Vol.1.Issue.1.;2013

Bulletin of Pharmaceutical and Medical Sciences (BOPAMS)
A Peer Reviewed International Journal http://www.bopams.com

RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL ACTIVITY OF CHITOSAN STABILIZED NANO ZERO VALANT IRON

GEORGE ROBIN.A.T, S.KARTHICK RAJA NAMASIVAYAM AND SATHYANARAYANA RAJU
Department Of Biotechnology, Sathyabama University, Chennai 600119, Tamil Nadu, India

S.KARTHICK RAJA NAMASIVAYAM

Article Info:
Received: 14/04/2013
Revised from: 15/04/2013
Accepted on: 02/05/2013

ABSTRACT:

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas ofsolar energy conversion, catalysis, medicine, and water treatment. This increasing demand must be accompanied by "green"synthesis methods. In the global efforts to reduce generated hazardous waste, "green" chemistry and chemical processes areprogressively integrating with modern developments in science and industry. Implementation of these sustainable processes should adoptthe 12 fundamental principles of green chemistry. These principles are geared to guide in minimizing the use of unsafe products and maximizing the efficiency of chemical processes. Hence, any synthetic route or chemical process should address these principles by using environmentally benign solvents and nontoxic chemicals. Among the different metallic nanoparticles nano zerovalent iron are quite popular because of the higher application in remediation of toxic heavy metals in soil and sewage. In the present study, nano zero valent iron synthesized from the crude green tea extract and the biogenic particles thus obtained coated with 0.1 % starch. The synthesized and coated nano zero valent iron characterized by scanning electron microscopy (SEM) and energy dispersive X ray spectroscopy (EDX). The characterized particles evaluated for antibacterial activity against human pathogenic bacteria such as *E.coli*, *Pseudomonas aeruginosa* and Staphylococcus *aureus* adopting dilution method. All the tested bacterial count was significantly reduced in the nanoparticles treatment which suggest the possible use of ZVI as the potential bactericidal agent

Keyword: Nano zero valent iron, anti bacterial activity

INTRODUCTION

The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment [1, 2].This increasing demand must be accompanied by "green" synthesis methods. In the global efforts to reduce generated hazardous waste, "green" chemistry and chemical processes are progressively integrating with modern developments in science and industry. Implementation of these sustainable processes should adopt the 12 fundamental principles of green chemistry [3] which are geared to guide in minimizing the use of unsafe products and maximizing the efficiency of chemical processes. Hence, any synthetic route or chemical process should address these principles by using environmentally benign solvents and non toxic chemicals. Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts. Synthesis of noble metal nanoparticles for applications such as catalysis, electronics, optics, environmental, and biotechnology is an area of constant interest. Zero-valent iron nanoparticle technology is becoming an increasingly popular choice for treatment of hazardous and toxic wastes, and for remediation of

contaminated sites. The diminutive size of the iron nanoparticles helps to foster effective subsurface dispersion whereas their large specific surface area corresponds to enhanced reactivity for rapid contaminant transformation. Recent innovations in nanoparticle synthesis and production have resulted in substantial cost reductions and increased availability of nanoscale zero-valent iron (nZVI) for large scale applications. In this work, methods of nZVI synthesis and characterization are highlighted. Applications of nZVI for treatment of both organic and inorganic contaminants are reviewed. The controlled synthesis of magnetic nanoparticles is of high scientific and technological interest. The ferrite material exhibits unique electric and magnetic properties based on the transfer of electrons between $Fe^{2+}$and Fe^{3+} in the octahedral sites. To synthesize such particles, several methods are used. The nanoparticles formed using each method show specific properties. G-nZVI can be biosynthesized from polyphenol rich plant materials. When iron and plant material are combined polyphenols reduce iron to Fe^{0} while simultaneously forming nanoparticles. Plant products are an especially effective medium for biosynthesis because they are very rich in polyphenols (up to 5% by weight) and other reducing compounds including phenolic acids and flavinoids which are incorporated into the NZVI structure upon formation. In addition to providing environmentally friendly and highly effective synthesis method it also available at an extremely low cost. The reducing plant compounds found naturally in the plant materials provide excellent stabilization [4]. In the present study, nano zero valent iron synthesized by green technology and the synthesized G – NZV iron stabilized with starch evaluated against pathogenic bacteria.

Materials and Methods

Green Synthesis of NZVI

Nano zero valent iron particles are synthesized from the tea extracts adapting the method of mallikarjuna *et al* [5].2g of commercially available dust tea powder (Red Label, Tata India ltd.99%) were extracted in 100 mL boiling hot water. The filtered solution was reacted with 0.2M $Fe(NO_3)_3$. The synthesized nano zero valent iron was characterized by TEM and EDX.

Stabilization of green synthesized nano zero valent iron with starch

5 ml of synthesized suspension and 10 mL of a solution of starch (6.92 mg mL-1) were mixed and stirred until homogenous. Then, the above-mentioned mixture was transferred to a cuvette and allowed to stand for 12 h at 95°C. The color of the solution progressed from colorless to light yellow, and finally to orange within hours after the reaction was initiated and the stabilized particles were characterized by SEM.

Evaluation of anti bacterial activity of G-NZV

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* was selected. The respective tested strain was inoculated in brain hot infusion broth for 12-24 hours and 100ul of respective test culture was incubated with 900ul of both nano zero valent iron, incubated at 37c for 24 hours. After the incubation 100ul of the culture was serially diluted and plated on nutrient agar plates. The plates were incubated at 37c for 24 hours. After the incubation, colony count was recorded in respective dilutions of both treated strain and the percentage of inhibition was calculated by the following formula

$$\text{Inhibitory effect (\%)} = \frac{\text{No. of colonies in control- No. of colonies in treatment}}{\text{Number of colonies in control}} \times 100$$

Table 1: colony count for NZVI (green synthesized) treated Escherichia coli.

	Colony count at different dilutions				
Dilutions	10^2	10^3	10^4	10^5	10^6
Control	131	101	41	17	9
Treatment	12	9	4	1	0
Percent Inhibition	90.83969	91.08911	90.2439	94.117647	100

Table 2: colony count for NZVI (green synthesized) treated Pseudomonas aeruginosa.

	Colony count at different dilutions				
Dilutions	10^2	10^3	10^4	10^5	10^6
Control	140	123	61	17	8
Treatment	11	9	5	1	0
Percent Inhibition	92.14286	92.68293	91.80328	94.117647	100

Table 3: colony count for NZVI (green synthesized) treated Staphylococcus aureus.

	Colony count at different dilutions				
Dilutions	10^2	10^3	10^4	10^5	10^6
Control	126	109	49	19	6
Treatment	17	11	7	4	0
Percent Inhibition	86.50794	89.90826	89.3617	89.473684	100

RESULT AND DISCUSSION

The primary identification of synthesized zero valent iron was confirmed by the colour change of the reaction mixture from reddish brown to black colour (fig 1) after the addition of tea extract to ferric chloride solution. The reduction of Fe was confirmed using UV spectra and. TEM images shows spherical partice with size range 25- 45 nm(Fig 2) and the starch stabilized nanoparticles confirmed by the colour change of the reaction mixture from colorless to light yellow, and finally to orange and SEM (Fig 3). Nano zero valent iron and starch nano conjugates was also demonstrated by TEM and SEM where electron dense nanoparticles core size was in the range of 10–12 nm, and electron scarce thin starch coating . The confirmation of the coating was also confirmed using EDAX, which shows the peak for Fe, C and O (Fig 4). The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Since the initial field demonstration of the nZVI technology in 2001, significant progress has been made in research and development of iron nanoparticles for soil and groundwater treatment. Several recent studies have reported on the antimicrobial activity of nano particulate zero valent iron (nZVI) [6,7,8].

New research and development efforts should be directed toward enhancing real-world performance and minimizing potential economic and environmental risks. Anti microbial activity of stabilized NZV iron against human pathogenic bacteria reveals all the tested bacteria susceptible to S-NZV.In all the tested bacteria, total count in respective dilution of G-NZV treatment was decreased (Fig 5). Maximum inhibition percentage by G-NZV was recorded on *Pseudomonas aeruginosa* (Table 2) with an inhibitory percentage of 92 and above followed by as *E.Coli* (Table 1) *with* more than 91% inhibition followed by *Staphylococcus aureus* (Table 3). There was a significant activity of the nanoparticles on all the dilutions. There are several factors that caused the presently studied IO nanoparticles to be bactericidal. The main mechanism by which antibacterial drugs and antibiotics work is via oxidative stress generated by ROS [9]. ROS, including superoxide radicals (O_2^-), hydroxyl radicals (−OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), can cause damage to proteins and DNA in bacteria [10]. Park et al [11] also demonstrated an antibacterial activity from silver metals because of reactive oxygen species (ROS) generation. In this case, metal oxide Fe_3O_4 could be the source that created ROS leading to the inhibition of *S. aureus*. A similar process was described by Keenan et al[12] in which Fe^{2+} reacted with oxygen to create hydrogen peroxide This H_2O_2 consequently reacted with ferrous irons via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macromolecules. Other research has demonstrated that the small size of nanoparticles can also contribute to bactericidal effects. For example, Lee et al[7] reported that the inactivation of *Escherichia coli* by zero-valent iron nanoparticles could be because of the penetration of the small particles (sizes ranging from 10–80 nm) into *E. coli* membranes. Nano-Fe^0 could then react with intracellular oxygen, leading to oxidative stress and eventually causing

disruption of the cell membrane. Several other studies on ZnO and MgO nanoparticles also concluded that antibacterial activity increased with decreasing particle size [13].The strong bactericidal activity of starch stabilized NZVI implies that Nano-Fe^0 can serve as a cost effective biocide for many of the applications in which silver is being used which is found to be toxic. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. However, further studies must be conducted to verify if the bacteria develop resistance towards the nanoparticles and to examine cytotoxicity of nanoparticles towards human cells before proposing their therapeutic use.

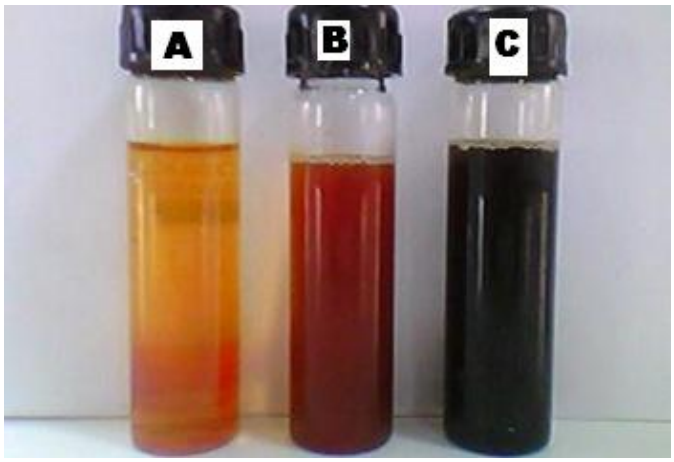

Fig 1: colour change during green ZNVI synthesis (A- $Fe(NO_3)_3$ solution , B-tea extract, C-ZNVI)

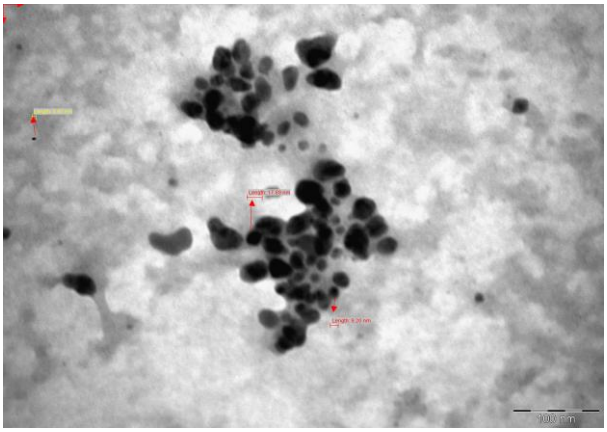

Fig 2: TEM image of the green synthesized nZVI

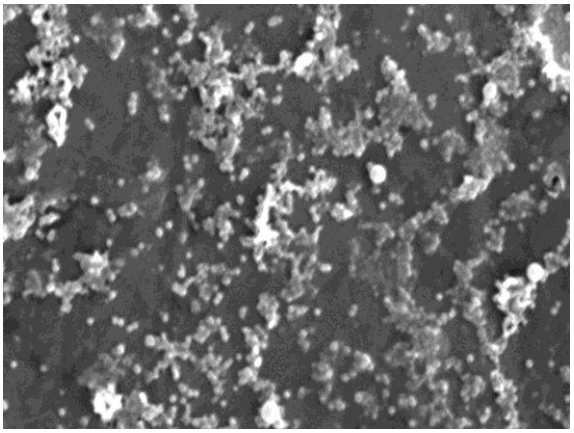

FIG 3: SEM image of the Starch stabilized nZVI

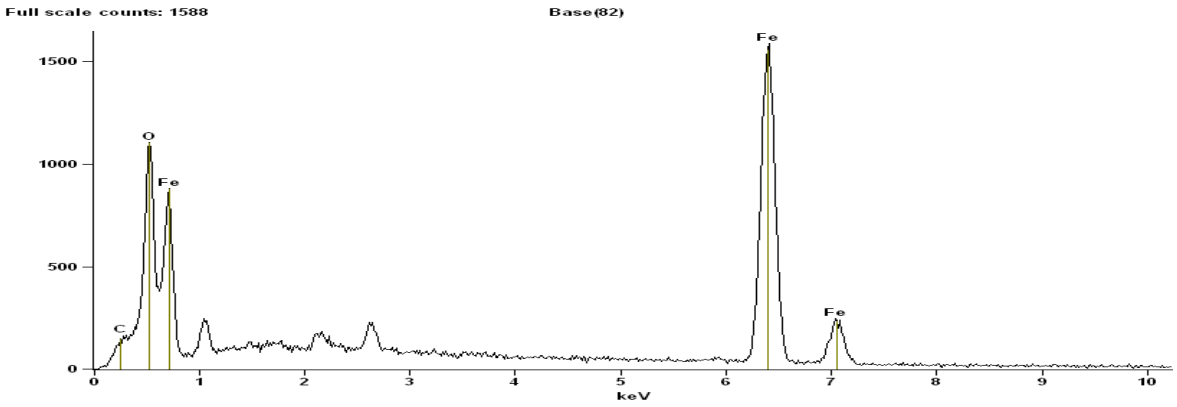

FIG 4: EDAX analysis of the starch stabilized nZVI

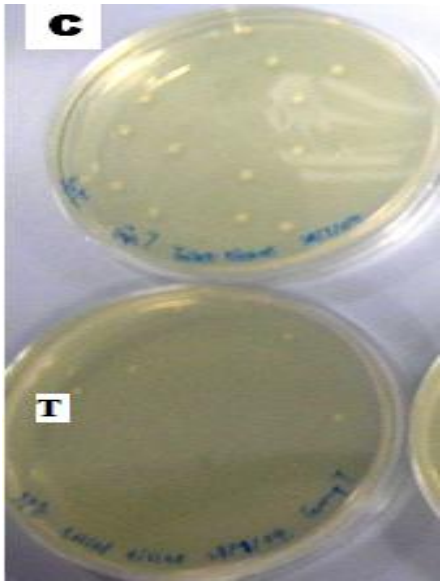

Fig. 5: Representative picture of the plate showing the colonies in treatment and control.

REFERENCES

[1]. Dahl, J.A; Maddux, B.L; Hutchison, J.E 2007..Towards greenter nanosynthesis. Chemical reviews,107, (6),2228-69.

[2]. Hutchison, J.E. 2008. Greener Nanoscience: A Proactive approach to Advancing Applications and reducing implications of Nanotechnology. ACS Nano,2,(3), 395-402.

[3]. Anastas, P.T; Warner, J.C. 1998. Green Chemistry: Theory and Practice, Oxford University Press, , p.30.

[4]. Petri-Fink A, Chastellain M, Juillerat-Jeanneret L, Ferrari A, Hofmann H .2005. Development of functionalized super paramagnetic iron oxide nanoparticles for interaction with human cancer cells. *Biomaterials.* **26**(15):2685–2694.

[5]. Mallikarjuna N. Nadagouda,*a* Alicia B. Castle,*b* Richard C. Murdock Saber M. Hussain*b* and Rajender S. Varma. 2010, *In vitro* biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols. 12, 114–122

[6]. Auffan, M., W. Achouak, J. Rose, M. Roncato, C. Chane´ac, D. T. Waite, A. Masion, J. C. Woicik, M. R. Wiesner, and J. Bottero. 2008. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward Escherichia coli. Environ. Sci. Technol. 42:6730–6735.

[7]. Lee, C., J. Y. Kim, W. I. Lee, K. L. Nelson, J. Yoon, and D. L. Sedlak. 2008. Bactericidal effect of zero-valent iron nanoparticles on Escherichia coli. Environ. Sci. Technol. 42:4927–4933.

[8]. Phenrat, T., T. C. Long, G. V. Lowry, and B. Veronesi. 2009. Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ. Sci. Technol. 43:195–200

[9]. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. Cell.;130(5):797–810.

[10]. Sies H. 1997. Oxidative stress: oxidants and antioxidants. Exp Physiol.;82(2):291–295.

[11]. Park HJ, Kim JY, Kim J, et al. 2009. Silver-ion-mediated reactive oxygen species generation affecting bactericidal activity. Water Res;43(4):1027–1032.

[12]. Keenan CR, Sedlak DL. 2008. Factors affecting the yield of oxidants from the reaction of nanoparticulate zero-valent iron and oxygen. Environ Technol.;42(4):1262–1267.

[13]. Zhang L, Jiang Y, Ding Y, Povey M, York D. 2007. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids) J Nanopart Res.;9(3):479–489.

.