Iravani was born in Iran. He has worked on several projects at the Isfahan University of Medical Sciences (Faculty of Pharmacy and Pharmaceutical Sciences), including green and ecofriendly synthesis of nanomaterials, phytochemical analysis of pine (*Pinus eldarica* and *P. pinaster*) bark extracts, technological and stability aspects of probiotic fermented milks, gene delivery systems for prostate cancer therapy, nanoparticles for drug delivery in cancer, nanocarriers and drug nanoparticles. He has worked in the areas of nanomaterials and green nanotechnology, analytical chemistry, green chemistry, cancer nanotechnology and bioinformatics. He has worked as research manager of the nanotechnology and nanobiotechnology group at the Isfahan Pharmaceutical Research Committee (project leader and communicator). His current research is focused on childhood acute lymphoblastic leukemia (ALL) treatment and nanotechnology. He has published over 20 scientific papers in reviewed journals.