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Biogenic Approach using Sheep Milk for the Synthesis of Platinum Nanoparticles: The Role of Milk Protein in Platinum Reduction and Stabilization

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Abstract
Bio-directed synthesis of nanoparticles is an interesting field of rapid advancement for biologists, chemists and materials scientists, especially in light of efforts to find out green methods of inorganic material synthesis. In the present study, green synthesis of platinum nanoparticles (PtNPs) using sheep milk is reported for the first time. By adjusting the concentrations of chloroplatinic acid (H_2PtCl_6) and milk in aqueous solutions, spherical PtNPs were obtained at room temperature. The nanoparticles obtained were characterized by UV-Vis spectroscopy, dynamic light scattering (DLS), high-resolution Transmission Electron Microscopy (TEM) and X-Ray diffraction (XRD). The spherical particles obtained have an average size 9.0 nm as shown by XRD pattern and TEM analysis. Fourier Transform Infra-Red (FTIR) measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the PtNPs synthesized by milk. The carboxylic acid group libration and amide I and II bands revealed the binding of protein with platinum surface through the amine group rather than the carboxyl group. Among a wide variety of biological resources which are introduced for reducing Pt ions into PtNPs, green approaches like using sheep milk have outstanding benefits for synthesize biological metal nanoparticles due to their eco-friendly phenomenon and very less amounts of cytotoxicity. PtNPs green synthesized in this study are potential candidates to use in drug discovery and gene transfer technologies.

Keywords: *Green synthesis, Platinum nanoparticles, Sheep milk, Electron microscopy, Physicochemical characterization.*

1. INTRODUCTION

Platinum nanoparticles (PtNPs) have been considered momentous area of research due to their unique and tunable surface plasmon resonance (SPR) and their applications in biomedical science including drug delivery, photo thermal therapy, tissue/tumor imaging and immunochromatographic identification of pathogens in clinical specimens [1-4]. There is no report on the synthesis of PtNPs using Sheep Milk. Milk is the probably the most nutritionally complete food found in nature. Thorough milk contains vitamins (principally thiamine, riboflavin, panthothenic acid and vitamins A, B_{12} and D), minerals (calcium, sodium, phosphorus, potassium, and trace minerals proteins (which include all the necessary amino acids), carbohydrates (mostly

lactose), and lipids (fats). The physicochemical and optoelectronic confidants of metallic nanoparticles are strongly dependent on the size and size-distribution, but also nanoparticles shape contributes significantly to the control of their properties [5]. Great varieties of physical and chemical procedures have been developed in order to synthesize nanoparticles of different compositions, sizes, shapes and controlled polydispersity. Nevertheless, the routinely physicochemical techniques for nanoparticle production such as photochemical reduction [6], laser ablation [7], electrochemistry [***8***], lithography [9] or high energy irradiation [10], either remain expensive or employ hazardous substances, such as organic solvents, and toxic reducing agents like sodium borohydride and N, N-dimethylformamide. In addition, due to the high surface energy of the nanoparticles, these tend to form aggregates; therefore, surface passive and capping reagents are frequently added to the reaction systems to avoid coalescence. The development of reliable, eco-friendly processes for the synthesis of nanomaterials is an important aspect of nanotechnology. Nanotechnology also requires the synthesis of nanomaterials of different chemical compositions, sizes and morphology with an excellent control over these characteristics. With the growing need to minimize or eliminate the use of environmental-risk substances, as the green chemistry principles describe [11], the synthesis of nanoparticles using biological entities has received increasing attention in the last decade [12, 13]. The biosynthetic procedures involve either living organisms such as bacteria, fungi and plants or biomass, like plant extracts [14-21]. Biological synthetic processes have emerged as a simple and viable alternative to more complex physicochemical approaches to obtain nanomaterials with adequate control of size and shape [22]. The use of the highly structured physical and biosynthetic activities of microbial cells for the synthesis of nano-sized materials has recently emerged as a novel approach for the synthesis of metal nanoparticles. The interactions between microorganisms and metals have been well documented [14, 16] and the ability of microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation [23]. Here we reported for the first time green synthesis of PtNPs using milk. The synthesized nanoparticles were characterized for shape, size and other physical characteristics using spectrometry, dynamic light scattering and electron microscopy analyses.

2. MATERIALS AND METHODS

2.1. Synthesis of platinum nanoparticles

Sheep milk was prepared from the local market and sterilized using a 22 µm filter syringe. H_2PtCl_6 was purchased from Sigma-Aldrich (USA). All steps of nanoparticle synthesis using sheep milk was carried out at a clean room under sterile conditions to maintain milk sterilization. Sheep milk (4 ml) was mixed with 96 ml of 1 mM H_2PtCl_6 solution, and the resulting mixture was incubated for 3 h in a rotary shaker (200 rpm) at room temperature. Reduction of platinum ions in the reaction mixture was monitored by change in color of the reaction mixture from milky white to brownish-red. The reaction product was separated by centrifugation at 12,000 rpm for 20 min and purified by redispersion of the pellet in autoclaved water. The processes of centrifugation and redispersion were repeated several times to ensure better separation of the free entities from the platinum nanoparticles. Platinum nanoparticles were finally collected by centrifugation at 80000 × g for 30 min, washed twice with distilled water and the unbound proteins were removed by treating with 80% (v/v) ethanol. The purified nanoparticles were freeze dried at −70 °C and used for characterization

studies. Autoclaved milk was used as the control for this experiment.

2.2. Characterizations of the green synthesized platinum nanoparticles:

2.2.1. UV-Vis spectral analysis

The color change in reaction mixture was recorded through visual observation. The bio-reduction of platinum ions in aqueous solution was monitored by periodic sampling of aliquots (1 ml) and subsequently measuring UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on UV-Vis spectrophotometer UV-1601 (Shimadzu).

2.2.2. XRD analysis

The platinum nanoparticles solution thus obtained was purified by repeated centrifugation at 6000 rpm for 10 min followed by redispersion of the pellet of PtNPs in 10 ml of deionized water. After freeze drying of the purified PtNPs, the structure and composition were analyzed by XRD and TEM. The dried mixture of PtNPs was collected for the determination of the formation of PtNPs by an X'Pert Pro x-ray diffract meter operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer's formula:

$D=0.94\ \lambda/\beta\ \mathrm{Cos}\ \theta$

1) where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained

Si sample: β corrected = $(FWHM^2sample-FWHM^2si)\ 1/2$

2) This modified formula is valid only when the crystallite size is smaller than 100 nm [24].

2.2.3. TEM analysis

The structural characterization of the PtNPs was carried out by Transmission Electron Microscopy (TEM). The sample was prepared by air-drying drops of diluted solutions of the preparations on carbon films supported by copper grids [25, 26, 27].

2.2.4. Zeta-Potential analysis

Zeta-potential measurements were performed on a Malvern Zetasizer 3000 at 25 ºC with an incident wavelength of 633 nm and a 170º backscattering angle. Clear disposable zeta potential cells (1 cm path length) were rinsed with ethanol, followed by deionized water prior to sample loading. The viscosity, refractive index, and absorption values were provided in the Malvern software for water (μ = 0.8872 cP, RI = 0.135). Twelve runs were averaged for each liquid sample for accurate determination of zeta potential measurements.

2.2.5. FTIR analysis

To remove any free residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 6000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by re-dispersion of the pellet of PtNPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 360 (Nicolet, USA). The FTIR were recorded in the range of 400–4000 cm^{-1}.

3. RESULTS

3.1. Synthesis of platinum nanoparticles

The platinum nanoparticles displayed brownish-red color, respectively, in water; these colors arose because of exciting surface plasmon vibrations in the metal nanoparticles. The color change is

attributed to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field in metallic nanoparticles, which is called SPR [28]. In the present study, the PtNPs were synthesized rapidly within 3h of the incubation period in the aqueous chloroplatinic acid solution, which turned to a brownish-red color in 2h of adding Sheep milk (Figure 1). The intensity of the brownish-red color increased in direct proportion to the incubation period because of the excited SPR effect and reduced H_2PtCl_6 [29, 30]. The control H_2PtCl_6 solution (without Sheep milk) showed no change of color with time.

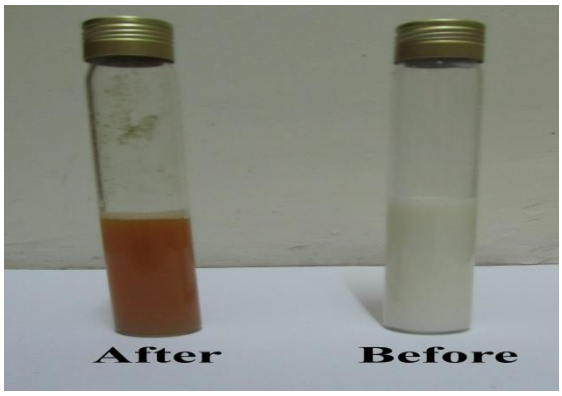

Figure 1. *Synthesized platinum nanoparticles show brownish-red color after 3 hours incubation at room temperature.*

3.2. UV-Vis spectrophotometer

The platinum ions immediately declined within 1h, which may have been due to the presence of milk soluble proteins. The reduction of platinum ions occurred rapidly and more than 90 % of the reduction of platinum ions was completed within 3 hours (at 1 and 5 ml of sheep milk, respectively) after adding the Sheep milk to the metal ion solutions. The characteristic absorption peak at 278 to 284 nm in UV–Vis spectrum (Figure 2) confirmed the formation of PtNPs. SPR patterns, which detail the characteristics of metal nanoparticles, strongly depend on particle size, stabilizing molecules or the surface of adsorbed particles, and the dielectric constant of the medium. The nanoparticles showed absorption peak around 278 to 284 nm after 1 hour of reaction, which is a characteristic SPR band of PtNPs, possibly because of exciting longitudinal plasmon vibrations in the PtNPs in the solution.

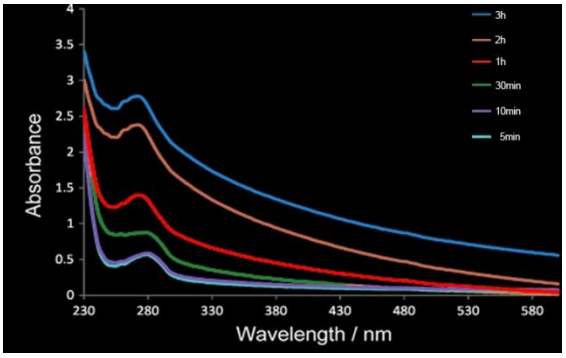

Figure 2. *UV-Vis absorption spectra of platinum nanoparticles synthesized by sheep milk.*

3.3. DLS analysis of platinum nanoparticles

Dynamic light scattering study well indicated that most of the PtNPs were in the range of 5-10 nm with zeta potential of -31 mV (Figure 3)

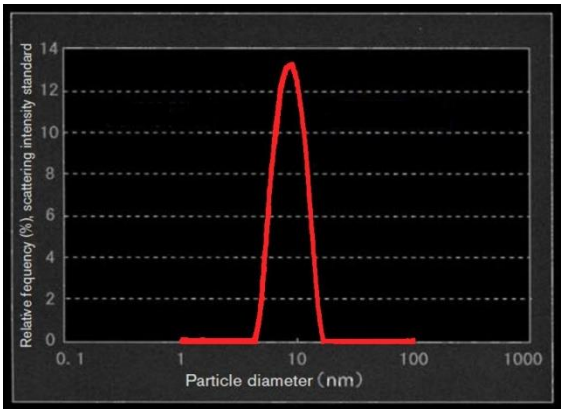

Figure 3. *Dynamic light scattering spectrum of platinum nanoparticles synthesized by sheep milk shows the maximum of scattering at 9 nm.*

3.4. TEM images of platinum nanoparticles

The TEM images clearly suggest that there was a thin layer of other material on the surface of the PtNPs because of the

capping platinum ions. The TEM analysis of the bio-reduced PtNPs confirmed that the size of the metal particles was in the nano range and were roughly spherical in shape. The size of the PtNPs was in the range of 8.5 nm after 3 hours and the representative TEM image is shown in Figure 4. Most of the nanoparticles were roughly spherical. The size of the particles agreed with the noted SPR band. Some nanoparticles had isotropic nanostructures with irregular contours as shown in Figure 4; also most of the PtNPs in the TEM images were in physical contact, but they were separated by a uniform inter-particle distance. From our previous reports, it has been observed that the spherical shape of nanoparticles is synthesized after bio-reduction.

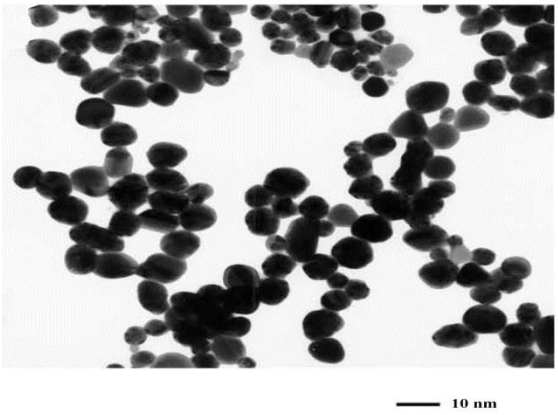

Figure 4. *Transmission electron microscopy of platinum nanoparticles synthesized by reduction of aqueous H_2PtCl_6 ions using sheep milk.*

3.5. FTIR analysis of platinum nanoparticles

FTIR analysis was used to identify the possible biomolecules responsible for the reduction and capping on nanoparticle surfaces. In the present study, FTIR spectra of both the sheep milk and synthesized PtNPs were recorded. FTIR measurements were carried out to identify the potential biomolecules in the sheep milk responsible for reducing the chloroplatinic acid ions. It was noted the capping reagent responsible for the stability of the bio-reduced PtNPs involved the secondary amines. The size distribution and characterization of the PtNPs was further corroborated by FTIR, as shown in Figure 5.

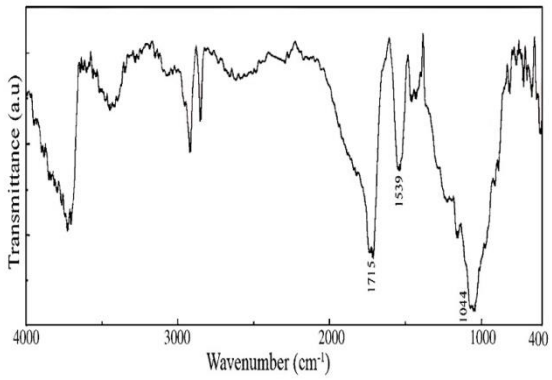

Figure 5. *Fourier transform infrared spectroscopy of platinum nanoparticles.*

The interaction of nanoparticles with proteins of sheep milk showed violent peaks at 1715, 1539, 1044 cm^{-1}. Relative shifts in position and intensity distribution were confirmed with FTIR. This clearly shows that the proteins capped the surface of the nanoparticles and kept them stable for longer periods. The IR band at 1715 $cm^{-1}$is characteristic of the C=O stretching mode [20, 31] of the carboxylic acid group. The band due to C–O stretching mode got merged in the very broad envelope centered on 1044 cm^{-1} arising from C–O–C symmetric stretching and C–O–H bending vibrations of protein in milk. The amide I and II bands of proteins [32] are expected to occur as prominent IR bands around 1655 and 1535 cm^{-1}, respectively. In the present case, the intense band observed at 1540 cm^{-1} arises from the amide II band and the amide I band got merged in the intense band around 1715 cm^{-1}. Proteins can bind to PtNPs through free amine groups or carboxylate ion of amino acid residue in it [33]. The presence of C=O stretching mode indicates the presence of –COOH group in the material bound to PtNPs. Thus, the bands at 1715 and 1044 cm^{-1} in IR indicate the possibility that PtNPs are bound to proteins through free amine groups.

3.6. XRD analysis of platinum nanoparticles

The X-ray structural diffraction pattern of the PtNPs produced using the milk was proved and confirmed by the characteristic peaks observed in the XRD images for platinum (Figure 6). The XRD pattern recorded for PtNPs showed intense peaks in the whole spectrum of 2θ values ranging from 20 to 80. The XRD analysis showed distinct diffraction peaks at 32°, 35°, 37°, 40°, 46°, 57°, 63°, and 68°, which indexed the planes (111) and (200) of the spherical face-centered platinum; whereas any peaks originating because of potential platinum oxide interference could not be observed and it could not be confirmed that the entire chloroplatinic acid was converted to nanoplatinum.

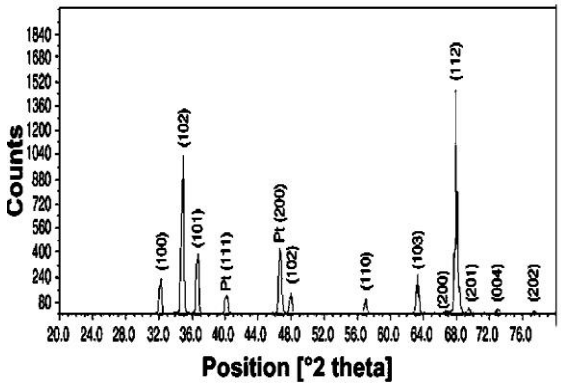

Figure 6. *XRD pattern of platinum nanoparticles synthesized using sheep milk.*

The average grain size of the PtNPs formed in the bio-reduction was determined using the Scherrer equation ($D=\frac{0.9\lambda}{\beta cos\theta}$) and estimated as 9 nm. The XRD pattern clearly explains the crystalline structure of the PtNPs formed by green biosynthesis.

4. DISCUSSION

Several studies have reported the synthesis of platinum nanoparticles using chemical methods [31-33]. However, the formation of these nanoparticles by biological methods has not been fully investigated. Synthesis of spherical PtNPs within bacterial cells of the filamentous cyanobacterium *Plectonema boryanum* UTEX 485 and bio synthesized PtNPs by the bacterium *Shewanella algae* are good examples of such green synthesis [34, 35]. Lee et al. synthesized silver nanoparticles using cow milk. The synthesized AgNPs were mostly circular in shape with an average size of 30–90 nm, and elemental composition confirmed the significant presence of Ag free of other contaminants [36]. The present study was an attempt to synthesize PtNPs using sheep milk. The color of reaction mixture turned from milky white to brownish-red after 3 h of reaction, indicating H_2PtCl_6 reduction. The intensity of the brown-red color increased after 3 h of incubation, giving it a darker look. It seems that proteins present in the milk could be responsible for the platinum ions reduction. Previous studies have reported PtNPs to exhibit a brownish-red color in water due to the excitation of surface Plasmon vibrations in metallic nanoparticles [26, 37]. Thus, change of color in our study is indicative of platinum ions reduction by sheep milk. Although UV– VIS spectroscopy is primarily use to confirm the PtNPs synthesis widely, in our study, it was not possible to use this technique because of high turbid nature of the reaction mixture. We successfully used other methods such as XRD, zeta sizer, FTIR and electron microscopy for PtNPs characterization. The synthesized PtNPs were mostly spherical in shape with an average size of 9 nm, and elemental composition was confirmed the significant amounts of pure platinum free of other contaminants.

5. CONCLUSION

In harmony with the essential for green chemistry, investigation has been attentive toward biological methods for the synthesis of metal nanoparticles [38]. A variety of biological resources were used to reduce Pt ions into PtNPs. However,

using milk for the synthesis of PtNPs has some benefits than other biological and chemical approaches. Milk does not have any toxic materials; so the synthesized PtNPs have a potential for application in pharmaceutical and medical sciences. On the other hand, application of milk for the nanoparticle synthesis is a safe approach and does not release any harmful chemical into the environment, incubation time for the synthesis of PtNPs is fewer; and even though milk is intended for nutritional purpose, the usage of minimum volume of milk for non-nutrient application may not limit the availability for human beings. Taken together, this study describes a simple, rapid and eco-friendly approach for synthesis of PtNPs using easily available bio-resource material named sheep milk. PtNPs green synthesized in this study are potential candidates to use in drug discovery and gene transfer technologies.

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