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Chemical Reduction Method of Penicillin G Derived Gold Nanoparticles for Antimicrobial Applications

S. Baskar[1], G. Selvan[1], R. Anbarasu[1] & V. Raja[1]

[1]*PG and Research Department of Physics, Thanthai Hans Roever College (Autonomous), Perambalur, Tamil Nadu, India.*

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Abstract

Nanotechnology has become one of the most interesting and advanced areas of research in this field. The main targets are to generate uniform gold nanoparticles in aqueous solutions via chemical reduction methods, which could generate final products in good quality. Standard antibiotic Penicillin G used to reduce the gold salt to gold nanoparticles. These particles have been widely used in various biomedical applications and drug delivery systems due to their inert nature, stability, high disparity, non-cytotoxicity and biocompatibility. Such the present investigation aims an efficient and convenient production routes to generate spherical and purified gold nanoparticles. These gold nanoparticles were characterized by different spectral data analysis. The antimicrobial activities of gold nanoparticles were confirmed by disc diffusion method against some human pathogens.

Keywords: Gold Nanoparticles, Chemical Reduction Method, Penicillin G, Antimicrobial Activity.

Introduction

Benzyl penicillin (INN, AAN, BAN), also known as penicillin G (USAN), is a narrow spectrum penicillin antibiotic that is given intravenously or intramuscularly as a treatment for syphilis, meningitis, endocarditis, pneumonia, lung abscesses and septicaemia in children (Ahmed John and Koperuncholan, 2012). Penicillin G is typically given by injection parenterally, bypassing the intestines, because it is unstable in the highly acidic stomach. Because the drug is given parenterally, higher tissue concentrations of penicillin G can be achieved than is possible with phenoxymethylpenicillin. These higher concentrations translate to increased antibacterial activity. The term "nano" is derived from the Greek word "nanos" which means small and it is used as the prefix for one billionth part (10^{-9}). Nanotechnology has emerged in the last decades, which is developed with high speed and is now undergoing a revolutionary. There is no doubt to say nanotechnology is preparing to play a significant and commercial role in our future society. According to American Society for Testing and Materials, nanoparticles are those particles which have two or more than two dimensions and are in the size range of 1 - 100nm (Ahmed John S and Koperuncholan M, 2012a). These particles have special and enhanced physical and chemical properties as compared to their bulk materials due to their large reactive and exposed surface area and quantum size effect as a result of specific electronic structures. These particles have been widely used in many fields such as electronics, photochemical, biomedicine and chemistry (Anitha et al. 2011; Beevi et al., 2012; Vignesh et al., 2014).

Gold nanoparticle is the most stable metal nanoparticles, and they present fascinating aspects such as their size-related electronic, magnetic and optical properties, biocompatible, non-cytotoxic properties, their assembly of multiple types, surface functionalization, and their applications to catalysis and biology (Fazal Mohamed et al. 2011). All these promise gold nanoparticles an important building block. By now the global market for gold nanoparticles is still in its infancy, although it can be foreseeing that a rapid development would show up in the next few years (Koperuncholan and Ahmed John, 2011a), we still lack the ability to deliver large amount of gold nanoparticles with stable good quality. There are certain short boards for the existence synthesis processes of gold nanoparticles. Therefore, an efficient, stable and convenient process for the production of gold nanoparticles is of importance. The development of a complete production route is challenge.

Correspondence
S.Baskar
E-mail: sivabaskar85@gmail.com, Ph.+9199941 58916

Materials and Methods

The standard antibiotic Penicillin G was purchased from Hi-Media. The 5 g of Penicillin G was taken and mixed with 100 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman No. 1 filter paper. The filtrate was further filtered through 0.6 µm sized filter paper. The filtrate was used for the present study.

Chemical Synthesis of Gold Nanoparticles

1mM of gold chloride was reduced using 100ml of 5% Penicillin G extract at room temperature for the synthesis of gold nanoparticles. Gold chloride has taken in similar quantities without adding antibiotic to maintain as a respective control. It was resulting in the pink solutions indicating the formation of gold nanoparticles. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations were recorded

Characterization of Nanoparticles

UV-VIS spectroscopy

The Au nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behaviour of Au nanoparticles. The scanning range of the samples was 200-800 nm at a scan speed of 480 mm/min. Baseline correction of the spectrophotometer was carried out by using a blank reference.

Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS)

In this research work, Joel JSM-6480 LV SEM machine was used to characterize the mean particle size and morphology of nanoparticles. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDS analysis of Au sample was done by the SEM (JEOLJSM 5800) machine. The EDS normally reveals the presence of phases.

X-ray Diffraction Method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherlands) using Cu radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. The Au sample was scanned in the range 10.0000 - 90.0000^{o} in continuous scan mode. The scan rate was 0.60/sec.

Antimicrobial Screening

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method (Koperuncholan and Ahmed John, 2011; Pandiyarajan et al., 2013; Lakshmi praba et al., 2013). This method was used to evaluate in vitro antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively (Vignesh et al. 2015a,b). A sterile cotton swab was used to inoculate the standardized bacterial suspension on surface of agar plate (Muthukumar et al., 2015). The 15 and 30 µL of test solutions were poured in each disc (6 mm diameter), separately. One separate disc was used for control study by taking sterile triple distilled water (without test sample) (Koperuncholan and Manogaran, 2015). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler/HiAntibiotic ZoneScale-C (Koperuncholan et al. 2010 and Vignesh et al., 2012a,b). The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control (Vignesh et al., 2013). All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

Results and Discussion

Chemical Synthesis of Gold Nanoparticles by Penicillin G Extract

The Penicillin G aqueous solution and gold chloride solutions were prepared separately. The antibiotic extract was mixed with gold chloride for the synthesis of gold nanoparticles. During this fabrication process, colour was changed from pale white to pink colour, suggested that formation of gold nanoparticles (Koperuncholan, 2015).

UV-VIS Spectroscopy

The UV-VIS spectroscopy studies revealed the presence of beard peaks at 541 nm (Figure 1). The absorption spectra of Au nanoparticles formed in the reaction media have absorbance maxima at 540 nm (Ramesh et al. 2014). A remarkable broadening of peak at around 480 nm to 580 nm indicates that the particles are polydispersed. During each time interval, the peak became distinct and rising. This peak rising clearly denoted the increasing nanoparticles synthesis as the time increases.

Scanning Electron Microscope with Energy Dispersive Spectroscopy (SEM/EDS)

The SEM image of gold nanoparticles synthesized by chemical synthesis process by using 5 % antibiotic extract and 1mM HAuCl4 concentration it gave a clear image of highly dense gold nanoparticles. The SEM image showing gold nanoparticles synthesized using antibiotic extract confirmed the growth of gold nanostructures (Figure 2). The EDS reading proved that the compulsory phase of gold (Au) and oxygen (O) is present in the sample. The graph also shows the presence of carbon (C), and silicon (Si) is present in the EDS picture of gold nanoparticles. It's revealed the presence of pure gold nanoparticles in higher percentages than other factors. This is likely due to the presence of

substrate over which the NP sample was held during SEM microscopy (Figure 3). As EDS equipment works at low vacuum (1-270 pa) it allows to observe non-conducting samples without the need to cover them with a thin conductive film, and consequently no evidence of noise by the coating material.

XRD Analysis

The XRD image of the sample after the addition of the gold chloride hydrate was depicted (Figure 4). Its represents the XRD pattern of the produced gold nanoparticles. In this result, peaks were observed at 2θ of 38, 44 and 64 are corresponding to the Bragg's reflections such as (111), (200) and (220). Other peaks were also observed along with the main peaks. The XRD patterns clearly show that the nanoparticles are crystalline in nature.

Antimicrobial Screening

The antimicrobial activity of gold nanoparticles was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities are summarized in Table 1. Found that the Au nanoparticles have exhibited considerable activity against some human pathogens. The antimicrobial property of Au is found to be the best among different metals in the following order Au > Zn > Fe > Mn > Mo > Sn (Sinthiya and Koperuncholan, 2015). In the present study, higher (30µL / disc) concentration of sample got greater sensitivity than (15µL / disc) lower concentration in all the tested microorganisms. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. The gold nanoparticles not only interact at the surface of cell membrane, but also enter inside the bacteria and cause damage of the cells by interacting with phosphorus/ sulphur containing DNA and its replication. In bacteria, the test sample was most effective against B5 while smaller effect was noticed from B4. In fungi, this was effective against F4 whereas smaller effect was observed in F2. All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6. Studied the increasing use of gold based products as antimicrobial agents and the concluded that the gold materials are an efficient alternative to antibiotics for the treatment this nanoparticles release

Conclusion

In our present investigation, we conducted an in-depth study on the synthesis and characterization of Au nanoparticles and their application on biological system. Antibacterial and Antifungal efficacy of the Au nanoparticles against some human pathogens has been performed. At nanoscale, gold exhibits remarkably unusual physical, chemical and biological properties. Effective chemical synthesis of nanoparticles will have greater implication and application in biomedical research. The mechanisms involved in the uptake of metal ions may be intracellular accumulation and surface adsorption. These biosynthesised gold nanoparticles can potentially be used for diff erent medical applications.

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Table I. Antimicrobial activity of AuNps derived from *B. cristata* leaves

S.No	Test Microorganisms		AuNPs (µL/disc)		PC
Bacteria			15	30	10 mcg
1.	*Aeromonas liquefaciens*	B1	13	14	14
2.	*Enterococcus fecalis*	B2	15	18	8
3.	*Klebsiella pneumoniae*	B3	16	19	28
4.	*Micrococcus luteus*	B4	14	18	38
5.	*Salmonella typhimurium*	B5	16	19	0
6.	*Vibrio cholarae*	B6	13	16	16
Fungi					
7.	*Candida albicans*	F1	13	17	10
8.	*Cryptococcus* sp.	F2	12	14	9
9.	*Microsporum canis*	F3	16	18	9
10.	*Trichophyton rubrum*	F4	11	13	7

PC - Positive Control

Using antibiotic disc = Bacteria – Methicillin (10mcg/disc);
Fungi – Itraconazole (10mcg/disc)

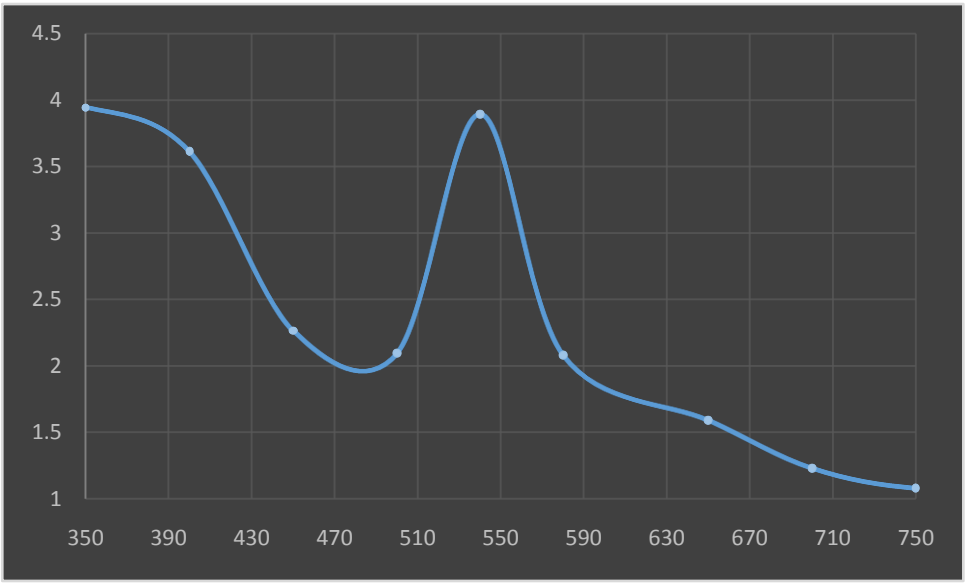

Figure I. UV-Spectrum of AuNPs

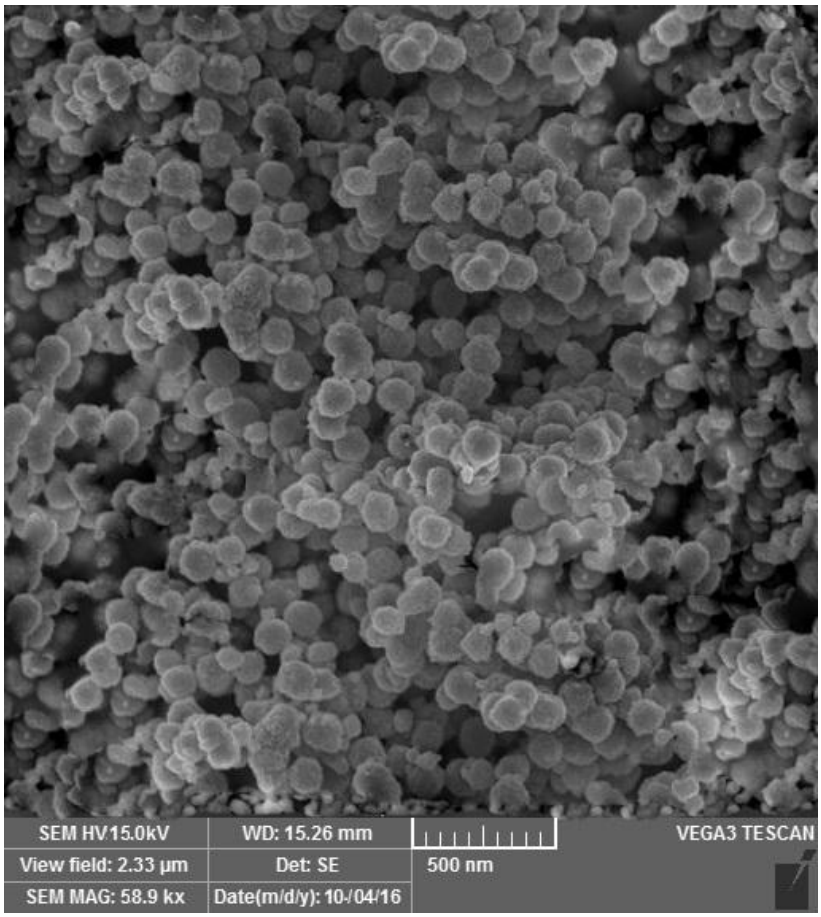

Figure II. SEM Image of AuNPs

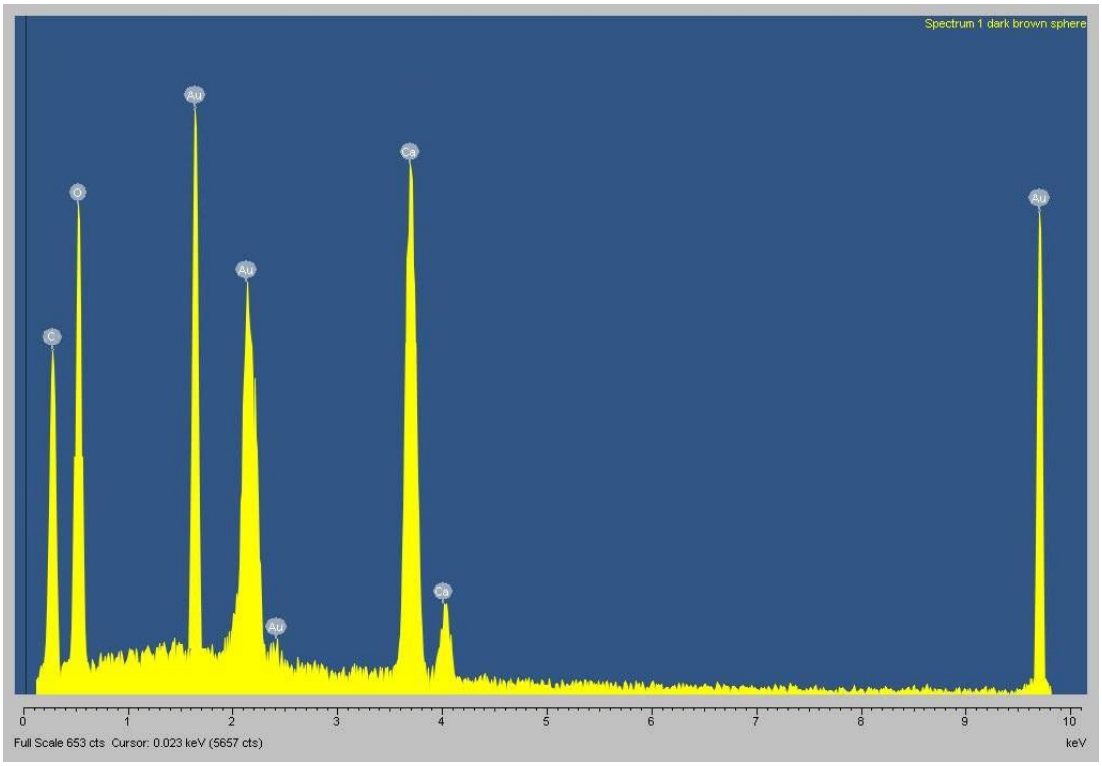

Figure III. EDAX spectrum of AuNPs

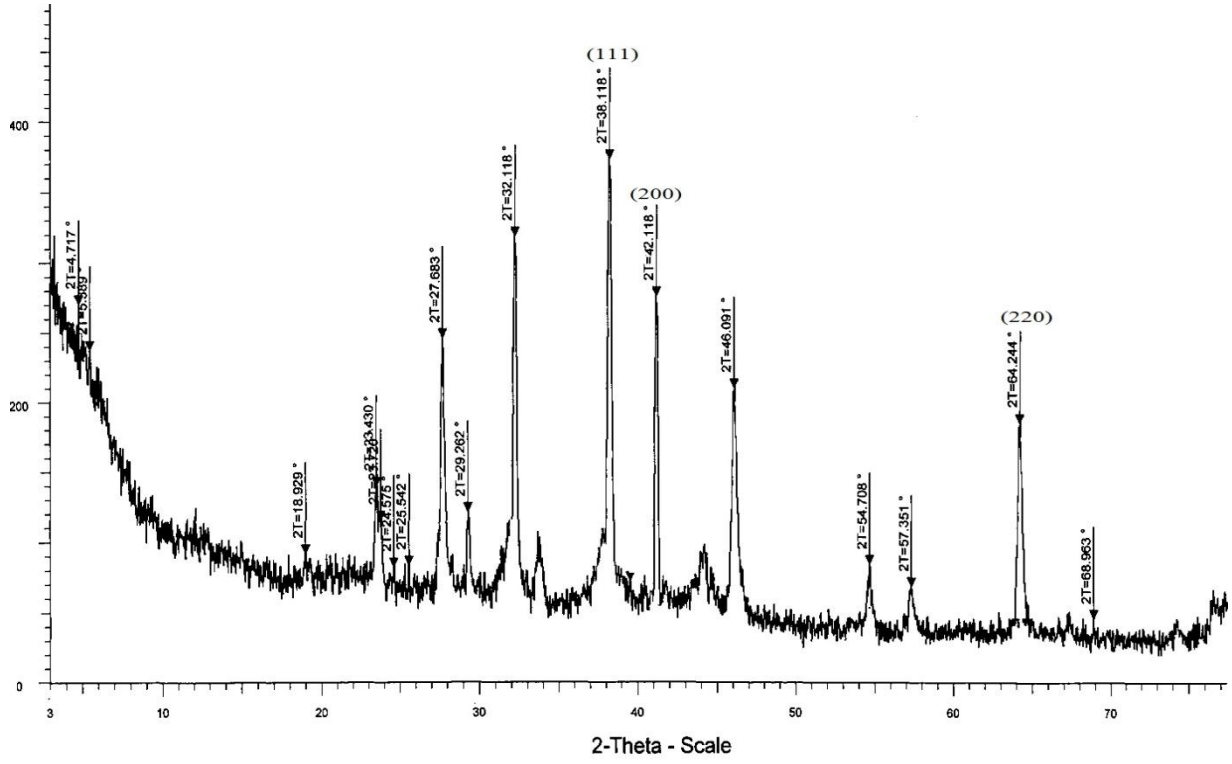

Figure IV. XRD Pattern of AuNPs