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NALIDIXIC ACID MEDIATED SILVER NANOPARTICLES SYNTHESIS, CHARACTERIZATION AND ITS PHARMACOLOGICAL APPLICATIONS

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Abstract:

In this research, the synthesis of stable silver nanoparticles by the standard antibiotic reduction method was investigated. The Nalidixic acid act as the reducing and stabilizing agent for the synthesis of silver nanoparticles. The aqueous extract of standard antibiotic Nalidixic acid was mixed with 1mM of Silver nitrate to reduce the metal salt to metal nanoparticles. Synthesized silver nanoparticles (AgNPs) particles were confirmed by analysing the excitation of surface plasmon resonance (SPR) using UV–vis spectrophotometer at 420 nm. The average sizes of the silver nanoparticles were 80 nm to 90 nm as determined through scanning electron microscopy (SEM). Energy-dispersive X-ray spectra (XRD) of silver nanoparticles revealed that the silver was in pure form. These synthesized nanoparticles were found to be highly toxic against human pathogens. All the microbial strains show higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6. The higher (30 µL/disc) concentration of sample got greater sensitivity than (15 µL/disc) lower concentration in all the tested microorganisms.

Key Words: Nalidixic acid, silver nanoparticles, Antimicrobial activity & metallic nanoparticles

Introduction:

Nalidixic acid is the first of the synthetic quinolone antibiotics. In a technical sense, it is a naphthyridone, not a quinolone: its ring structure is a 1,8-naphthyridine nucleus that contains two nitrogen atoms, unlike quinoline, which has a single nitrogen atom. Nalidixic acid is effective primarily against gram-negative bacteria, with minor anti-gram-positive activity. In lower concentrations, it acts in a bacteriostatic manner; that is, it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning that it kills bacteria instead of merely inhibiting their growth. It has historically been used for treating urinary tract infections, caused, for example, by *Escherichia coli*, *Proteus*, *Shigella*, *Enterobacter*, and *Klebsiella*. It is no longer clinically used for this indication in the USA as less toxic and more effective agents are available (Ahmed John and Koperuncholan, 2012a). It is also a tool in studies as a regulation of bacterial division. It selectively and reversibly blocks DNA replication in susceptible bacteria (Fazal Mohamed et L. 2011). Nalidixic acid and related antibiotics inhibit a subunit of DNA gyrase and topoisomerase IV and induce formation of cleavage complexes. It also inhibits the nicking-closing activity on the subunit of DNA gyrase that releases the positive binding stress on the supercoiled DNA.

Nanotechnology is rapidly growing by producing nano-products and nanoparticles (NPs) that can have novel and size-related physico-chemical properties differing significantly from larger matter (Anitha et al. 2011). The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (Beevi et al. 2012). Nanoparticles are particles that have one dimension that is 100

nanometers or less in size. The properties of many conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles; this causes them to be more reactive to certain other molecules. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials generally change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material starts to become significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in comparison to the number of atoms in the bulk of the material. Hence, the field of nanotechnology is one of the most active research areas in modern material/ medical science. There have been impressive developments in the field of nanotechnology in the recent past year, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements (Ahmed John and Koperuncholan 2012). Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100 nm) materials for the development of science. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology.

Materials and Methods:

Aqueous Extraction:

The standard antibiotic Nalidixic acid was purchased from Hi-Media. The 5 g of Nalidixic acid 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.

Biosynthesis of Silver Nanoparticles:

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

Characterization of Nanoparticles:

UV-VIS Spectroscopy:

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

Dynamic Light Scattering Particle Size Analyzer:

In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor [Vibronics, model: VPLP1]. Then experiment was carried out in computer controlled particle size analyzer [ZETA Sizers Nanoseries (Malvern Instruments Nano ZS)] to find out the particles size distribution.

Dynamic Light Scattering Zeta Potential Measurement:

Zeta potential describes the electrical potential in the double layer of ions surrounding a particle at the boundary of the particle surface and the adsorbed ions in

the diffuse layer (8&9). Zeta potentials were determined with a Zetaphorementer IV (CAD, France).

Scanning Electron Microscopy (SEM):

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

Fourier Transform-Infra Red (FT-IR) Spectroscopy:

The analysis of bio-reducing agent present in each of the extracts was measured by FT-IR. After the reaction, a small aliquot of the concentrated reaction mixture was measured in the transmittance mode at 400 to 4000 cm^{-1}. The spectra of the extracts taken after the biosynthesis of nanoparticles were analysed.

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 Netherland) using Cu Ká radiation. The generator voltage and current was set at 35 KV and 25 mA respectively. The Ag samples were scanned in the 2è ranges 15 to 700C range in continuous scan mode. The scan rate was 0.04^{o}/sec.

Antimicrobial Screening:

Microorganisms:

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 M and Ahmed John S. 2011). 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. A sterile cotton swab was used to inoculate the standardized bacterial suspension on surface of agar plate. 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). 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. 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. All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

Result and Discussion:

Synthesis of Ag Nanoparticles:

The Nalidixic acid aqueous solution and silver nitrate solutions were prepared separately. A quantity of 1.5 ml of Nalidixic acid extract was mixed with 30 ml of 10^{-3} M of silver nitrate for the synthesis of silver nano particles. During silver nanoparticles synthesis, the change of colour from pale white to brownish colour suggested the formation of silver nanoparticles (Koperuncholan and Ahmed John 2011a and Vignesh et al. 2011).

UV-VIS Spectral Analysis:

The UV-VIS spectroscopic studies revealed the presence of beard peaks at 420 nm Figure 1. The absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 420 nm. The plasmon resonance of the silver nanoparticles was recorded. When the precursor silver nitrate solution has mixed with the Nalidixic acid extracts they were reduced into silver (Ag) nanoparticles (Koperuncholan and Manogaran, 2015). The absorption spectra of Ag nanoparticles formed in the reaction media has absorbance maxima at 420 nm (Koperuncholan, 2015a). A remarkable broadening of peak at around 350 nm to 600 nm indicates that the particles are polydispersed. It was observed that the peak was blue shifted in the absorption spectrum from 350nm to 600 nm with increasing reaction time. (Figure 1).

Scanning Electron Microscope (SEM):

SEM absorption of the products was recorded as synthesis of nanoparticles spherical in structure of about 60 nm in diameter in the case of silver nanoparticles (Figure 2).

Energy Dispersive Spectroscopy (EDS):

EDS revealed the presence of pure silver (Figure 3) nanoparticles in higher percentages. Silver peak is higher than other peak. The EDX reading proved that the required phase of silver (Ag) is present in the sample.

Dynamic Light Scattering of Particle Size Analyzer:

The Figure 4 shows the particle size of the nanoparticles samples. After analyzing data, it was found that Ag nanoparticles size were in the range of 30-80nm. However, beyond 100 nm range the percentage of nanoparticles present is normally. The highest fraction of AgNPs present in the solution was of 58nm. From the plot it was evident that the solution was consist of nanoparticles having various sizes which are indeed in agreement of the result obtained by SEM analysis (Koperuncholan 2010).

Dynamic Light Scattering of Zeta Potential Measurement:

The Figure 5 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the Nano silver Vignesh et al. 2012b). The overall absorbance of Zeta Potential revealed the incipient instability nature occurred in this sample.

Fourier Transform Infra-Red Spectroscopy:

The FTIR spectrum of the Nalidixic acid extract with Silver nitrate solution was given Figure 6. Wherein some pronounced absorbance was recorded in the region between 4000 and 400 cm^{-1}. They include 3436(secondary amine, free, N-H asymmetric stretching), 2064 (Diazo, RCH=N=N Stretching), 1646 (etartiN, O-NO_2 Stretching asymm) and 684(C-S, R-C-CH_3 stretching for sulphur compounds), cm^{-1} (Lakshmi praba. et al. 2013)

X-Ray Diffraction Study:

Silver nanoparticles were synthesized using Nalidixic acid and the synthesis was confirmed by observable colour change in the reaction mixture and also by UV-VIS spectrum. Subsequently, X-ray diffraction (XRD) pattern of synthesized particles were analysed and found peak profile of relevant particles. In this result, peaks were observed at 2θ of 38, 44, 64 and 78 are corresponding to the Bragg's reflections such as (111), (200), (220) and (311). Other peaks were also observed along with the main peaks. This may be due to the crude nature of the extracts containing other metabolites and salts (Muthukumar et al. 2015 and Vignesh et al. 2014) These components would

have reacted with the ionic silver during the synthesis reaction. These compounds might be reason for the formation of other peaks (Figure 7).

Antibacterial and Antifungal Screening:

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test (7). found that the Ag nanoparticles have exhibited considerable activity against some human pathogens. The antimicrobial property of silver is found to be the best among different metals in the following order Ag >Hg > Cu >Cd>Cr>Pb>Co>Au>Zn>Fe>Mn>Mo>Sn (12). The results of the antimicrobial activities are summarized in Table 1. In the present study, higher (30 µL/disc) concentration of sample got greater sensitivity than (15 µL/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 silver 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/ sulfur containing DNA and its replication (Pandiyarajan et al. 2013 and Ramesh et al. 2014). 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 (Sinthiya and Koperuncholan, 2015 and Vignesh et al. 2013). Studied the increasing use of silver based products as antimicrobial agents and he concluded that the silver materials are an efficient alternative to antibiotics for the treatment (Vignesh et al. 2015b). This nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity (Vignesh et al. 2012a, Vignesh et al. 2015) There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample.

Conclusion:

The present investigation is highly warranted to through more light upon the Ag nanoparticles from medicinal Nalidixic acid will helpful to investigate the active principle action for biochemical and molecular studies. At nanoscale, silver exhibits remarkably unusual physical, chemical properties. Effective synthesis of nanoparticles will have greater implication and application in biomedical research. In this study nanoparticles of 50 ± 10 nm were synthesized by using *Nalidixic acid,* as confirmed by SEM and DLS. These nanoparticles showed characteristic absorption peak at 420 nm in UV spectra. The possibility of chemical compound as a stabilizing material in silver nanoparticles is revealed by FTIR analysis. The crystalline structure of silver nanoparticles was confirmed by XRD. The antimicrobial study was confirmed that Ag biosynthesized nanoparticles will act as an alternative antibiotic in future.

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Table 1: Antimicrobial activity of the silver nanoparticles

S.No	Test Microorganisms		AgNPs -µL/disc		PC	Diseases	Route of Transmission
	Bacteria		15	30	10 mcg		
1.	*Aeromonas liquefaciens*	B1	12	13	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis*	B2	15	18	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae*	B3	18	24	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus*	B4	18	24	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium*	B5	13	15	0	Typhoid	Water / Food
6.	*Vibrio cholarae*	B6	11	14	16	Cholera	Water / Food
	Fungi						
7.	*Candida albicans*	F1	12	14	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp.	F2	10	11	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis*	F3	13	15	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum*	F4	12	13	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc); Samples – 15, 30 µL/disc

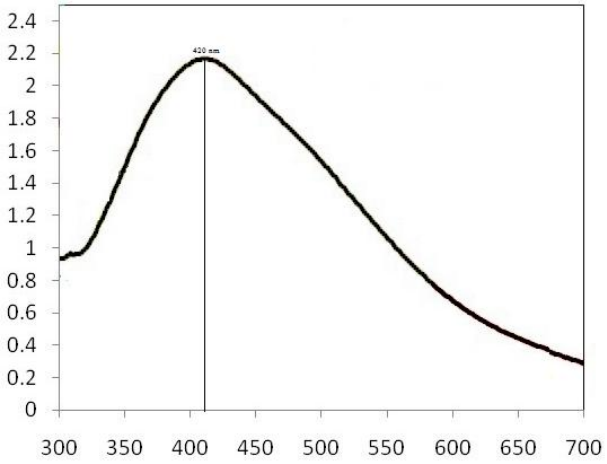

Figure 1: UV characterization of AgNPs

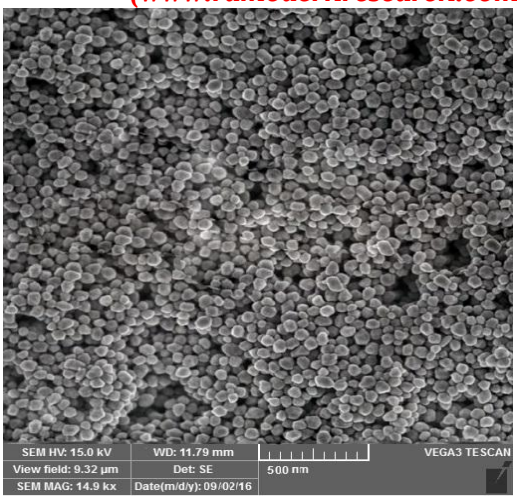

Figure 2: SEM characterization of AgNps

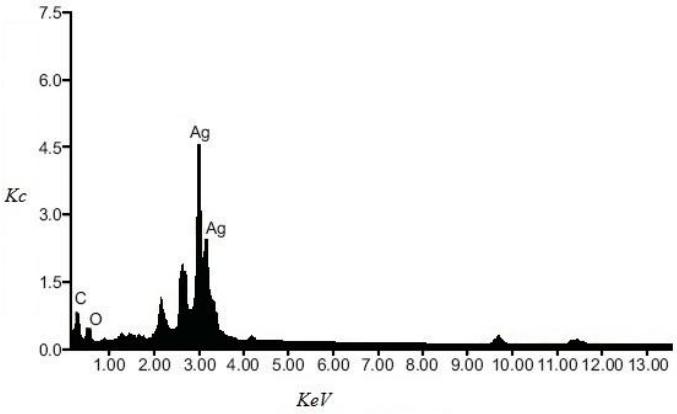

Figure 3: EDAX characterization of AgNPs

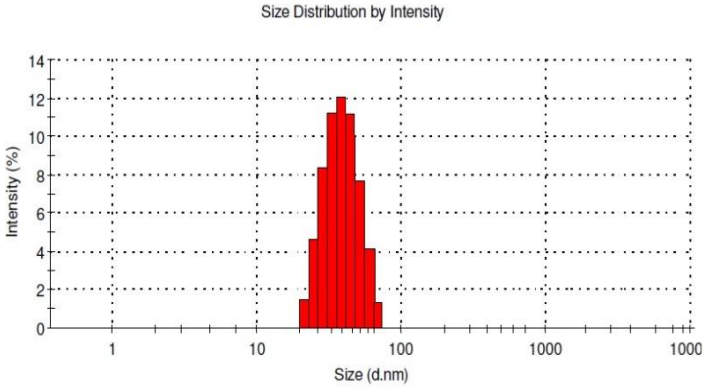

Figure 4: DLS Size distribution characterization of AgNPs

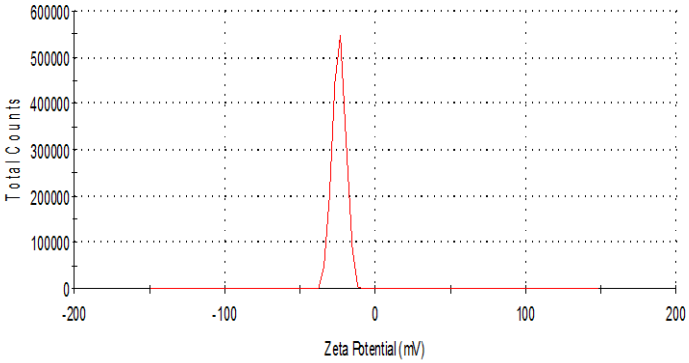

Figure 5: DLS Zeta potential characterization of AgNPs

Figure 6: FTIR characterization of Nalidixic acid broth and AgNPs

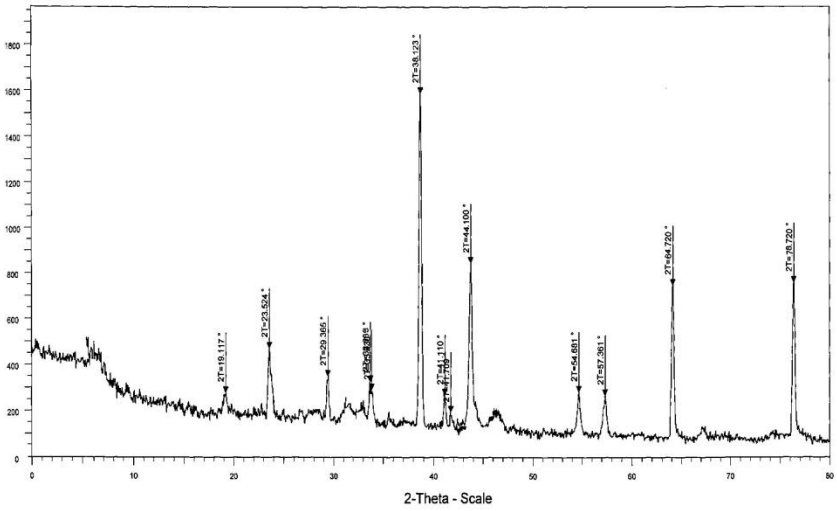

Figure 7: XRD characterization of AgNPs