



Bio-inspired synthesis of catalytically and biologically active palladium nanoparticles using *Bos taurus* urine

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Abstract

Physiologically processed bio-molecules present in cow excreta can be used to reduce metal ions into metal nanoparticles in a single step by novel synthetic routes. This biogenic reduction of metal ion to base metal is quite rapid and easily scaled up. These NPs exhibit excellent catalytic activities for studied organic reactions. X-ray diffraction pattern of reaction product confirmed the formation of palladium nanoparticles. The synthesized PdNPs shows potential activity for organic transformation. Antimicrobial activity of nanoparticles determines biogenic potential of the nanoparticles against different microorganisms and antioxidant activity determined the potential of free radicals. Both of the antimicrobial and antioxidant activity helps in study application of nanoparticles in medical and veterinary sciences.

Keywords Green-synthesis · PdNPs · FE-SEM · Catalyst · ABTS · Antimicrobial

1 Introduction

Since the ancient times material scientists are actively engaged in designing the new materials. In earlier days in order to develop new properties two or more materials were mixed together in different proportions. The resulting material exhibits new properties. Also some physical process were imparted to offer new properties in the materials. Therefore many times developmental scenario of human civilization is recognized by the invention of materials such as stone age, bronze age, iron age, plastic age etc. Today's researcher is mainly interested to develop materials at nanoscale. Nanotechnology is one such innovation that has become the indispensable tool for researchers from various wings of pure and applied sciences. Actually nanotechnology has dissolved the boarders of various disciplines of sciences. Nanoparticles do not obey the law of classical physics. American physicist Sir Richard Feynman is regarded as father of nanotechnology

[1–3]. According to Nario Taniguchi 'Nanotechnology mainly deals with separation, consolidation and deformation of the materials at atomic or molecular level' [4–6]. Nanoparticles are recognized as building blocks to make materials with tailored optical, magnetic, electronic, mechanical, electrical, catalytic and biological properties. The examples include different types of sensors such as gas sensors, bio-sensors, memristors, metamaterials, supercapacitors, bioactive materials, catalysts, batteries such as lithium ion batteries, electrochromic materials and recording media. The nano-structured materials enjoy novel size and shape dependent attributes which are distinct from that of bulk materials counterparts. This is because of some reasons such as high aspect ratio, ineffectiveness of gravitational force, sensitive electrostatic force, presence of dangling bonds, effective random molecular motion, more significant surface tension and Van der Waal's attraction etc. [7, 8]. At nano scale, as mass becomes extremely small and thus the gravitational force

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becomes negligible. Though the size becomes small, the charge remains same. Thus, electrostatic force remains same as in bulk condition. Thus, magical properties are developed in materials in nano regime. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures [9–11]. A bulk material has constant physical properties regardless of its size, but at nano-scale this is often not the case. Several well-characterized bulk materials have been found to possess most interesting properties when studied in nanoscale. Therefore, many scientists believe nano regime as a separate state of matter.

2 Laboratory synthesis of nanoparticles

Attempts have been made to synthesize nanomaterials by different routes of synthesis. The synthetic route can be broadly classified into two categories such as: (1) top down approach and (2) bottom up approach. In the top down approach, a suitable starting larger material is reduced in size of nano range using physical or chemical methods [12]. Though top down method is simple and easily understandable but it suffers from certain drawbacks such as the imperfection of the surface structure. Such defects in the surface structure can have significant impact on physical properties and surface chemistry of metallic nanoparticles due to high aspect ratio. Also the cost of top down method is very high e.g. photolithography, electron beam lithography. Therefore, due to simplicity and financial consideration bottom up approach is now a day commonly adopted. Here miniaturization of material component leads to formation of nano-structured materials. The bottom up synthesis mostly relies on chemical and biological methods of production [13, 14]. Generally the chemical methods are capable of voluminous production at low cost; however their drawback includes contamination from precursor chemicals, use of toxic solvents and generation of hazardous by-products. Bio-nanotechnology has emerged up as

integration between biotechnology and nanotechnology for developing biosynthesis and environmentally friendly technology for synthesis of nonmaterial's and to check the biological effectiveness of the nanoparticles and to use in medicinal applications [15, 16].

Therefore, there is a certain need for development of clean, biocompatible, non-toxic, eco-friendly, safe, cost effective, and sustainable process. The major problems associated with biologically synthesized nanoparticles are that they are not mono dispersed and rate of production is slow. Several researchers have attempted to synthesize palladium nanoparticles using biological routes. Literature review reveals that the researchers have so far synthesized nanoparticles using various plant extracts. The phytochemicals like polyphenols and anti-oxidants are supposed to be responsible for reduction process and formation of palladium nanoparticles. The research work of some of the earlier researchers is summarized (Table 1).

Herein an attempt has been made to synthesize palladium nanoparticles using A-2 type cow urine. In *Ayurveda* literature there is a long tradition of using cow products for positive health effects. It is constituent of *Panchagavya* (a combination of cow urine, milk, clarified butter, curd and dung). According to ancient *Ayurvedic* literature it is useful to control various diseases such as skin disease, liver, kidney, diabetes mellitus, anticonvulsant drug etc.

3 Materials and methods

3.1 Biosynthesis of palladium nanoparticles

Nanoparticles of palladium were synthesized using urine of indigenous Indian *Khilar* cow, which is also known as A-2 type. The cow urine is collected from local animal rear Hupari, India. The freshly collected cow urine is stored in clean glass bottle at room temperature. Urine routine analysis reveals the nature of cow urine as mentioned ahead: color-dark yellow, pH-alkaline, albumin-present,

Table 1 Biosynthesis of palladium nanoparticles using plant extracts

Plant	Part used	Size	Shape	Reference
<i>Anogeissus latifolia</i>	Gum	4.8	Spherical	[17]
<i>Cinnamomum zeylanicum</i>	Bark	15–20	Crystalline	[18]
<i>Cinnamomum camphora</i>	Leaves	3.2–6	–	[19]
<i>Curcuma longa</i>	Tuber	10–15	Spherical	[20]
<i>Euphorbia granulata</i>	Leaves	25–35	–	[21]
<i>Gardenia jasminoides</i>	Leaves	3–5	–	[22]
<i>Glycine max</i>	Leaves	15	Spherical	[23]
<i>Moringa oleifera</i>	Waste petal	10–50	Spherical	[24]
<i>Moringa oleifera</i>	Peel extract	27	Spherical	[25]
<i>Musa paradisiaca</i>	Peeled banana	50	Crystalline irregular	[26]

sugar-absent, ketone bodies-absent. For the synthesis of palladium nanoparticles palladium chloride precursor is procured from Sigma Aldrich which is used without further purification. Here 200 mL of 0.01 M PdCl₂ solution was prepared by dissolving palladium chloride in double distilled water. Then pinch of cetyl trimethyl ammonium bromide (CTAB) a cationic surfactant was dissolved in 5 mL of double distilled water and slowly added in above solution with constant stirring. After this, 50 mL of cow urine was taken in burette and drop wise added with constant stirring in the reaction mixture. The reaction mixture was maintained at about 80 °C temperature. As soon as the cow urine was added dark blackish colored precipitate appeared in the solution. After complete addition of 50 mL of cow urine a sufficient amount of the precipitate was observed. Then the reaction mixture was continuously heated till complete dryness. Then, solid mass remaining in the beaker was separated using spatula and then crushed into fine powder in mortar. The fine powder obtained was used for characterization and also studied catalytic efficacies for organic transformation and biological activities [27, 28].

3.2 Catalytic activity of palladium nanoparticles

As stated in literature, palladium nanoparticles are one of the excellent catalysts for reductive degradation of organic dyes [29–31]. Herein, we have tried to study palladium catalyzed reduction reactions. Analytical grade 4-Nitrophenol (C₆H₅NO₂), Sodium Borohydride (NaBH₄), were used for this experimentation process without further purification. The palladium catalyzed reduction of 4-Nitrophenol has been spectrophotometrically monitored. The catalytic reduction reaction was carried out in 3 mL capacity quartz cuvette. The cuvette was filled with 3 mL de-ionized water. Then, about 20 µL ice cold solution of 7.5 M NaBH₄ was slowly added. Then, in the above reaction mixture about 20 µL 0.015 M 4-Nitrophenol solutions was drop-wise added. To this reaction mixture water suspension of 30 µL (1 mg/10 mL) palladium nanoparticles were added. The progress of reaction was studied using Cary 60 UV–visible spectrophotometer in the spectra ranging from 200 to 600 nm.

3.3 Biological activities of palladium nanoparticles

3.3.1 Minimal inhibitory concentration (MIC)

MICs of prepared palladium nanoparticles were assessed using broth micro dilution method [9]. The palladium nanoparticles master stock of 1 mg/mL was used to prepare working solutions of different concentrations such as 50 µg/mL, 100 µg/mL, 150 µg/mL and 200 µg/mL. Fresh inoculums of two Gram-positive bacterial strains:

Staphylococcus aureus (NCIM 5276), *Bacillus cereus* (NCIM 2217) and three Gram-negative bacterial strains: *Escherichia coli* (NCIM 2662), *Salmonella typhimurium* (NCIM 5278) and *Pseudomonas aeruginosa* (NCIM 2037) were used in this experimentation process. All bacterial strains were inoculated in 3 mL nutrient broth containing 100 µL of various concentrations of palladium nanoparticles separately and were incubated for 20–24 h on shaking incubator (REMI) for 100 rpm at 37 °C. Microbial growth inhibition or stimulation was observed by measuring the OD of each tube at 625 nm using a UV–visible spectrophotometer. A negative control, of distilled water was maintained. Streptomycin of similar concentration was used as reference substances and was labeled as positive control. Inhibition of bacterial growth might most probably occur due to accumulation of palladium nanoparticles in bacterial membrane. MIC was defined as the lowest nanoparticles concentrations at which growth of bacterial cells were inhibited.

3.3.2 Agar well diffusion techniques

The antibacterial and anti-fungal activity is determined using common agar well diffusion method. Also these activities are determined using instrumental technique i.e. spectrophotometric method.

3.3.2.1 Antibacterial activity Herein simple agar well diffusion techniques was employed to estimate antimicrobial efficacy of palladium nanoparticles against five different microbes with small modification [10]. 100 µL of various concentrations of aqueous colloidal solution of palladium nanoparticles were loaded in well on nutrient agar plate. Then plates were maintained at 4 °C for 30–40 min for sample diffusion in culture medium agar and then were transferred to incubator overnight at 37 °C. Then, after 48 h the plates were observed for zone of inhibition. Furthermore the obtained results are compared with the well containing 100 µg/mL streptomycin as reference substance.

3.3.2.2 Antifungal activity Well diffusion method is widely used to evaluate the antifungal and antimicrobial activity of different nanoparticles prepared of silver and palladium. Similarly to the procedure used in agar well-diffusion technique, the PDA plates were prepared using submerged inoculation using two fungal strains: *Aspergillus niger* (NCIM 1360) and *Fusarium solani* JALPK [11]. The palladium nanoparticles (100 µL), of different concentrations were introduced in the well, these agar plates were then incubated under desire temperature. The zone of

inhibition was observed and zone diameter was measured.

3.3.3 Antioxidant activity

Anti-oxidant activities of synthesized nanoparticles are determined using two different techniques viz. (1) ABTS and (2) DPPH radical scavenging assay. The results obtained are in good agreement with one other.

3.3.3.1 ABTS radical scavenging assay ABTS radical scavenging assay was performed as described by Re et al. [12] with minor modifications. About 5 mL of 7 mM ABTS ammonium aqueous solution was mixed with 88 μ L of potassium peroxydisulfate ($K_2S_2O_8$) 140 mM. The mixture was allowed to stand for 12–16 h at room temperature to yield a dark blue solution. Then it was mixed with 99.5% of ethanol which gave 0.7 ± 0.02 units absorbance at 734 nm to get working solution. 2950 μ L of working solution was mixed with 50 μ L of samples extract and then incubated at 30 °C for 2 h under dark condition. Then, the absorbance of the mixture reaction was measured at 734 nm to produce A_{sample} . 99.5% Methanol was used as a blank solution and its absorbance was measured to produce control. Ascorbic acid was used as positive control. The inhibition rate was calculated using the formula:

$$\text{ABTS RSA(\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100. \quad (1)$$

3.3.3.2 DPPH radical scavenging assay DPPH assay was carried out using the method as described by Brand-Williams et al. [13] with slight modification. DPPH radical-scavenging activity of various concentrations of palladium nanoparticles (40 μ L) and 160 μ L of water were mixed with 3 mL of methanolic solution containing DPPH radicals. The mixture was vigorously shaken and left to stand for 30 min in the dark (to avoid light oxidation). The reduction of the DPPH radical was determined by measuring the spectrophotometric absorption at 517 nm. Ascorbic acid was used as standard. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the formula

$$\text{DPPH RSA(\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

where A_{sample} —is the absorbance of the solution when the sample has been added at a particular level, A_{control} —is the absorbance of the DPPH solution.

4 Results and discussion

4.1 Characterizations of synthesized nanoparticles

The characterization of synthesized product is incredibly important because it ensures the formation of NPs and morphology. Nanoparticles are generally characterized by their size, shape, absorbance, and disparity. The common techniques of characterizations adopted are spectroscopic and microscopic. The characterization techniques used are summarized below.

At the end of experimentation we are getting fine black colored powder. The properties of nanoparticle largely depend upon their size and shapes. As nanoparticles are beyond the perception of human eye it becomes essential to use advanced characterization techniques. Herein we have used spectroscopic and microscopic techniques for the study of nature and morphologies of the nanoparticles.

4.1.1 UV–visible spectroscopic study

At the end of experimentation process we are getting blackish colored powder. This powder is insoluble in water, thus sonicated in a bath sonicator and dispersed. Then the dispersed solution of nanoparticle was taken in quartz cuvette and exposed to UV–visible radiation and absorption was observed. Here, maximum absorption is observed at 410 nm which is in agreement with reported values for PdNPs. However, SPR absorbance is sensitive to the nature, size and shape of particles present in the solution. It also depends upon inter particle distance and the surrounding media.

4.1.2 XRD pattern

To investigate crystal structure of PdNPs, XRD measurements were performed. The diffraction pattern was carried out using Bruker Powder Diffractometer at Shivaji University, Kolhapur, India. A $\text{CuK}\alpha$ X-ray beam of wavelength 0.1540 nm was used. All the data were collected with standard X-ray generator setting of 40Kev and 30 mA. As shown in Fig. 1 all the diffraction peaks observed can be assigned to the (001), (002), (100), (011), (102), (004), (103), (110), (111), (112), (200), (113) and (022) diffraction peak of hexagonal structure. The peak position explains about the translational symmetry namely size and shape of the unit cell whereas the peak intensities give details about the electron density inside the unit cell. Thus the XRD pattern confirms the

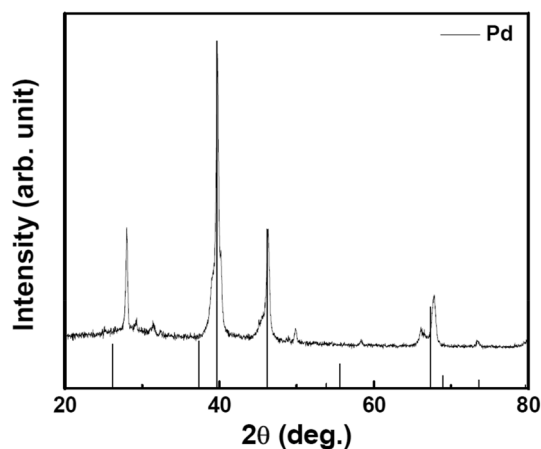


Fig. 1 XRD pattern of palladium nanoparticle

crystalline nature with hexagonal structure of the synthesized nanoparticles which is in agreement with the JCPDS file No. 01-071-0710. The crystalline size can be calculated by Scherer formula as under Table 2.

4.1.3 FE-SEM

Electron microscopy is the powerful tool to study size and shape of nanoparticles. Field emission scanning electron microscopic photograph of synthesized product is shown in Fig. 2 which reveals the formation of cylindrical, poly-dispersed cluster morphology in nano scale. The FE-SEM image reveals formation of 3D crystallite structure of palladium nanoparticles.

4.1.4 Zeta potential and DLS

Zeta potential from Fig. 3 signifies the stability of the synthesized nanoparticles. The particle size can be determined using dynamic light scattering. The DLS has been

Table 2 Crystallite size of poly-dispersed palladium nanoparticles

Full width half maxima	Peak position: 2θ (°)	Crystallite size (nm)
0.4723	14.43	16.96
0.1574	28.07	5.20
0.2362	29.33	3.47
0.3149	31.48	2.62
0.1200	39.72	7.04
0.0984	39.83	8.59
0.1574	40.22	5.37
0.3542	46.30	2.44
0.2755	49.89	3.18
0.4723	58.33	19.27

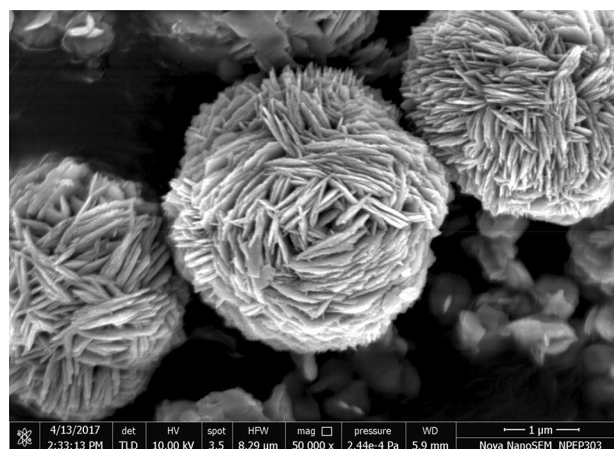


Fig. 2 FE-SEM image of palladium nanoparticle

determined using Brookhaven Instruments Corp. The poly-dispersed nanoparticles have been synthesized. The DLS analysis from Fig. 4 reveals the hydrodynamic diameter of the synthesized nanoparticles.

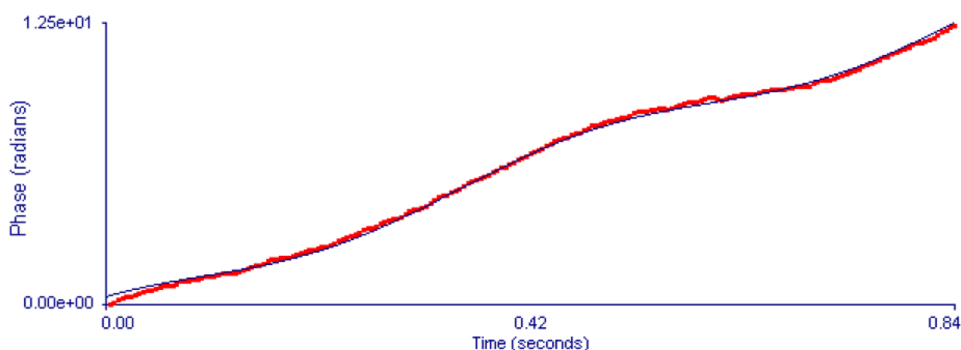
4.2 Catalytic activity of Pd nanoparticles for organic reactions

Palladium nanoparticles is considered as one of the best catalyst for hydrogenation chemical. In this work efficacy of palladium nanoparticles for the reduction of 4-Nitrophenol into 4-Aminophenol were determined. Initially the absorbance peak was observed at 400 nm. With the progress of time the intensity of absorbance continuously decreased which indicates the decrease in the concentration of reactant with progress of time. There is new peak at 300 nm and with progress of time intensity of this peak also increase. The peak at 300 nm indicates formation of 4-Aminophenol. The concentration of the product 4-Aminophenol is observed to increase with progress of time. About 97% of 4-Nitrophenol was converted into 4-Aminophenol within 10 min. Heterogeneous catalysis reactions are extensively studied using the classical Langmuir–Hinshelwood equation, which is depended on the reactant being chemisorbed on surface of catalyst [18–20]. In our study, assuming both reactants adsorbed on the surface of as-synthesized Pd-nanoparticles, Langmuir–Hinshelwood equation was used to study the rate of reduction reaction, the general equation for rate of heterogeneous catalysis reduction reaction in presence of NaBH_4 is given by

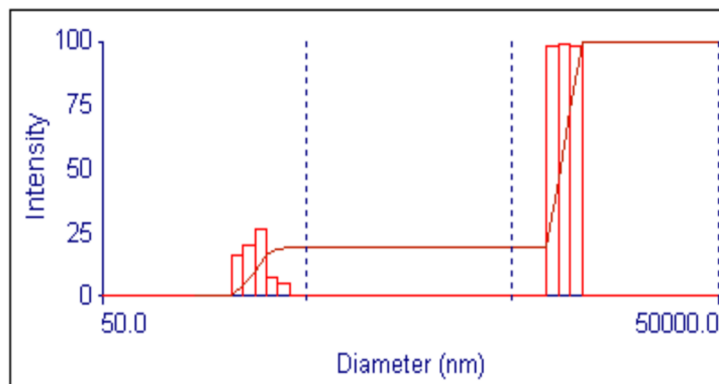
$$-\frac{d[R]}{dt} = k[4NP]^a [BH_4^-]^b \quad (3)$$

The conversion of 4-Nitrophenol into 4-Aminophenol can be calculated by apparent pseudo first order rate constant (k_{app}) which is determined by combining Eq. (1) and when

Fig. 3 Zeta potential of palladium nanoparticle



Elapsed Time	00:00:28
Mean Diam.	7149.0 nm
Rel. Var.	0.239
Skew	-1.316



Multimodal Size Distribution

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
153.0	0	0	643.7	0	20	2708.3	0	20
174.3	0	0	733.5	0	20	3086.2	0	20
198.6	0	0	835.8	0	20	3516.8	0	20
226.4	15	4	952.5	0	20	4007.6	0	20
257.9	20	10	1085.4	0	20	4566.8	0	20
293.9	26	16	1236.8	0	20	5204.1	0	20
335.0	7	18	1409.4	0	20	5930.3	0	20
381.7	4	20	1606.1	0	20	6757.8	0	20
435.0	0	20	1830.2	0	20	7700.8	99	46
495.7	0	20	2085.6	0	20	8775.4	100	73
564.8	0	20	2376.6	0	20	10000.0	99	100

Fig. 4 Dynamic light scattering of palladium nanoparticle

concentration of 4-Nitrophenol is very less as compared with the concentration of sodium borohydride, can be narrowed down to

$$\ln\left(\frac{C_0}{C}\right) = k_{app}t = k S t \tag{4}$$

where, C_0 and C are initial and final concentration having absorbance at fixed wavelength (λ) and at fixed time (t), k_{app} is considered as first order rate constant and is inferred to be equivalent to the accessible surface area

(S) of Pd-Nanoparticles. The surface area normalized rate constant k is calculated by

$$k = k_{app}/S \tag{5}$$

Thus, for constant catalyst concentration and uniform accessible surface of the reactants, a plot of $\ln(C_0/C)$ versus time gives a straight line, whose slope is k_{app} (Figs. 5, 6).

As shown in Fig. 7 graph of $\ln(C_0/C_t)$ versus time shows good correlation, and rate constant is calculated as 0.36667 min^{-1} for palladium Nanoparticles.

Brookhaven Instruments Corp.
90Plus Particle Sizing Software Ver. 5.34

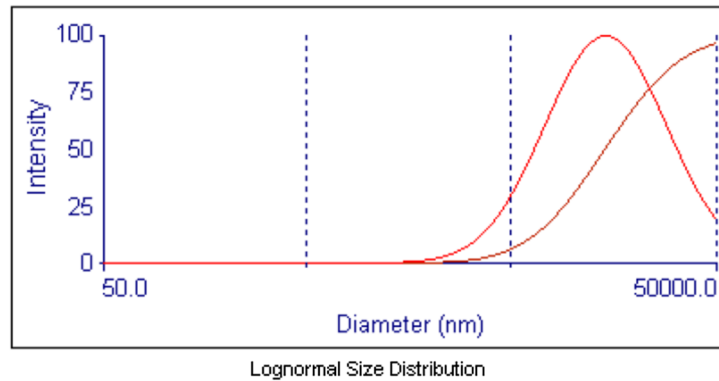
Date: May 7, 2015

Time: 11:38:20

Batch: 0

Sample ID **y3**
Operator ID **smita**
Notes

Elapsed Time	00:00:28
Median Diam.	14403.3 nm
Mean Diam.	18189.1 nm
Polydispersity	0.595
GSD	1.980



d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
4681.5	26	5	12117.0	97	40	22826.4	80	75
5999.1	44	10	13215.3	99	45	25602.6	70	80
7097.1	58	15	14403.3	100	50	29231.0	58	85
8102.9	70	20	15698.0	99	55	34580.6	44	90
9088.3	80	25	17120.9	97	60	44313.4	26	95
10069.1	87	30	18736.6	93	65			
11072.1	93	35	20603.1	87	70			

Fig. 5 Size distribution graph of palladium nanoparticle

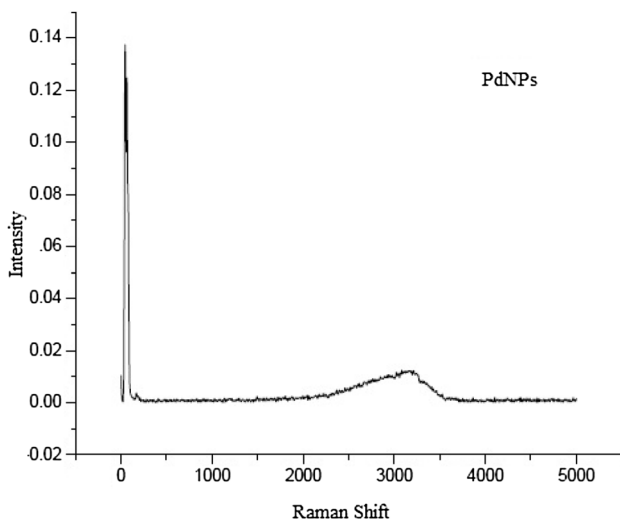


Fig. 6 Raman spectra of palladium nanoparticle

4.3 Determination of biogenic activities

Herein an attempt has been made to study the biological effectiveness of synthesized palladium nanoparticles. In the current experimentation antimicrobial activity with MIC and anti-oxidant activity of the synthesized nanoparticles are determined by two different techniques. The results obtained are in good agreement with one another.

4.3.1 Determination of minimum inhibitory concentrations (MIC)

The MIC of palladium nanoparticles were studied using spectrophotometric method [27], where turbidity was measured at 625 nm. No growth was observed under spectrophotometer at concentration of 100–200 µg/mL. This concludes that concentration of 100–200 µg/mL

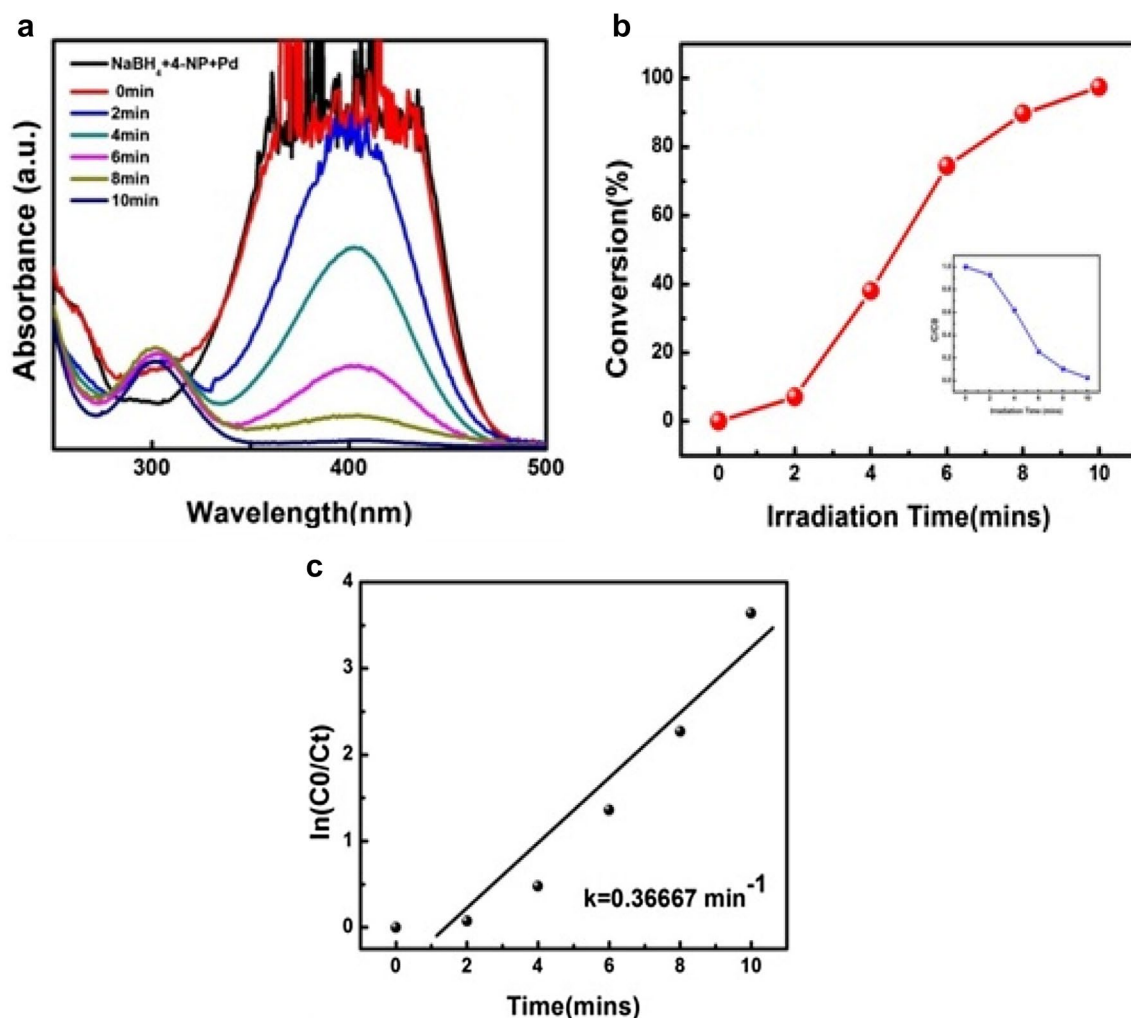


Fig. 7 **a** UV-visible spectra indicating reduction of 4-Nitrophenol to 4-Aminophenol with time, **b** percentage conversion of 4-Nitrophenol, **c** kinetics of catalytic reduction

Table 3 Bacterial growth at different concentrations of Palladium nanoparticles after 24 h

Conc µg/mL	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>
25	+	+	+	+
50	+	-	+	-
100	-	-	+	-
150	-	-	-	-
200	-	-	-	-

Positive (+): Presence of turbidity in medium due to microbial growth, Negative (-): Absence of turbidity in medium due to no microbial growth

have good bactericidal activity which is important in the making antibacterial agents. Results determine presence or absence of turbidity which was determined by + or - respectively in Table 3. Lower concentration of palladium nanoparticles seems to be turbid due to bacterial growths which represents smaller concentration of nanoparticles do not have any antibacterial activity. Results also concluded that bacterial strains *Pseudomonas aeruginosa* and *Salmonella typhi* were highly sensitive to nanoparticles were as *Bacillus cereus* and *Escherichia coli* bacterial strains are lower bactericidal activity seen by visible growth.

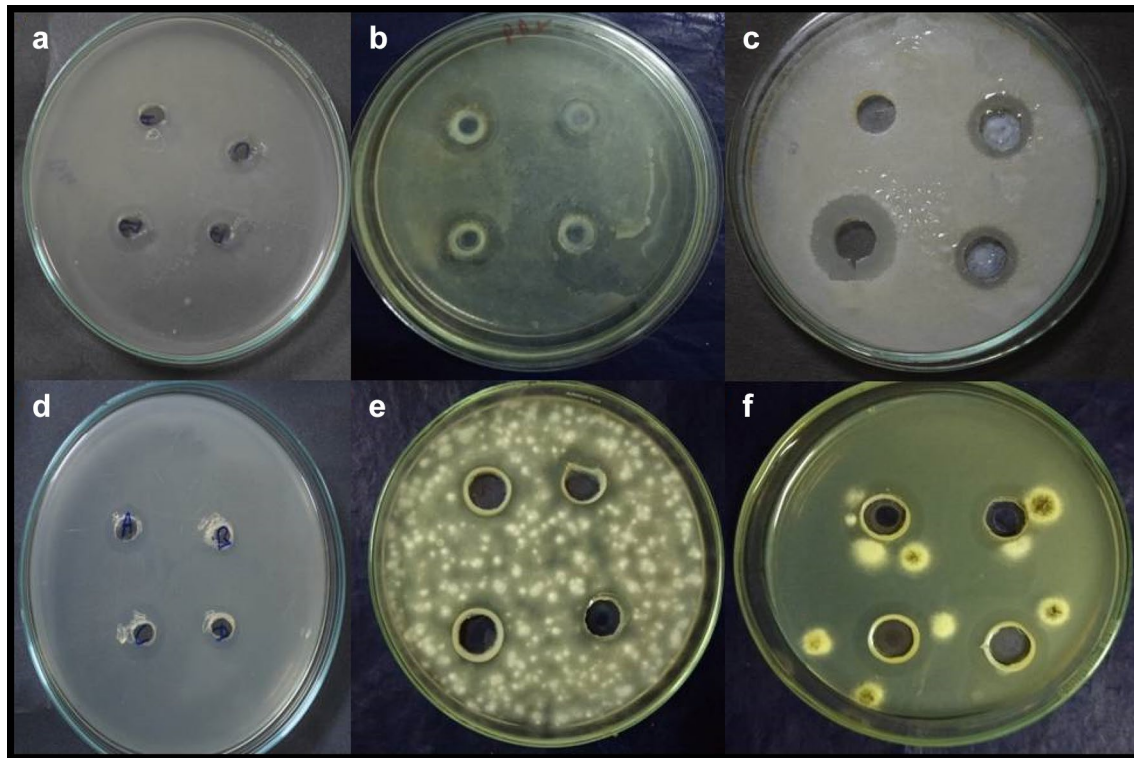


Fig. 8 Antimicrobial and antifungal action of palladium nanoparticle on **a** *Salmonella typhi*, **b** *Pseudomonas aeruginosa*, **c** *Bacillus cereus*, **d** *Escherichia coli*, **e** *Fusarium solani*, **f** *Aspergillus niger*

Table 4 Anti-bacterial and Anti-fungal effect exhibited by Palladium nanoparticles on various test microbes

Sample name	Conc $\mu\text{g/mL}$	Bacterial strains				Fungal stains	
		<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>	<i>Aspergillus niger</i>	<i>Fusarium solani</i>
Palladium nanoparticles	50	10	12	13	11	10	10
	100	13	14	14	12	10	11
	150	14	15	14	14	11	12
	200	15	16	15	16	12	11

Zone of in inhibition in in mm

4.3.2 Antibacterial activity and antifungal activity

Previous report on synthesis and application of nanoparticles from palladium were studied by Chen et al. [28]. The antibacterial and antifungal activities of palladium nanoparticles prepared were evaluated against a set of 4 microorganisms and 2 fungal strains are displayed in Fig. 8. Their potency was assessed qualitatively by the presence or absence of inhibition zone diameter in mm. The results are presented in Table 4 and it indicates that, the given concentrations of nanoparticles extract is having considerable antimicrobial activity against tested

microorganisms. Results showed that synthesized nanoparticles exhibit moderately good antibacterial activities against *E. coli* and *Bacillus cereus*. Similar results are observed in experiments performed by Sondi et al. [29]. *P. aeruginosa* and *S. typhi* also exhibit good antimicrobial activity. The zone of inhibition observed is 16 mm for both microbes. The small antimicrobial spectrum of zone of inhibition was found in *A. niger* than *Fusarium solani* which indicate lowest antifungal activity. Size-dependent antimicrobial effects of novel palladium nanoparticles are earlier studied by Adam et al. [30].

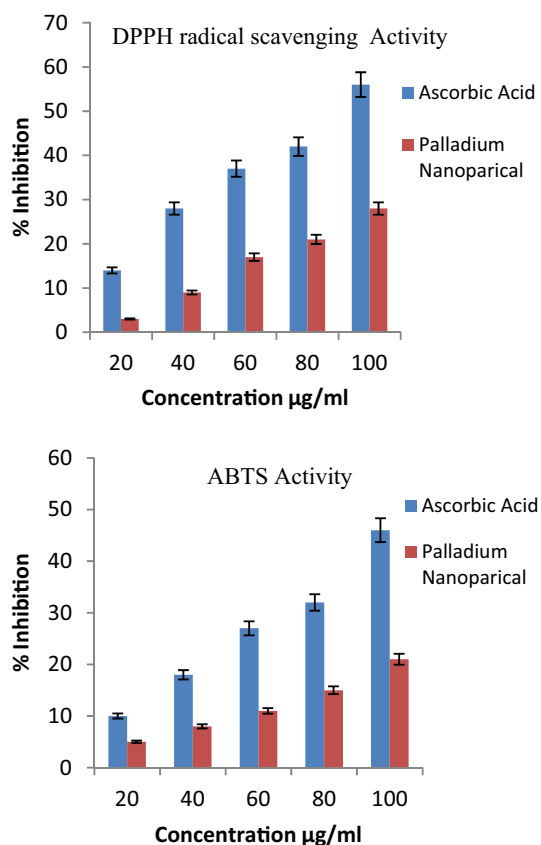


Fig. 9 Comparative study antioxidant activity of ascorbic acid with antioxidant activity of PdNPs prepared using cow urine

4.3.3 Antioxidant activity

Antioxidants are free radicals molecules produced from many systems which have capability to damage biological cellular system. ABTS and DPPH are commonly used or convenient method to measure the free radical damaging activity. DPPH and ABTS scavenging activities of palladium nanoparticles in comparison with standard antioxidant ascorbic acid are shown in Fig. 9. DPPH shows 28% radical scavaging activity and ABTS shows 21% radical scavenging activity at 100 µg/mL. The good scavenging activities were observed in the ABTS and DPPH, due to its capability of good oxidant, electron loosing and capping agent present on the surface of different nanoparticles. Palladium nanoparticles have different application with various different chemical method of preparations are reported previously by Saldan et al. [31]. Significantly, biogenic synthesis of palladium nanoparticles demonstrate a broad-range of spectrum for antimicrobial and good antioxidant action therefore it signify promising antioxidants and antimicrobial agents with possible use in preparation of biomedical compound (Fig. 9).

4.4 Possible reaction pathway

Mammalian animal urine contains abundant amount of urea. Basically urea is a neutral compound. Therefore, colloidal synthesis due to reduction reaction is not possible merely due to addition of urea. Thus one of the possible reactions is as under:



Thus, here formation of ammonia takes place. This is a slow reaction. Now ammonia contains lone pair of electron on Nitrogen atom. This can be donated in a chemical reaction. Thus metal ions can accept the electron and formation of metal in zero oxidation state takes places.

5 Conclusion

This work represents successful synthesis of PdNPs using cow urine. During the synthesis of palladium nanoparticles cow urine acts as reducing agent. Therefore it is concluded that presence of bio-components in cow urine is responsible for reduction of palladium ions and formations of nanoparticles. This is a rapid and eco-friendly method which has added advantage of reduced reaction time and better control over size and morphology. This is very simple and does not require sophisticated instrumentation and high skilled man power. This is very economical as cow excreta are used as reducing agent which is a waste product. The nanoparticles can be synthesized at physiological pH. Pd nanoparticles have been characterized using UV-visible spectroscopy, XRD, and FE-SEM to determine their size, shape, and structure. These results reveals that the synthesized nanoparticles are mostly cylindrical, and poly-disperse. The synthesized PdNPs shows catalytic efficacy for studied organic transformation. This was the first report on Biological activities of PdNPs using cow urine. This antimicrobial activity helps to synthesis the antimicrobial agents and use of nanoparticles in medicinal science. Free radical activity of nanoparticles helps to cure the damages to the cell due to oxidative stress. It also help in drugs delivery and drugs development.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest for the publication of article.

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