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Synthesis of Cobalt Nanoparticles by Chemical Routes and its Antimicrobial Activity.

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ABSTRACT

Rapid synthesis of Cobalt nanoparticles through economically feasible chemical reduction approach is highly desirable. In this study we have developed a method to synthesize Cobalt nanoparticles by mixing $CoCl_2$ solution with Hydrarzine hydrate as a reducing agent and Sodium Dodecyl sulphate as a surfactant. In this method, physiologically stable, bio-compatible Co nanoparticles were formed. These functionalized CoNPs could be used for targeted drug delivery with enhanced therapeutic efficacy and minimal side effects. These nanoparticles were analyzed by various characterization techniques to reveal their morphology, chemical composition, and antimicrobial activity. TEM image of these NPs indicated the formation of spherical, non-uniform, poly dispersed nanoparticles. A detailed study of anti-microbial activity of nanoparticles was carried out.

Keywords: Chemical synthesis, Co Nanoparticles, PSD, TEM, SEM

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INTRODUCTION

Nanomaterials have a long list of applicability in improving human life and its environment. The first relationship between human life and nanoscale was established in *Ayurveda*, a 5000 years old Indian system of medicine. It has the knowledge of nanoscience and technology in some form or other even before the term nanotechnology was coined [1].

One of the first and most natural questions asked about nanoparticle is: Why are nanoparticles so fascinating? Why work with these extremely small structures that are challenging to handle and synthesize especially when compared with their macroscopic counterparts? The answer lies in the unique properties possessed by these nanoparticles [2].

The matter changes its properties when it enters the nano regime, and more specifically they become size and shape dependent. In particular, several interesting quantum mechanical or sub-domain phenomenon are observed which may be absent either in bulk or molecular systems [3]. The unique size and shape dependent properties of nanometre sized particles render nanotechnology as a potentially important branch of science and technology. Accordingly, several applications such as solar cells, targeted drug delievery, selective chemical sensors, light emitting diodes, single electron transistors and memory devices have been demonstrated in recent past [4].

The term *nano* is adapted from Greek word meaning dwarf. When used as a prefix, it implies 10^{-9}. A nano meter is one billionth of a meter, or roughly the length of three atoms side by side. A DNA molecule is 2.5nm wide, a protein approximately 50nm and a flu virus about 100nm. A nanoparticle is a microscopic particle with less than 100nm size atleast in one dimension and atleast one property different from their bulk counterpart [5]. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. Several well characterized bulk materials have been found to possess most interesting properties when studied in the nanoscale. There are number of factors responsible for property change at nanoscale such as high aspect ratio, ineffective gravitational force, and significant van der Wall's force. This effect is extremely robust and as little as 1gm of AgNPs is known to impart antibacterial properties to hundreds of kilograms when used in bulk form [6].

Metal nanoparticles are intensively studied due to their unique optical, electrical and catalytic properties. A large spectrum of research has been focused to control the size and shape of nanoparticles which is crucial in tuning and optimizing their physical, chemical and optical properties. Various techniques such as chemical reduction, electrochemical reduction, sol-gel, laser ablation, photochemical reduction etc have been developed to synthesize nanoparticles. Recently biological methods are being investigated for the synthesis of nanoparticles [7-10].

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EXPERIMENTAL METHODS

All the chemicals used were analytical grade and used without further purification. The chemicals were purchased from Sigma Aldrich Chemicals Pvt. Ltd. The aqueous solutions were prepared using doubly distilled de-ionized water.

General procedure for the preparation of Co Nanoparticles

2gm of $CoCl_2.6H_2O$ is dissolved in 20 ml of water by constant stirring. Then sodium succinate solution is prepared by dissolving 7.2gm of sodium succinate in 100ml of water. This 100ml of sodium succinate solution is added into 20ml of Cobalt Chloride solution and heated at 70°C for 10 minutes. Polyvinyl alcohol solution is prepared by dissolving 0.1gm of PVA in 10ml of water and added in above mixture. The reaction mixture is heated for 5 minutes. Then a solution of hydrazine hydrate is prepared by dissolving 3ml of hydrazine hydrate in 10ml of water. The solution is drop-wise added with constant stirring. The solution is filtered through Whatsman filter paper and washed several times with deionized water. It is kept in oven over night at 120°C temperature for drying. Then the dried powder is collected and sample is analysed by using various characterization techniques.

Characterization

To investigate the formation of CoNPs, the dried powder was analysed by various characterization techniques at room temperature of about 27^oC in atmospheric air. Then prepared sample was analysed first by dynamic light scattering for obtaining particle size distribution of the prepared sample. Then the sample was characterized by transition electron microscope (TEM) to reveal particle size. The morphological features of the sample have been analysed through scanning electron microscope (SEM). The antimicrobial activity of the prepared sample is tested by agar diffusion method.

RESULTS AND DISCUSSION

UV-Visible Spectroscopic Image:

The pinkish coloured sample powder was dissolved in deionized water and sonicated. 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 phenomena resonant peak occurs at different wavelength for different nanoparticles solution. As per the theory of resonance maximum wavelength is absorbed at210nm resonant wavelength. It is reported in the literature that typical CoNPs shows the characteristic SPR at the wavelength in the range of 190-240nmnm. The SPR absorbance is sensitive to the nature, size and shape of particles present in the solution and also it depends upon their inner particle distance and the surrounding media.

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As shown in fig.1. we observed SPR for the sample solution to occur at the wavelength of 210 nm which confirms the presence of Cobalt nanoparticles in the prepared solution. The MATLAB analysis of this UV image shows that the image format is JPEG with bit-depth of 24 bits and image resolution is found to be 492 x 492 and the file-size is 13.7 KB. Also it is seen that the image class is uint8. The UV characterization has been carried out at Department of Chemistry, Shivaji University, Kolhapur.

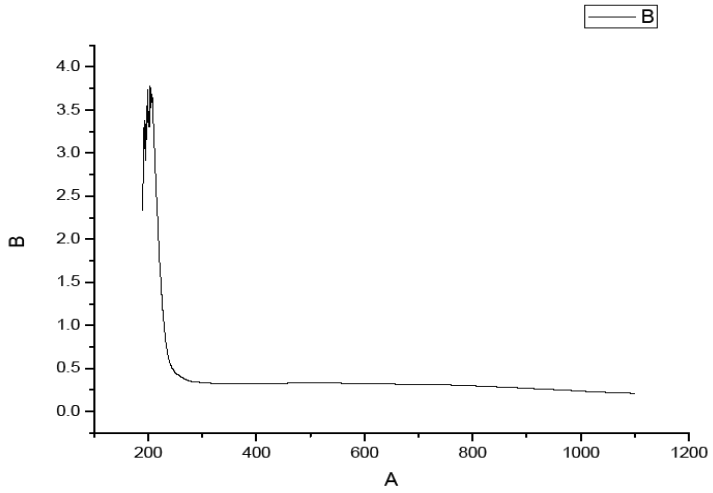

Figure 1 UV-Visible Image of Synthesized nanoparticles

XRD Pattern of Synthesized Nanoparticles

XRD patterns of synthesized CoNPs are shown in fig. no. 2 where four major peaks 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. The synthesized nanoparticles are crystalline in nature. These peaks are of cubic structure which is in agreement with the JCPDS file.

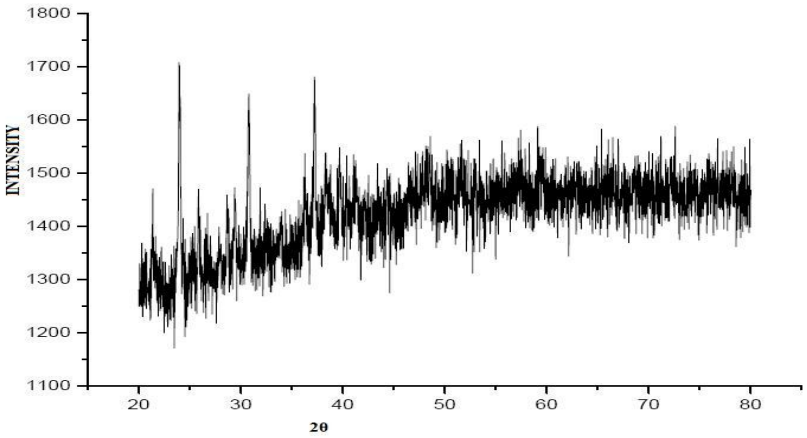

Figure 3 XRD-Pattern of synthesized nanoparticles

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Particle Size Distribution

Particle size distribution histogram in fig.1 indicates that 15.95% particles size is around 530nm with a standard deviation of 18.28% while the remaining particles are around 2.8k nm with a standard deviation of 21.61%. The difference in size as observed from TEM and PDS may be due to presence of water droplets and other chemicals on the CoNPs surface. Similar observation is recorded by Prarthana *et al.* The MATLAB analysis of this Particle size distribution image shows the bit-depth of 24 bits and image resolution of 1024 x 768. Also the image file-size was 124.9KB and image format JPEG with image class as uint8. The particle size distribution graph is taken from C-MET, Pune, India.

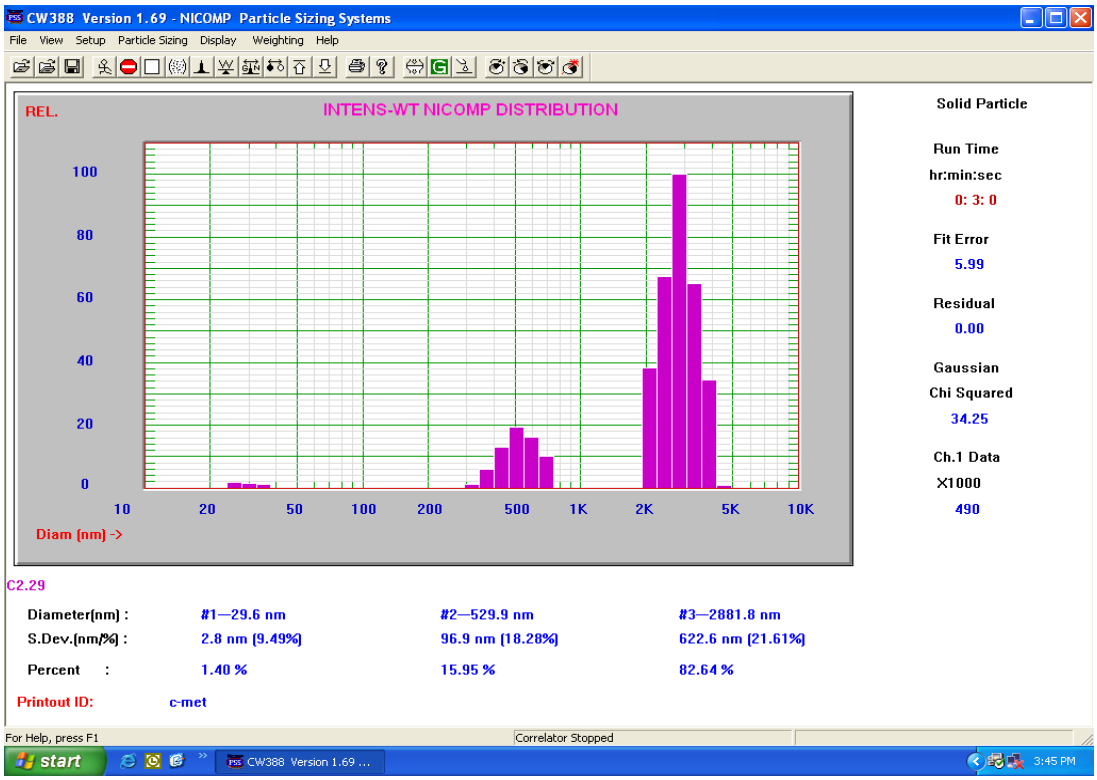

Figure 3 Particle Size Distribution Histogram of Cobalt Nanoparticles

SEM Image of Silver nanoparticles

The SEM image in fig.2 reveals the formation of cluster of spherical cauliflower-like structure of CoNPs with non uniform distribution. The MATLAB analysis gives the pixel depth of the image equal to 8 bits and the image resolution of 1280 x 960. Also the image file-size was found to be 1.23MB and the image format as TIFF. The SEM image has been taken with JSM-6360 instrument which uses accelerating voltage of 20KV. The SEM Image has been taken at Department of Physics, Shivaji University, Kolhapur, India.

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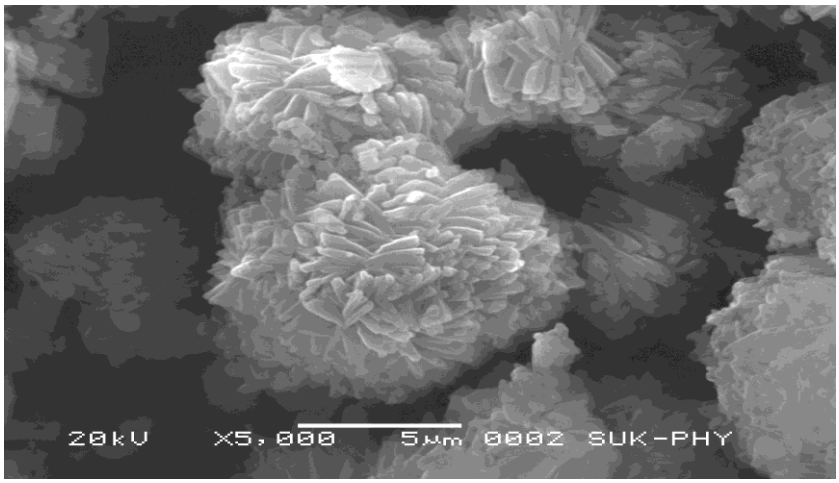

Figure 4.SEM image of Silver nanoparticles

TEM Image of Silver nanoparticles

TEM Images in fig.3 reveal that there was poly-disperse spherical particles with non uniform distribution in the prepared sample. For TEM measurements, a drop of solution containing the particle was deposited on a copper grid covered with amorphous carbon. After allowing the film to stand for 2 minutes the extract solution was removed by means of blotting paper and the grid allowed drying before the measurement. It was observed that the nanoparticles formed were of different sizes and particle size was found to be 9.75nm, 6.33nm, and 11.33nm and the mean size of about 9.13nm which lies in the nano range. The TEM measurement was done with JEOL model 1200Ex instrument operated at an accelerating voltage of 80kV. The TEM image was of very high resolution and MATLAB analysis gives the pixel depth of the image equal to 24bits and the image format as JPEG. The TEM Images have been taken at National Chemical Laboratory, Pune, India.

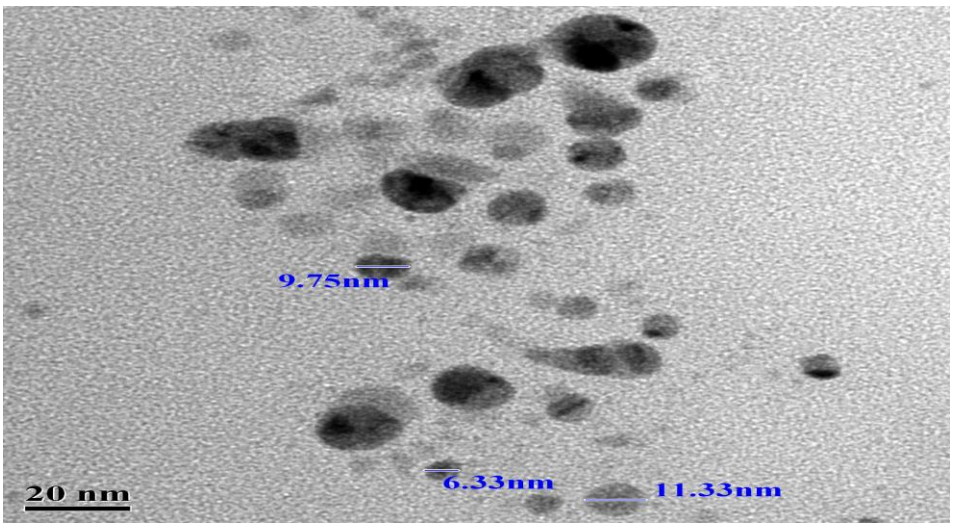

Figure 5. TEM Image of Cobalt NP showing size of nanoparticles

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Antimicrobial Activity of Silver nanoparticles

The antimicrobial activity of CoNPs was evaluated against pathogenic bacteria as per below mentioned agar diffusion method. In this method first the bacterial cultures were developed on nutrient agar (NA) slants which contained different ingredients such as peptones (5.0g), agar (15.0g), meat extract (q.s.), sodium chloride (q.s.), and yeast extract (q.s.) per litre of distilled water. After solidification of prepared culture medium the stainless steel cylinder were placed on the surface and holes were punched in the medium. Freshly prepared CoNPs were added into the wells that were made in the culture medium plates. The samples were initially incubated for 15 minutes at 4°C temperature and later were incubated for 24 hours at 37°C temperature. The antimicrobial activity has been tested against gram-positive and gram-negative bacteria. A Gram-positive bacterium used for experimentation was *Bacillus subtilis* NCIM 2635 and gram negative bacterium used was *Salmonella typhimorium*. The antimicrobial study for *Bacillus subtilis* NCIM 2635 is shown in fig.4 where the well size is found to be 8mm and zone of inhibition of about 26mm. Thus it can be claimed that these CoNPs are exhibiting antibacterial property against *Bacillus subtilis* NCIM 2635. However, CoNPs didn't show antimicrobial activity against *Salmonella typhimorium*. This distinction of CoNPs in antimicrobial activity can be attributed to their attachment to the bacterial surface. Because of their antibacterial property CoNPs are widely used in antimicrobial coatings in medical instruments and in textiles. The photograph was captured by Kodak Camera and shown in the picture. The MATLAB analysis of this antimicrobial culture image shows that the image format is JPEG with bit-depth of 24 bits and image resolution was found to be 4000 x 3000 and the file-size is 1.55MB. Also it is seen that the image class is uint8.

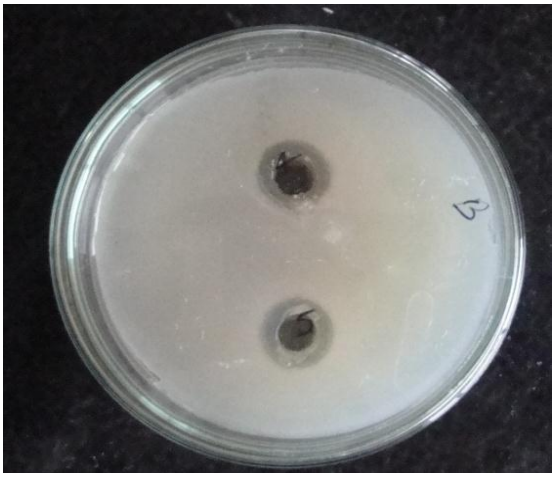

Figure 6. Image of culture plate showing antimicrobial activity of Cobalt nanoparticles

Experimental Outcome

The present chemical synthetic method is a low cost and rapid approach and capable of synthesizing CoNPs at room temperature. The size and structure of obtained NPs were characterized by PSD, TEM, and SEM. The synthesized CoNPs had significant antimicrobial

activity against gram-positive bacteria *Bacillus subtilus*. This kind of study may also make a future platform for preparing nano-medicines, and targeted drug delivery etc. The future studies may include the use of synthesized nanoparticles alongwith routinely used antibiotics to see its synergistic effects. It confirms that CoNPs are capable of rendering high antibacterial efficacy and hence has great potential in the preparation of drugs against bacteria.

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