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Synergetic effect of Ag-Cu bimetallic nanoparticles on antimicrobial activity

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ABSTRACT

In recent years the application of nanoparticles in various disciplines has expanded considerably. Nanoparticles possess unique physical properties and high reactivity viz high surface to volume ratio. An attempt has been made to determine the antimicrobial activity of copper and silver nanoparticles; also the antimicrobial activity of Ag-Cu bimetallic nanoparticles is determined. It is found that the antimicrobial activityincreases when the bimetallic nanoparticles are used.

Keywords: Bimetallic nanoparticles, Reducing agents, Capping agents, Antimicrobial activity.

INTRODUCTION

Miniaturization is a concept nurtured by nature since the process of evolution and with time, the control of biological processes at small length scales has become immaculate. The origin of the field of nano science and nanotechnology has primarily been a motivation to mimic the programmed synthesis and manipulation of materials at similar length scale, an art perfected by nature [1]. Richard Feynaman was the visionary who first drew attention towards this possibility in his speech titled “There’s plenty of room at the bottom” [2]. Since then, several significant achievements have been made towards the process of miniaturization,even though control at complexity levels manifested by biological systems is still a dream.

Richard Feynman in 1959, has initiated a new branch of science which is presently known as ‘Nanoscience Nanotechnology’, where everything depends on our ability to manipulate and design materials atom by atom and molecule by molecule at the nanoscale. These nanoscale materials possess novel size and shape dependent properties useful for diverse applications in various fields of science and engineering. Accordingly, recent technological development makes it possible to generate structures or devices less than 100 nanometers in size with noteworthy functional advantages over conventional devices leading to the threshold of a revolution having the potential to change the entire scenario of present technology [3-6]. Indeed, promises are so high that it can even cope up with the millennium goal of achieving affordable amenities to all human beings from equally distributed technological developments touching.

Nanotechnology deals with the art of designing new materials at the nano domain wherein the size, shape and structure of the material dictate the properties of the same as much as the composition. The concepts and ideas derived from chemistry, physics and engineering are married to design a novel material with desired properties. Recently the interesting properties and appealing structures of biomaterials have inspired the synthesis of modern nano-materials with precise control over their morphology and dimension [7-8]. Structural arrangement of atoms and the length scales of the material are the two parameters, which when tuned properly at the nanometer scale, could lead to variation in the properties of the material, compared to its bulk [9].

Definition and Classification of Nano-structured Materials
According to Hunt, "Nanomaterials are an enabling component of the popularly labelled area of 'nanotechnology', but are generally not well understood by the materials' community at large" [10]. Although a variety of prosaic definitions of nanotechnology is available, almost all lack definitional precision despite providing a deeper insight into the subject. One of such definitions described in Scientific American states: "The field is a vast grab bag of stuff that has to do with creating tiny things that sometimes just happen to be useful. It borrows liberally from condensed-matter physics, engineering, molecular biology and large swaths of chemistry" [11]. However, a more comprehensive definition is given by the US National Nanotechnology Initiative (NNI) as: (i) research and technology development at the atomic, molecular, or macromolecular levels, approximately 1-100 nano-meters in length, (ii) creation and use of structures, devices, and systems that have novel properties and functions because of their small and/or intermediate size, and (iii) ability to control or manipulate on the atomic scale [12]. Another definition given by 'The Royal Society' and 'The Royal Academy of Engineering' states: 'nanotechnology is the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometre scale' [13]. Thus, improvement of properties of materials by controlling their nanoscale structures is the heart of nanotechnology or can be better called as incremental nanotechnology. Similarly, another term radical nanotechnology means the development of nanoscale machines that would exist at the convergence of nanotechnology, biotechnology, information technology, and cognitive technology [11].

Metallic nanoparticles can be classified as bi-metallic or tri-metallic depending on the number of constituent metallic parts [14]. Most of the cases, they form alloys, and commonly known as alloy nanoparticles. Similarly, core-shell nanoparticles constitute another class of nanomaterials which are cross-linked nanosized particles with a chemical composition that is different on the surface compared to the core region. Non-metallic nanoparticles (nanotubes) are another class consisting of non metals and organic molecules with interesting electrical behaviour which can be tuned to be insulating or metallic based on size and composition [15]. The most familiar example of non-metallic nanoparticle is Fullerenes which constitute a different class of nanomaterials. Carbon nanotubes constitute another celebrated class of nanomaterials that can be metallic or semiconducting depending on their diameter and chirality [15-16].

Another way of classifying nanomaterials is based on their dimensionality. If we consider the three dimensional space vectors for a specific nanomaterial and length scales are in the critical regime of 1-100 nm, they can be known as 0-dimensional particles or quantum dots, typical examples being spherical (1-10 nm diameter) nanoparticles of Au, Ag, CdS, CdSe etc. Similarly, if two spatial vectors are restricted in that critical length scale allowing nanomaterial to grow only in the third direction, then the resultant nanomaterial is known as 1D nanostructure (quantum wires), specific examples being nanorods, nanowires, etc. Single walled carbon nanotubes can be considered as an important example of such material. In 2D nanostructures, only one dimension is restricted to that critical length scale and these nanomaterials (quantum sheets) are allowed to grow along the other two spatial directions [17].

A more interesting method of classifying nanomaterials is based on their shape. Thus, nanomaterials are known as quantum dots or nanoparticles (monolayer protected nanoclusters, MPCs or simply NPs), nanowires, nanorods, nanoribbons, nanobelts, nanobipods, nanotetrapods, nanocubes, nanoboxes, nanotriangles, etc. Nanoparticles that consist of homogeneous material, especially those that are almost spherical or cubical in shape are called quantum dots. Nanostructures that are shaped like long sticks or dowels, with a diameter in the nanoscale and a length very much longer are known as nanorods or nanowires [18]. If a normal nanorod is coated with a different metal layer, then it is known as core-sell nanorod or coaxial nanocables. Similarly, if the inside of a nanorod is hollow, then it is known as nanotubes or nanorods with hollow interiors. Nanoparticles of triangular or prismatic shape are called nanoprisms or nanotriangles [19]. All such nanomaterials having aspect ratio (R, division of length by diameter or more precisely, division of length along the major axis by the length along the minor axis), $R > 1$ come under a broad term high aspect ratio nanostructures. An enormous number of differently shaped nanomaterials of metals, non metals, and semiconductors have been categorized as high aspect ratio nanomaterials and reviewed recently.The present work, however, discusses only the metallic high aspect ratio nanostructures, especially nanomaterials of copper and silver since our studies are mainly focused on these two metals. Nanoscience deals with the design of materials whose length scale falls inthe size regime of 1 nm to 100 nm. Reduction in size of a material leads to changes in properties such aselectrical conductivity, colour, mechanical strength and melting point. The concept of making materials ofnanometre size is fundamentally interesting for the following reason. As the sizeapproaches atomic dimensions, energy level bands are slowly transformed intoquantized discrete energy levels. Since the changes in the electronic structureoccur in the nanometre region, it gives an insight as to how the properties evolvefrom the molecular or atomic level to the bulk [20]. Also the reduction in size wouldconfine the electronic motion, which will affect the physical and chemicalproperties of the material. As the size of the metal approaches to the nanometre regime, the changein their properties, takes place.

Size and shape dependant catalytic properties
As the particle size decreases, the fraction of surface atoms significantlyincreases, for example a 3 nm particle would have 45% of its atoms on thesurface and a 1 nm particle would have 76% of the atoms on its surface [21].As anyreaction takes place at the surface and the high percentage of surface atoms inmetal nanoparticles viable them as good catalysts.The unique surface structure, electronic states, lower work function andhigh surface area of a nanoparticle become great advantages for promoting therates of chemical reactions. For example, gold is considered to be a noble metalin bulk state, but the nanoparticles of gold dispersed in alumina or iron oxidewas found to be excellent catalysts for carbon monoxide oxidation [22]. The shape of the nanoparticles along with size influences the surfacereactivates. The reactant molecules show differential affinity in adsorptiontowards different faces of the catalyst [23]. Hence metal nanoparticles of differentshapes covered by different faces could be used to increase the selectivity of a catalyst. Hence surface reactivity can be tailored in such a way by varyingthe shape of the nanoparticle, could help in designing molecular specificcatalysis [24].Mechanical Properties of a material depend strongly on the density ofdislocations, grain size and the surface / interface-to-volume ratio. The strengthand hardness of the material could be severely affected if any decrease in grainsize. As compared to the bulk, a nanoparticle has more defects due to the highsurface to volume ratio. However the capability of a nanomaterial to undergoextensive tensile deformation without destroying the structure is well reported and is called super-plasticity [25]. Grain boundary diffusion and sliding magnetic properties of nanoparticles differ from those of bulk in twoways. The large surface to volume ratio results in a different local environmentfor the surface atoms in their magnetic coupling / interaction with neighbouringatoms, leading to the mixed volume and surface magnetic characteristics [26]. Unlikebulk ferromagnetic materials, which usually form multiple magnetic domains,several small ferromagnetic particles could consist of only a single magneticdomain [27].In the case of a single particle being a single domain, thesuperparamagnetism occurs, in which the magnetizations of the particles arerandomly distributed and they are aligned only under an applied magnetic field, and the alignment disappears once the external field is withdrawn[28]. These couldhave important implications and for example, in ultra-compact informationstorage where the size of the domain determines the limit of storage density

Optical Properties
The optical properties of these nanoparticles are spectacular and,therefore, have stimulated a great deal of excitement during the last fewdecades [29]. The colour variations arising from changes in the composition, size, andshape of nanoparticles, surrounding medium and the very high absorption crosssectionpromoted these materials as inorganic chromophores from visible to nearinfrared region [30]. Due to this reason they find applications as sensors andimaging agents.These effects are due to the phenomena called surface plasmon resonance,the frequency at which conduction electrons oscillate in response toalternating electric field of incident electromagnetic radiation [31].

In recent years there has been growing interest in the preparation and study of silver nanoparticles (Ag-NP) because those nanoparticles have been found to exhibit antimicrobial activities. Production of nano sized metallic silver particles with different morphologies and size using different routes has been reported [32]. The exact antimicrobial activity of Ag NPs is not completely understood. There are reports in literature that shows that electrostatic attraction between negatively charged bacterial cell and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal material [33]. Human beings are often infected by micro organisms such as bacteria, moulds, yeast and virus in living environment [34]. Research in antimicrobial material containing various natural and inorganic substances have been extensive nanoparticles (Me-NP) which have a high surface area and high fraction of surface atoms have been extensively studied such as,physic-chemical characteristics including catalytic activity, optical properties, electronic properties, antimicrobial activities and magnetic properties [35].

Because of biologically produced silver nanoparticles are attached with the microscale surface of the bacterium on which they were formed. They are prevented aggregation making them an interesting alternative for chemically produced silver nanoparticles [36]. Silver nanoparticles have been demonstrated to exhibit biocidal activity towards a broad range of Gram positive and Gram negative bacteria, viruses and fungi. Moreover compared to other metals the toxicity of silver is very low towards human [37]. This makes nanosilver an interesting candidate for a number of applications such water decontaminations and surface disinfection method such as chlorination and ozone can produce toxic by-products.

Chemical methods: It involves the reduction of metal ions with different reducing agentsin the presence of a capping agent. Though chemical synthesisdates back to the middle ages, Faraday method of making goldnanoparticles in carbon disulphide by the reduction of chloroaurateions by phosphorous vapours is the well known example for thismethod.

Chemical methods involve thereduction of metal ions by a suitable reducing agent in the presence of a cappingagent. It is similar to the conventional colloids preparation, where aprecipitating agent is added to induce the colloid formation [38].In a similar manner nanoparticles are formed by an initial nucleationstage in which tiny seed particles precipitate spontaneously from solution and a subsequent growth phase in which the newly formed seeds capture dissolvedNanoparticles are small and are not thermodynamically stable due totheir very high surface to volume ratio [39]. The atoms present on the surface arecoordinatively unsaturated and are too reactive. They try to combine with otherparticles, which would lead to bulk structures if the surface is not protected.

Biological Methods: It involves employing fungus, bacteria and leaf extract to reduce metal ions.

MATERIALS AND METHODS

2.1. Synthesis of Cu Nanoparticles

7.2 gm of sodium succinate is dissolved in 100 ml of deionized water. This solution is added to 2 gm $CuCl_2$ $2H_2O$ solution in 20 ml of deionized water. Then this mixture is heated at 70^oC for 10 minutes. 0.1 gm PVA solution is prepared in 10 ml of deionized water. It is added in it. The whole solution is heated for 5 minute. And a solution of 3 ml hydrazine in 10 ml of deionized water is prepared. This is added slowly with constant stirring at 70^oC for 1 hour.The solution is filtered through Whatsman filter paper No. 1. It is washed several times with deionized water and kept in oven for drying. Next day the sample is removed from oven and powdered in mortar. We got 0.8623 gms of copper nanoparticles from 2 gms of $CuCl_2$ $2H_2O$

2.2. Synthesis of Ag Nanoparticles

7.2 gm of sodium succinate is dissolved in 100 ml of deionized water. This solution is added to 2 gm $AgNO_3$ solution in 20 ml of deionized water. Then this mixture is heated at 70oC for 10 minutes. 0.1 gm PVA solution is prepared in 10 ml of deionized water. It is added in it. The whole solution is heated for 5 minute. And a solution of 3 ml hydrazine in 10 ml of deionized water is prepared. This is added slowly with constant stirring at 70^oC for 1 hour.The solution is filtered through Whatsman filter paper No. 1. It is washed several times with deionized water and kept in oven for drying .Next day the sample is removed from oven and powdered in mortar We got 0.8623 gms of silver nanoparticles from 2 gms of $CuCl_2$ $2H_2O$.

2.3. Synthesis of Ag-Cu bimetallic Nanoparticles

7.2 gm of sodium succinate is dissolved in 100 ml of deionized water. This solution is added to 1gm $AgNO_3$ solution in 10 ml of deionized water. Also 1 gm of $CuCl_2$ $2H_2O$ solution is prepared in 10 ml of deionized water. Then this mixture is heated at 70^oC for 10 minutes. 0.1 gm PVA solution is prepared in 10 ml of deionized water. It is added in it. The whole solution is heated for 5 minute. And a solution of 3 ml hydrazine in 10 ml of deionized water is prepared. His is added slowly with constant stirring at 70^oC for 1 hour. The solution is filtered through Whatsman filter paper No. 1. It is washed several times with deionized water and kept in oven for drying.Nextday the sample is removed from oven and powdered in mortar we got 0.9300 gms of silver copper bimetallic nanoparticles from 2 gms.

Antimicrobial Activity of nanoparticles:

The antimicrobial activity of AgNPs, Cu NPs and Ag-Cu NPs were evaluated against pathogenic bacteria such as *Bacillus subtilis* and *Salmonella typhimurium* 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 wells were prepared by using cork borer having diameter 8mm. Then 100µl freshly prepared AgNPs, CuNPs and Ag-Cu bimetallic NPs were added into the wells that were made in the culture medium plates. The plates were kept in refrigerator for 15 minutes at 4°C temperature for diffusion and later were incubated for 24 hours at 37°C temperature in incubator.

RESULTS AND DISCUSSION

Particle Size Distribution: Particle size distribution histogram in fig.1 indicates that 16.46% particles size is around 83.3nm with a standard deviation of 15.06% size while the remaining particles are around 419.2nm with a standard deviation of 17.62%. The difference in size as observed from TEM and PDS may be due to presence of water molecules on Ag-CuNPs surface. The MATLAB analysis of this Particle size distribution image shows the bit-depth of 24 bits and image resolution of 797 x 473. 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.

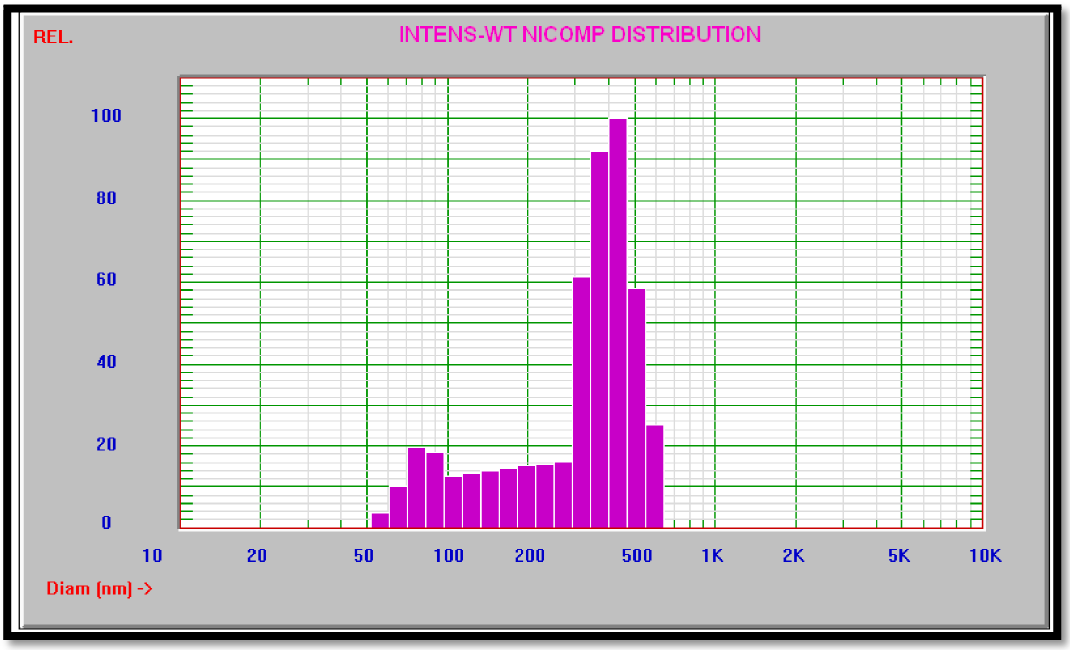

Fig.1. Image of Particle Size Distribution graph showing silver-copper bimetallic nanoparticles

SEM Image of Silver Copper bimetallic nanoparticles: The SEM image in fig.2 reveals the formation of cluster of spherical beadlike structure of silver copper bimetallic nanoparticles with non uniform distribution. The MATLAB analysis gives the image class as unit8 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, ShivajiUniversity, KolhapurIndia.

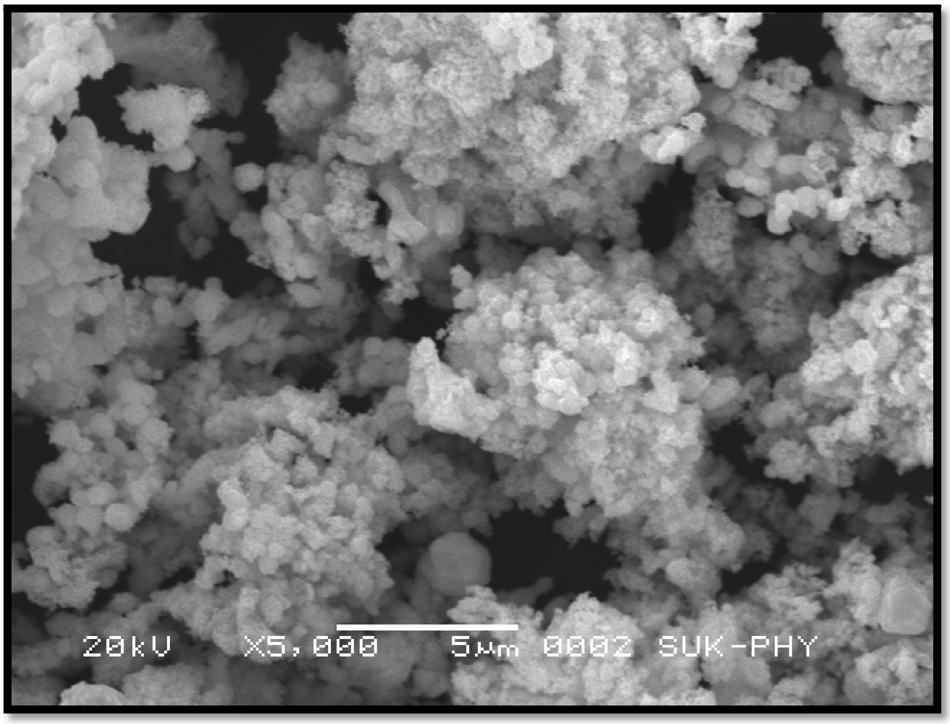

Fig.2. SEM Image for silver-copper Bimetallic Nanoparticles

TEM Image of Silver Copper bimetallic nanoparticles: TEM Images in fig.3 reveal that there was poly-disperse spherical particles with non uniform distribution of nanoparticles 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 3.98nm, 5.97nm, 6.63nm and 6.66 and the mean size of about 5.81nm 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 taken with very high resolution and MATLAB analysis gives the image resolution as 2048 x 2048. The image class is uint8 and the image format as JPEG. The TEM Images have been taken from National Chemical Laboratory, Pune, India.

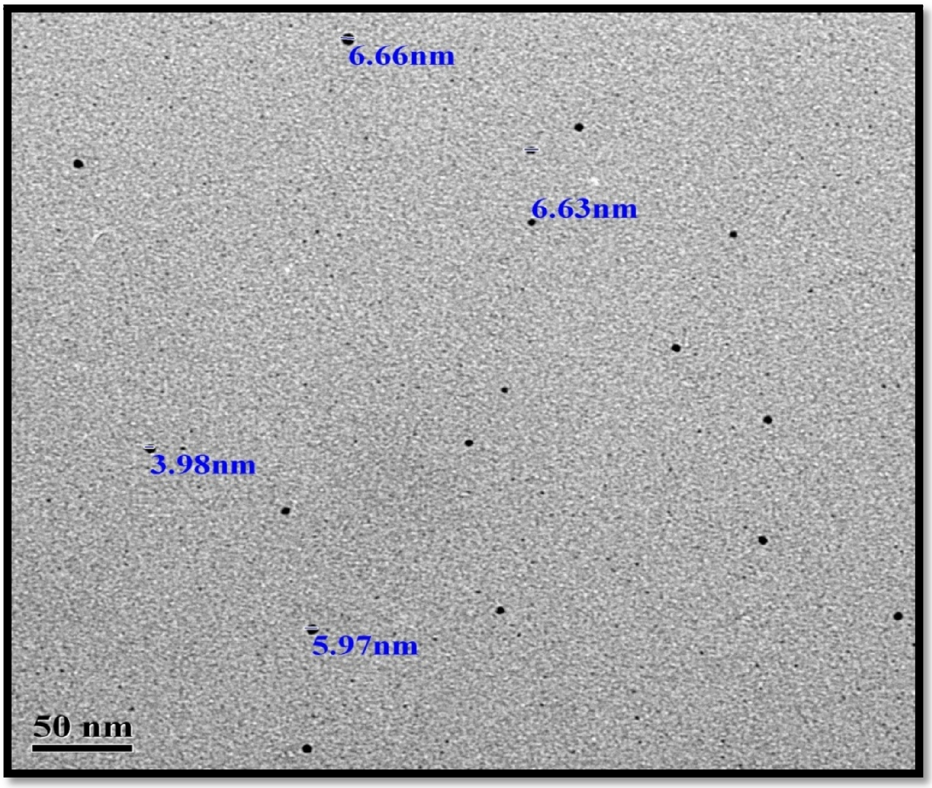

Fig.3.TEM Image for silver-copper Bimetallic Nano-particles

Antimicrobial Activity

A primary interest in the idea of nanoscience comes from its associationswith biology. The size of the nano-particle is comparable to the size of thebiomolecules such as DNA, enzymes, antibodies and polysaccharides. Noblemetal nanoparticles like gold and silver are found to be bio-compatible.Hence biomolecules could be anchored onto the nano-particle surface in such away that they form units with complex biological functions, which could includecombinations of recognition, inhibition, synthesis and signalling.

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. In the antimicrobial study shownin fig.4 the well size is found to be 8mm and zone of inhibition of about 15mm for AgNPs, 18mm and 28mm respectively. Thus it can be claimed that these AgNPsand CuNPsare exhibiting antibacterial property against *Bacillus subtilis* NCIM 2635. It is clearly observed that the antimicrobial activity increases tremendously when Ag-Cu bimetallic nanoparticles were used. Thus it is the synergetic effect of two metals against gram positive pathogenic bacterium *Bacillus subtilis*. Ag Cu bimetallic NPs can be widely used in antimicrobial coatings in medical instruments and in textiles. The photograph was captured by Kodak Camera and shown in the picture.

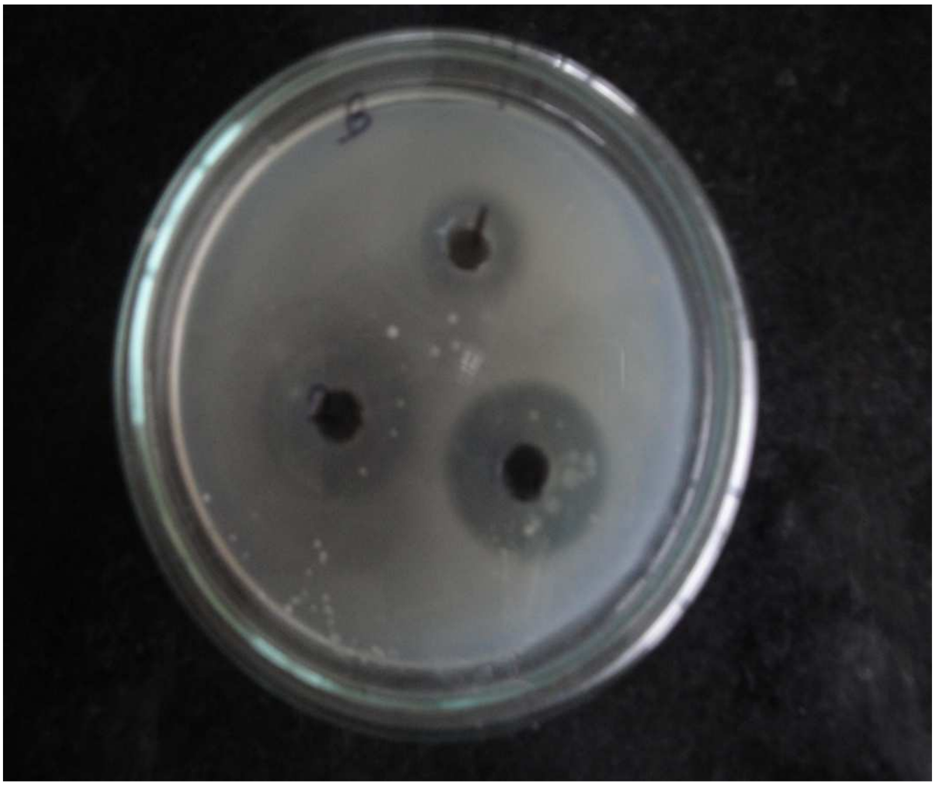

Fig.4.Image showing antimicrobial activity of Copper silver bimetallic nanoparticles

CONCLUSION

The present synthetic method is a low cost approach and capable of synthesizing AgNPs, Cu NPs and Ag-Cu bimetallic nanoparticles. The size and structure of obtained NPs were characterized by TEM, SEM, and Particle Size Distribution histogram. Our results have shown that the metallic nanoparticles are showing antimicrobial activity against gram positive bacterium *Bacillus subtilus*. Ag-Cu bimetallic nano-particle is showing high antimicrobial activity. 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 and sulpha drugs to see its Synergeticeffects. It confirms that Ag-Cu bimetallic NPs are capable of rendering high antibacterial efficacy and hence has great potential in the preparation of drugs against bacteria.

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