Journal of Photochemistry & Photobiology, B: Biology 170 (2017) 241–246

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Antibacterial activity of biochemically capped iron oxide nanoparticles: A view towards green chemistry

Rabia Irshad[a], Kamran Tahir[a,b], Baoshan Li[a,*], Aftab Ahmad[a], Azka R. Siddiqui[a], Sadia Nazir[b]

[a] State Key Laboratory of Chemical Resource Engineering, School of Science, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] Institute of Chemical Sciences, Gomal University, D. I. Khan, KP, Pakistan

ARTICLE INFO

Keywords:
Antibacterial activity
Green synthesis
Iron oxide nanoparticles
Punica granatum

ABSTRACT

A green approach to fabricate nanoparticles has been evolved as a revolutionary discipline. Eco-compatible reaction set ups, use of non-toxic materials and production of highly active biological and photocatalytic products are few benefits of this greener approach. Here, we introduce a green method to synthesize Fe oxide NPs using *Punica granatum* peel extract. The formation of Fe oxide NPs was optimized using different concentrations of peel extract (20 mL, 40 mL and 60 mL) to achieve small size and better morphology. The results indicate that the FeNPs, obtained using 40 mL concentration of peel extract possess the smallest size. The morphology, size and crystallinity of NPs was confirmed by implementing various techniques i.e. UV–Vis spectroscopy, X-ray diffraction, Scanning Electron Microscopy and Electron Diffraction Spectroscopy. The biochemicals responsible for reduction and stabilization of FeNPs were confirmed by FT-IR analysis. The biogenic FeNPs were tested for their size dependent antibacterial activity. The biogenic FeNPs prepared in 40 mL extract concentrations exhibited strongest antibacterial activity against *Pseudomonas aeruginosa* i.e. 22 (± 0.5) mm than FeNPs with 20 mL and 60 mL extract concentrations i.e. 18 (± 0.4) mm and 14 (± 0.3) mm respectively. The optimized FeNPs with 40 mL peel extract are not only highly active for ROS generation but also show no hemolytic activity. Thus, FeNPs synthesized using the greener approach are found to have high antibacterial activity along with biocompatibility. This high antibacterial activity can be referred to small size and large surface area.

1. Introduction

Large scale urbanization and industrialization have contributed to today's environmental calamities principally in aquatic domain. Nanoparticle synthesis is one of the most emerging processes to cope with various organic and inorganic toxic pollutants [1–3]. In recent years, iron nanoparticles due to their diversified applications are being actively looked into. Iron nanoparticles are characterized as active agents against many organic and inorganic pollutants. These iron-based nanoparticles have been reported in different states i.e. zero valent iron [3], Fe-ball clay [4], iron oxide nanoparticles [5]. Minuscule size, large surface area and high degree of dispersion of nanoparticles make them unique for their catalytic activity. Owing to high magnetic susceptibility and biocompatibility, iron nanoparticles have been magnificently employed in various therapeutics for cancer treatment and radiation oncology [6].

Various distinctive methods have been in practice to manufacture nanoparticles. The methods used for their production fall under chemical, physical and biosynthetic domains. Some of these chemical processes include thermal decomposition [7–8], co-precipitation [9], sol-gel method [10], polyol methods [11] and hydrothermal method [12]. However, these conventional methods are not as enticing as they lead to degradation of the ecosystem, exhibit low dispersion rates, are expensive, exhibit low uniformity in dispersion and are inconvenient to work with in scaled-up applications. Moreover, these aforementioned processes tend to operate under prime critical conditions i.e. temperature and pH.

Contemporary to these methods, Green synthesis stands out showcasing the encouraging results and a wide range of flexible effects which include no demand of optimum operating conditions, stable economical perspectives and their hospitable approach to environment. Green synthetic process has already been used to fabricate various metal nanoparticles and nanocomposites such as silver, palladium, gold nanoparticles, Au/TiO_2 nanocomposite, ZnO nanoparticles and Cu/ZnO nanoparticles [13–20].

In the present work, *Punica granatum*'s (generally called as pomegranate, family *Punicaceae*) peel extract is used to synthesize iron oxide nanoparticles. *Punica granatum* is a primeval fruit and is widely

* Corresponding author.
E-mail address: bsli@mail.buct.edu.cn (B. Li).

http://dx.doi.org/10.1016/j.jphotobiol.2017.04.020
Received 29 December 2016; Received in revised form 12 April 2017; Accepted 17 April 2017
Available online 19 April 2017

cultivated. Its peel extract has been found to have divergent applications in various medical fields i.e. drugs and medicine [18] and exhibits a potential ability against certain bacteria [21] and other microbes. In comparison to the pulp pomegranate, peel contains thrice the amount of polyphenols [22]. *Punica granatum* peel may contains different phenolic compounds i.e. ellagic and ellagic acid derivatives like punicalagin [23–24]. These compounds contribute in the stability of nanoparticles and have a reducing nature. The present work holds dual profits i.e. an innovative and eco-friendly method to synthesize iron nanoparticles as well as a movement to decline the pitch of pollution by using the peels which are disposed of as waste material. The green synthetic iron oxide nanoparticles have been examined for their antibacterial activity. The result illustrated that the nanoparticles prepared at 40 mL extract concentration have high antibacterial activity as compared to nanoparticles prepared at 20 mL and 60 mL peel extract.

2. Materials and Methods

2.1. Preparation of Punica granatum Peel Extract

Punica granatum was collected from local market in Beijing. Peels were thoroughly washed several times with distilled water to remove the dust particles, dried in dark and ground in minute sized granules. 7 g of this sample was taken in 250 mL beaker and enough distilled water was added to make the total volume up to 150 mL. The resulting solution was heated initially at 80 °C for about 30 min and then further stirred 60 min at 1000 rpm for. The peel extract was filtered using Whatman filter paper no. 3 and stored at 4 °C for further use.

2.2. Synthesis of Fe Nanoparticles Using Punica granatum Peel Extract

Fe nanoparticles were synthesized by adding different concentrations of peel extract i.e. 20 mL, 40 mL and 60 mL in 150 mL of 0.15 M solution of ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) in a 500 mL beaker. The color of salt solution turned from brown to black immediately. Then it was autoclaved for 5 h at a constant 200 °C. After the formation of Fe oxide nanoparticles, it was centrifuged at 10,000 rpm. Later, it was dried in 6ES freeze drier for 4 days.

2.3. Screening for Antibacterial Activity by Agar Well Diffusion Method

Agar well protocol was applied to check the antibacterial activity of greener Fe oxide nanoparticles [25–26]. A bacterial culture was prepared in nutrient broth at 37 °C for 24 h in an incubator. Inocula of underlined bacteria was marked on Muller Hinton agar plates, using sterile swab. It ensured an even dense lawn of culture following incubation. Wells of 6 mm diameter were made on nutrient agar plates, using sterile cork borer. A solution of 1 mg Fe oxide nanoparticles in 1 mL distilled water was prepared and 50 µL of this solution was poured into the wells formed on nutrient agar plates. The agar plates then left to stay for 1 h at 25 °C. Finally, the plates were incubated for 24 h at 37 °C. The resultant diameter of zone of inhibition was measured cautiously. Streptomycin was used as standard.

2.4. Determining Minimum Inhibitory Concentration

Serial dilution method was adopted to determine MIC of Fe oxide nanoparticles. 1 mL of biogenic FeNPs, with different concentrations of peel extract i.e. 20 mL, 40 mL and 60 mL, were taken in sterilized test tubes containing 1 mL of bacterial (*Pseudomonas aeruginosa*) solution having turbidity of 0.5 Mcfarland turbidity standard. The test tubes were mixed and then kept in incubator at 37 °C for 24 h. These test tubes containing culture were taken as control. The concentration range of FeNPs used was from 2 mg mL^{-1} to 0.031 mg mL^{-1}. MIC can be considered as the minimum concentration of compound which inhibits the growth of microorganism. The assay was carried out in triplicates.

2.5. Reactive Oxygen Species Generation by FeNPs

2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) kit, a method for oxidative stress assessment of FeNPs treated microbes, was employed to verify the intracellular generation of reactive oxygen species (ROS). This fluorogenic organic dye is quite advantageous to detect hydroxyl, per hydroxyl and other reactive oxygen species (ROS) within the cell. Fe nanoparticles with 40 mL *Punica granatum* peel extract concentrations were incubated for 4 h at 250 rpm along with the tested bacterial (*Pseudomonas aeruginosa*) strain. After incubation, the suspension of bacterial cells (*Pseudomonas aeruginosa*) was collected (8000 rpm, 5 min) and the obtained pellet was washed thrice with phosphate buffer saline (PBS). A suspension of pellet in 1 mL of buffer solution (PBS) was subsequently treated with 1 mL of 20 mM 2, 7-dichlorodihydrofluorescein diacetate reagent for 40 min. The DCFH-DA treated cells were washed thrice with PBS to get rid of the excess dye from outer surface of cells. The fluorescence image of the suspension was determined by a fluorescence microscope (Olympus 1 × 51) at two wavelengths i.e. excitation wavelength of 488 nm and the emission wavelength of 535 nm [27].

2.6. Hemolytic Activity Assay

To check the hemolytic property of green synthesized FeNPs, the amount of hemoglobin released from red blood cells (RBCs) on treatment with biogenic FeNPs was measured. The blood was obtained from a male albino rat and was taken in a sterile Lithium Heparin Vacutainer. The test tube was then centrifuged at 1500 rpm for 20 min. The supernatant was removed cautiously and the pellet was sterilized three times with phosphate buffered saline (PBS). The pH of PBS was maintained at 7.4. Different amounts of FeNPs synthesized at optimized condition i.e. using 40 mL peel extract (20, 40, 60, 80, 100 and 120 mg) taken in PBS solution and cells (5% v/v) in PBS were added in each tube to make the total volume up to 1 mL. RBCs in PBS were taken as negative control whereas RBCs in 1% Triton X-100 solution were taken as positive control. A shaking incubator maintained at 37 °C was used to incubate the reaction mixtures for 1 h with gentle shaking. The tubes were then centrifuged at 1500 rpm for 10 min and the supernatant was observed keenly at 540 nm against their blank [28].

3. Results and Discussion

3.1. Characterizations

3.1.1. UV Spectroscopic Analysis

UV-spectrophotometer (Shimadzo UV-2400) was used to verify the formation of Fe oxide nanoparticles. The UV spectrum obtained is shown in (Fig. 1). The spectrum obtained noticeably specified the formation of Fe nanoparticles during the course of synthesis. Biogenic FeNPs showed maximum absorbance at wavelength of 300 nm that is in complete harmony with UV spectral analysis of metallic iron. Such UV spectral results have been already reported [29].

3.1.2. Fourier Transform Infrared Spectroscopic (FT-IR) Analysis

The presence of phytochemicals in peel extract of *Punica granatum* was revealed by FT-IR. IR spectrum was obtained using an ABB MB 3000 spectrophotometer. These phytochemicals play significant role as stabilizing and reducing agents. Fig. 2 represents the results of FT-IR spectrum of green Fe nanoparticles. The spectrum obtained specifies some prominent peaks at 3426 cm^{-1}, 2924 cm^{-1}, 2848 cm^{-1}, 1719 cm^{-1}, 1616 cm^{-1}, 1327 cm^{-1} and 1228 cm^{-1} respectively. Hydroxyl group (OH) is usually characterized by the presence of a broad peak i.e. at 3426 cm^{-1}. The two other distinctive peaks obtained at 2924 cm^{-1} and 2848 cm^{-1} represent the C—H stretching frequen-