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Bos taurus Urine Assisted Synthesis of Cadmium Nanoparticles

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ABSTRACT

We are reporting for the first time the synthesis of cadmium nanoparticles assisted by cow urine. These NPs exhibit excellent antimicrobial activity against test micro-organism such as Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa. Also the synthesized nanoparticles show synergetic effectiveness with common broad spectrum antibiotic tetracycline. X-ray diffraction pattern of reaction product confirmed the formation of cadmium nanoparticles.

Keywords: Green-synthesis, CdNPs, Anti-microbial activity, MIC, Synergetic effectiveness

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INTRODUCTION

Material scientists are actively engaged in designing the new materials with the application of nanotechnology. Nanotechnology is one such innovation that has become the indispensable tool for researchers from various fields such as Physics, Chemistry, Mechanical Engineering, Microbiology, Medical Sciences etc. Nanotechnology has dissolved the boarders of science. Nanoparticles do not obey the law of classical mechanics. American physicist Richard Feynman is regarded as father of nanotechnology [1-3]. According to him 'Nanotechnology mainly deals with separation, consolidation and deformation of the materials at atomic or molecular level. The nano-structured materials possess novel size and shape dependent properties which are different from that of bulk materials because of some reasons such as high aspect ratio, ineffectiveness of gravitational force, electrostatic force, presence of dangling bonds etc. [4-6]. At nano scale, mass becomes extremely small and thus the gravitational force becomes negligible. Though the size becomes small, the charge remains same. Thus, electrostatic force remains same as in bulk condition. Thus, exotic properties are developed in materials in nano regime. Therefore, many scientists believe nano regime as a separate state of matter.

The methods of making nanoparticles generally involve either top down approach or bottom up approach. Due to simplicity and economy bottom up method is now a day commonly used. The bottom up synthesis mostly relies on chemical and biological methods of production. The traditional and most widely used methods for synthesis of metallic nanoparticles use wet chemical procedures. To prevent the agglomeration of metallic nanoparticles, the stabilizing agent such as sodium dodecyl sulphate or polyvinyl pyrrolidane can be added into their reaction mixture. 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.

Therefore, there is a 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. Here an attempt has been made to synthesize cadmium nanoparticles by using cow urine.

MATERIALS AND METHODS

Nanoparticles of Cadmium were prepared by using urine of indigenous Indian cow, which is also called as A-2 type. The 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, sugar-absent, and ketone bodies-absent. 100 ml of 0.1 M $CdSO_4$ solution was prepared by dissolving cadmium sulfate in double distilled water.

Then pinch of N,N cetyl trimethyl ammonium bromide (CTAB) was dissolved in 5 ml of double distilled water and slowly added in above solution. 50 ml of cow urine was taken in burette and drop wise added at about 80ºC with constant stirring in the reaction mixture. As soon as the cow urine was added dark yellowish 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 and powdered in mortar. The fine powder obtained was used for characterization and for determination of anti-microbial activity.

RESULTS AND DISCUSSION

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

UV-visible spectroscopic study

The yellowish colored sample powder was sonicated in de-ionized water. Then this solution was taken in cuvette and exposed to UV-visible radiation and the absorbance of the solution was recorded. Because of the Surface Plasmon Resonance, resonant peak occurs at different wavelength for different nanoparticles. As per the theory of resonance maximum absorbance occurs at resonant wavelength. Here, maximum absorption is observed at 410 nm which is in agreement with reported values for CdNPs. 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.

X-RD pattern

XRD patterns of synthesized CdNPs are shown in Figure 1 where five major peaks have appeared. 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 crystalline nature with cubic structure of the synthesized nanoparticles which is in agreement with the PCPDS file No. 85-1328. The PCPDS data indicates the melting point of the sample to be 960.6ºC.

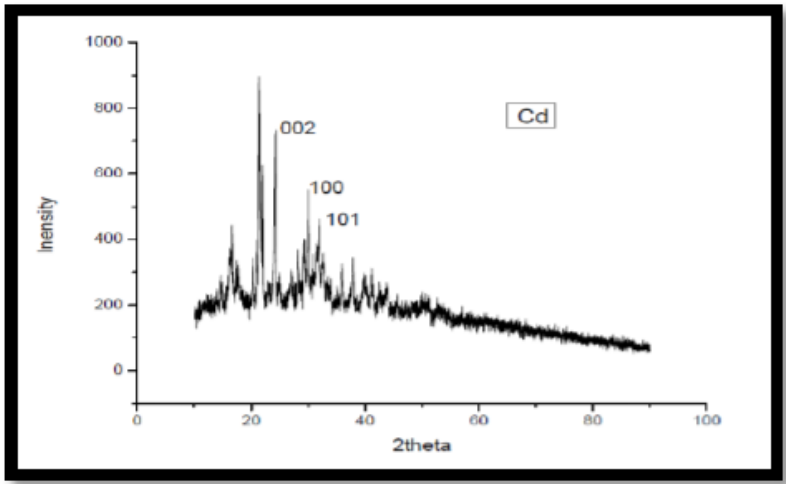

Figure 1: XRD pattern

SEM

SEM photograph of synthesized product is shown in Figure 2 which reveals the formation of cylindrical, poly-dispersed morphology in nano scale.

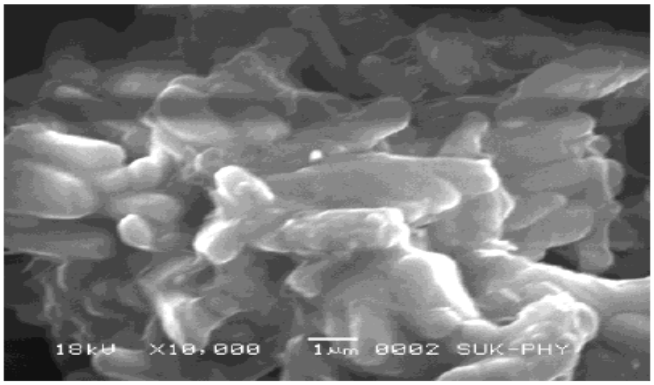

Figure 2: SEM image

The various characterization techniques reveal the formation and morphology of cadmium nanoparticles.

Zeta potential and DLS

Zeta potential signifies the stability of the synthesized nanoparticles. The particle size can be determined using dynamic light scattering. The DLS has been determined using Brookhaven Instruments Carp. The poly-dispersed nanoparticles have been synthesized. The smallest particle size is found to be 62.2 nm which lies in nano range (Figures 3 and 4).

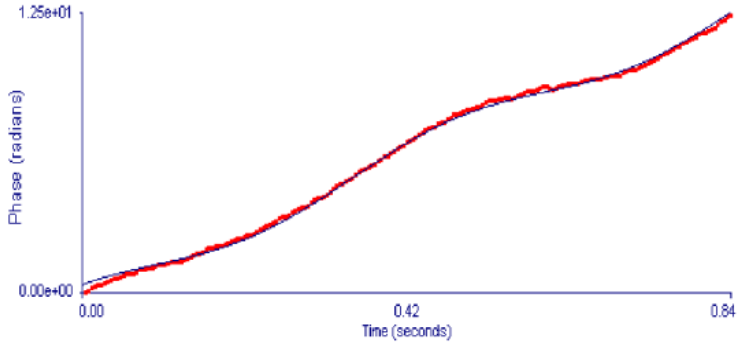

Figure 3: Zeta potential

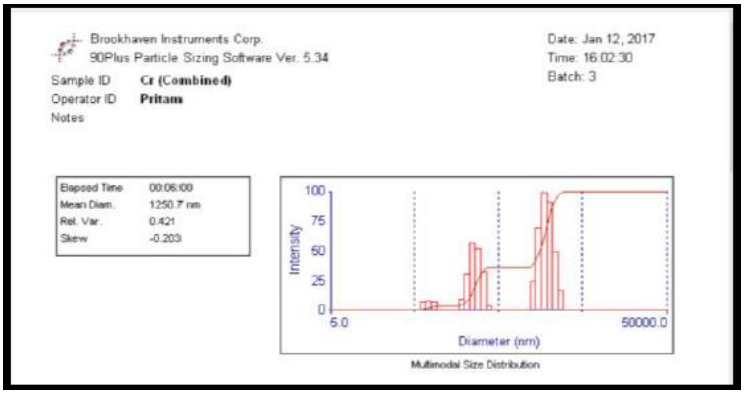

Figure 4: Dynamic light scattering

Antimicrobial efficacy of Cd nanoparticles alone and in combination with anti-biotic tetracycline

The antimicrobial activity of Cd nanoparticles was determined by agar cap diffusion method against three pathogens namely *Staph aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* responsible for wound infections, respiratory tract diseases, urinary tract infections and septicemia. The media used for anti-microbial study was nutrient agar [7-9]. A sterile cork borer of standard 8 mm diameter was used to cut the well on agar surface and 500 mL solution of the nanoparticle was added in the well. After diffusion of nanoparticle solution for 20 min at 10ºC, all the plates were incubated at 37ºC for 24 h. The results were observed and the diameters of zone of inhibition were measured in cm. As the experiment was performed in triplicate, the mean values of zone of inhibition were recorded. The minimum inhibition concentration was also determined by using concentration range 0.2 mg/ml to 50 mg/ml.

The Cd nanoparticles can change cell membrane permeability and may allow maximum transport of anti-biotic within cell when applied in combination. So to study the synergetic effect of nanoparticles with known broad spectrum antibiotic the same experiment was repeated by using tetracycline conc. 10 µg/L to 1000 µg//L alone and in combination with Cd nanoparticles. The antimicrobial activity is demonstrated in Figure 5. Literature survey reveals that antibacterial activity is derived through the electrostatic attraction between negatively charged cell membrane of micro-organism and positively charged metal nanoparticles.

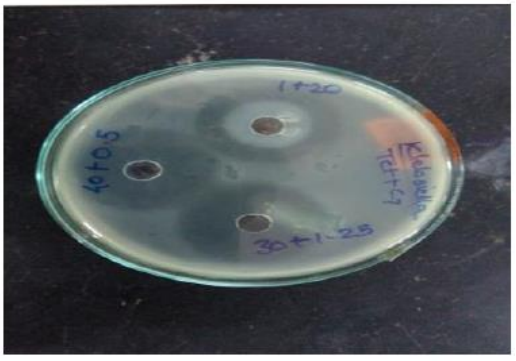

Figure 5: Antimicrobial activity

Table 1 reveals that among the three tested pathogens all are sensitive to Cd nanoparticles. However *Staph aureus* was found to be most sensitive as compared to *Klebsiella pneumonia* and *Pseudomonas aeruginosa* for Cd nanoparticles. MIC values are as shown ahead.

Table 1: Antimicrobial Efficacy at various concentrations

S. No	CdNPs				Tetracycline				Synergetic			
	Conc.	*Staph aureus*	*Klebsiella pneumonia*	*Pseudomonas aerogenosa*	Conc.	*Staph aureus*	*Klebsiella pneumonia*	*Pseudomonas aerogenosa*	Conc.	*Staph aureus*	*Klebsiella pneumonia*	*P. aero-Genosa*
1	0.1	R	R	R	1 µg/ml	R	R	R	1 µg/ml+1 mg/ml	2.7	-	-
2	0.2	R	R	R	5 µg/ml	R	R	R	5 µg/ml+0.8 mg/ml	2.5	-	-
3	0.4	1 cm	R	R	10 µg/ml	R	R	R	10 µg/ml+0.6 mg/ml	1.7	-	-
4	0.6	1.2	R	R	20 µg/ml	1.5	1.2	R	15 µg/ml+0.4 mg/ml	1.5	-	-
5	0.8	1.3	R	R	30 µg/ml	1.7	1.5	R	20 µg/ml+0.2 mg/ml	2.5	-	-
6	1	1.5	R	R	40 µg/ml	2.2	2	R	30 µg/ml+1.25 mg/ml	-	3	-
7	1.25	-	1.1	R	60 µg/ml	2.5	2	R	40 µg/ml+0.5 mg/ml	-	3.2	-
8	2.5	-	1.5	R	80 µg/ml	-	-	R	100 µg/ml+50 mg/ml	-	-	2.9
9	5	-	1.7	1.2	100 µg/ml	-	-	R	200 µg/ml+40 mg/ml	-	-	3
10	10	-	2	1.8	200 µg/ml	-	-	R	300 µg/ml + 30 mg/ml	-	-	2.5
11	20	-	2.3	2.1	300 µg/ml	-	-	R	400 µg/ml+ 20 µg/ml	-	-	3
12	30	-	-	2.5	400 µg/ml	-	-	R	500 µg/ml+10 mg/ml	-	-	2.8
13	40	-	-	2.7	500 µg/ml	-	-	1	600 µg/ml+5 mg/ml	-	-	2.5
14	50	-	-	2.8	600 µg/ml	-	-	1.2	-	-	-	-
15	-	-	-	-	700 µg/ml	-	-	1.4	-	-	-	-
16	-	-	-	-	800 µg/ml	-	-	1.5	-	-	-	-

For CdNPs: *Staph aureus* 0.2 mg/L, *Klebsiella pneumonia* 1 mg/ml and *Pseudomonas aeruginosa* 2.5 mg/L. b) For tetracycline MIC values are *Staph aureus* 10 µg/L, *Klebsiella pneumonia* 15 µg/L and *Pseudomonas aeruginosa* 400 µg/L.

It is also observed that when nanoparticles and antibiotics are used in combination then effectiveness of antibiotic increased. MIC values under this synergetic condition for three pathogens are 1 µg/ml, 5 µg/ml and 10 µg/ml for *Staph aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively.

Possible reaction pathway

Basically urea is a neutral compound. The colloidal synthesis due to reduction reaction is not possible merely due to urea. But one of the possible reactions is as under.

$$2NH_2CONH_2 \rightarrow H_2NCONHCONH_2 + NH_3$$

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.

CONCLUSION

This work presents successful synthesis of CdNPs using cow urine. This reveals cow urine as a novel reducing agent. The presence of different bio-components in cow urine could be responsible for reducing the cadmium ion and formation of nanoparticles. This is a rapid and environmentally benign method which has added advantage of reduced reaction time and better control over size and shape. The present green synthetic method is an easy, economical and eco-friendly way to synthesize CdNPs at physiological pH. The synthesized CdNPs have shown significant antimicrobial activity against *Staph aureus, Klebsiella pneumonia and Pseudomonas aeruginosa* bacteria. We have shown that CdNPs may come up as promising candidate in the medical field. The use of antibiotics in combination with nanoparticles can help in treatment of multidrug resistant bacteria. This research work may also make a future platform for preparing nano-medicines, targeted drug delivery.

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