THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

Synthesis of 5-Methyl 2-mercapto benzimidazole Capped Silver Nanoparticles via chemical reduction method & Study of their Biological properties

Abstract:
The morphology and anti bacterial activity of capped Silver Nanoparticles of 5-Methyl 2-mercapto benzimidazole prepared via chemical reduction is studied. Spherical shaped capped Ag-NPs with size of 40nm are obtained by treatment of aqueous silver ions with ethanolic solution of 5-Methyl 2-mercapto benzimidazole as capping agent .The synthesized nanoparticles are characterized by SEM, TEM, XRD, FTIR studies and were tested for antibacterial activity. It is observed that the obtained silver nanoparticles are uniform in their shape and sizes and also shown good antibacterial properties.
Key words: Silver nanoparticles, benzimidazole , capping Agent and Antimicrobial.

Introduction:

Chemical reduction is the most frequently applied method for the preparation of silver nano particles (Ag-NPs) as stable, colloidal dispersions in water or organic solvents.[1, 2] The commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen.[3–11] The reduction of silver ions (Ag^+) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers.

Previous studies showed that, use of a strong reductant such as borohydride, resulted in small particles that were somewhat monodisperse, but the generation of larger particles was difficult to control.[16,17] Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but the size distribution was far from narrow.[3, 4, 18] Controlled synthesis of Ag-NPs is based on a two-step reduction process.[17] In this technique a strong reducing agent is used to produce small Ag particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent.[3] Different studies reported the enlargement of particles in the secondary step from about 20–45 nm to 120–170 nm.[19–21] Moreover, the initial sol was not reproducible and specialized equipment was needed.[5] The syntheses of nanoparticles by chemical reduction methods are therefore often performed in the presence of stabilizers in order to prevent unwanted agglomeration of the colloids.

Experimental:

Synthesis of Silver Nanoparticles via chemical reduction method:

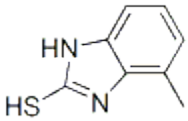
Synthesis of 5-Methyl 2-mercaptobenzimidazole:

3,4-Diaminotoulene (1g, 0.0082mol) and KOH (0.55g, 0.01mol) was dissolved in 10ml ethanol, then the reaction mixture was kept in ice bath. To the stirred solution of this reaction mixture, CS_2 was added (0.6 ml, 0.01mol) in drop wise manner and stirring is continued for 15min. Latter the reaction mixture was refluxed at 70^oC for 2h. The progress of the reaction was monitored by TLC. After consumption of all the reactants, ethanol was evaporated under reduced pressure to obtain pale brown coloured residue. The obtained residue was treated with 20% glacial acetic acid to give glistening pale white crystals, and the mixture is placed in a refrigerator for three hours to complete the crystallization. The product was collected on a Buckner funnel and dried over night at 40^0 C. The dried product was re-crystallized using ethanol.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170



Structure of 5-Methyl, 2-mercaptobenzimadazole

Synthesis of 5-Methyl 2-Mercapto benzimidazole capped silver Nanoparticles:

$AgNO_3$ (0.1g, 0.00059mol) is dissolved in de ionised water (4ml), and 5-methyl 2-Mercapto benzimidazole (0.14g, 0.00089mol) is dissolved in 4ml ethanol. To the stirred solution of $AgNO_3$ the solution of 5-Methyl 2-Mercapto benzimidazole is added and stirring is continued for 1h, then freshly prepared aqueous $NaBH_4$ (0.04g, 0.0011mol, 1mL) is added drop by drop. The colour change of the reaction mixture indicates the formation of thiol capped silver nanoparticles shown in fig-1 and continue vigorous stirring for 2h. Formed silver capped NPs can be isolated by simple filtration followed by washing with ethanol to remove excess capping agent.

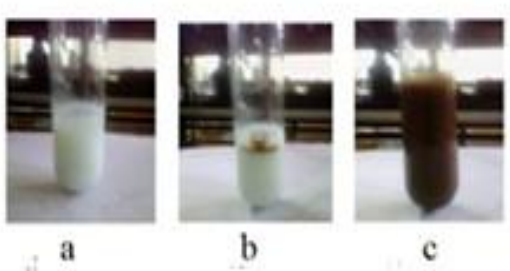


Fig-1: Synthesis of (I) 5-Methyl 2-Mercapto benzimidazole capped Silver nanoparticles

Reagents and Instruments:

All the reagents used were of AR grade. Silver nitrate was obtained from National Refinery Pvt Ltd, and a 0.1 M aqueous solution was used as stock solution. Sodium borohydride was obtained from Merck, India. Organic-free water was used throughout the experiment.

The UV-visible spectra were recorded on a Schimadzu UV-vis spectrophotometer, and the solutions were taken in a 1 cm well-stoppered quartz cuvette. Fourier transform infrared (FTIR) spectral characteristics of the samples were collected on a Schimadzu FTIR spectrometer with the samples as KBr pellets. The FTIR spectrum was recorded over 45 scans of each sample, and the background spectrum was automatically subtracted. The formation of single-phase compound was checked by X-ray diffraction (XRD) technique. The XRD pattern was taken with X-ray diffractometer (XPERT-PRO) at room temperature, using CuK_α radiation λ=1.5406 A˚ over a wide range of Bragg angles ($30^0 \leq 2\theta \leq 85^0$). SEM micrograph of 5-Methyl 2-Mercapto benzimidazole capped Ag - NP was obtained at 20 K data on a F20 Tecnai High Resolution microscope (Philips, Netherlands). For SEM analysis, the specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under Infrared lamp.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

Results and discussion

Evolution and Characterization of Silver Nanoparticle Aggregates:

UV–visible spectroscopy Study:

The aggregates of silver nanoparticles have been synthesized using silver nitrate as the precursor salt and 5-Methyl 2-Mercapto benzimidazole as the capping agent. The successive changes of the absorption spectra of synthesized silver nanoparticles aggregates are shown in Figure 2. The color of the solutions changes from colorless to light yellow & light yellow to brown by the addition of Sodium borohydride indicates the nucleation of the silver particles at their infancy. The UV absorption measurements of the synthesized nano particles were studied by dissolving them in solvent DMSO.

Figure 2 shows the UV-visible spectra of nano colloids obtained. The Surface Plasmon Resonance (SPR) band is broad indicating poly-dispersed nanoparticles. A smooth and narrow absorption bands at 421 nm is observed in DMSO solvent which clearly indicates the formation of silver colloids. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of Nanoparticles. The optical absorption spectra of metal nanoparticles are dominated by Surface Plasmon Resonances (SPR), which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species.

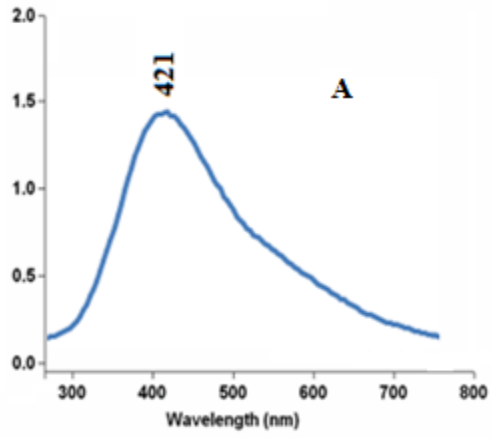


Fig. 2. UV–vis absorbance spectra of Ag- NP in DMSO

Scanning electron microscopy study:

The SEM images obtained for colloid is shown in figure 3. It is clear from the SEM images in figures 3 that the particles are nearly crystalline and spherical particles respectively.

The Scherrer rings, characteristic of fcc silver is clearly observed, showing that the structure seen in the SEM image are nano crystalline in nature. It is observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under SEM. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

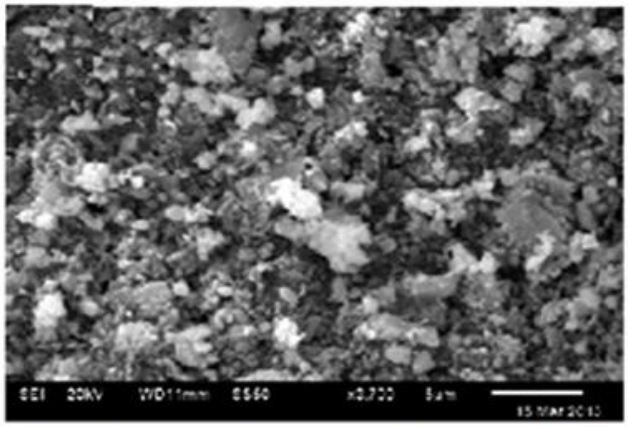


Figure3: Scanning electron micrographs of capped Ag Np

X-ray diffraction study:

XRD spectra of Capped Ag - NPs measured in the small and wide angle region are shown in Fig.4. The XRD spectrum confirms the tendency of nanoparticles to form the organized structures, as seen from the peaks in the small angle XRD spectrum in Fig. 4. The peaks are broadened because of the nano crystalline nature of Nanoparticles. By comparing with standard database values, all the peaks can be indexed to face-centered cubic (fcc) crystal structure.

From the figures, it can be noticed that the particles appeared basically amorphous and abroad. Size-dependent and structure-specific features in diffraction patterns can be quite striking in nano meter-sized particles. Small particles have fairly distinct diffraction patterns, both as a function of size and as a function of structure type. In general, regardless of structure, there is a steady evolution in the aspect of diffraction profiles: as particles become larger, abrupt changes do not occur, features grow continuously from the diffraction profile and more details are resolved. These observations form the basis for a direct technique of diffraction pattern analysis that can be used to obtain structural information from experimental diffraction data. The Capped Ag-NPs are almost crystallized with the appearance of diffraction peaks at the scattering angles (2y), which is in agreement with the results of Leff et al. It is well known that with diminishing crystallite size the measured XRD pattern exhibits broadening, and very often overlapping reflections. The broadening of the reflections is inversely proportional to the crystallite size (i.e. size of coherently diffracting domains). The relation is known as Scherrer's equation where "y" is the diffraction angle of a particular reflection. The total diffracted intensity for a given Bragg reflection from a crystallite is the sum of independently diffracted intensities by each of the unit-cell columns making-up the crystallite. It means that the calculated size distribution is in fact a distribution of diffraction column lengths in a given crystallographic direction perpendicular to the diffraction planes and not of crystallite (coherently diffracting domains) sizes. Theoretical considerations show that the interference function of a polycrystalline or nano crystalline solid is identical to that of an arrangement of isolated particles with the same size or size distribution as those of the polycrystalline or nano crystalline solid. Thus the values of Scherrer's formula are solely an estimate of a volume-weight average column length. This explains the difference between the experimental and theoretical values, and the values from the Scherrer's formula is termed ''apparent crystallite size''.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

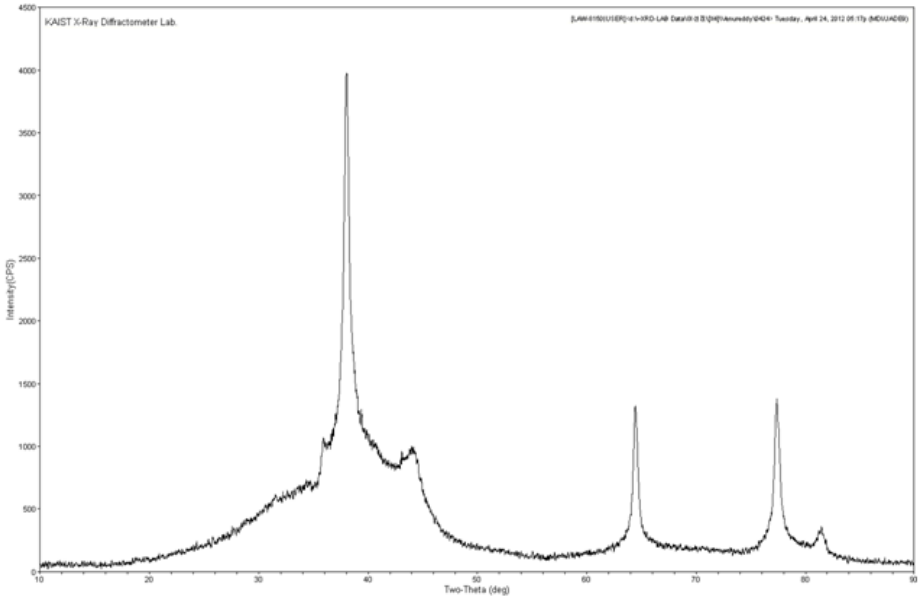


Figure 4: XRD patterns of capped Ag-Np.

FT-IR analysis:

The FT-IR spectra of Ag-NPs were obtained from the powdered particles as KBr discs. Fig-5 describes the interaction between the metals and the bounded ligand in Nanoparticles. The IR spectra of free 5-methyl 2-mercaptobenzimidazole differs from those of metal capped NPs. The thiol S-H stretching peak appear at 2549 cm^{-1} which is absent in metal capped NPs indicating the capping of metal with 5-methyl 2- mercaptobenzimidazole. The N-H stretching peak appear at 3122 cm^{-1} where as in Ag capped it shifts to 3302 cm^{-1}.

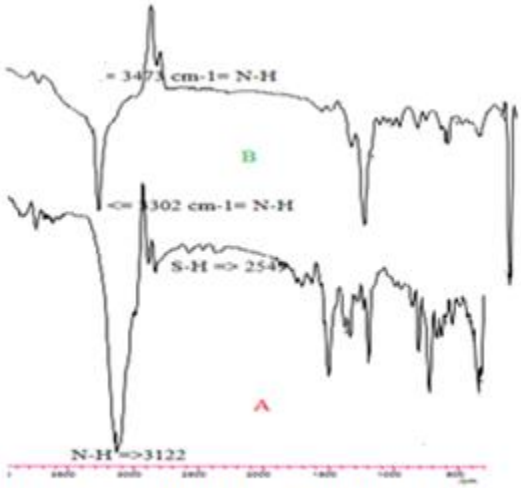


Figure 5: FT-IR Spectr of Capped Ag -NP

However, there is a remarkable difference in the peak intensity found between the peaks of the transverse mode (rocking mode, bending mode, etc.) in 5-Methyl 2-mercaptobenzimidazole and Ag-Np capped by 5-Methyl 2-

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

mercaptobenzimidazole. The reason for the intensity difference between the spectras is believed to be that the thiolate molecules on the nanoparticle form are relatively close packed thiol layer and molecular motion is constrained. Thus, this steric constraining effect on the transverse mode is stronger than that on the longitudinal mode. Therefore, the change of the peak intensity of the longitudinal modes is smaller than that of the transverse mode. The small peak at 2360 cm^{-1} which corresponds to the S–H stretching vibration mode, disappears when the 5-Methyl 2-mercaptobenzimidazole molecules adsorb on the silver particle surface (comparing spectra of free 5-Methyl 2-mercaptobenzimidazole (5A) and Ag-NP capped by 5-Methyl 2-mercaptobenzimidazole (5B), giving strong evidence that 5-Methyl 2-mercaptobenzimidazole anchors on the silver surface through the sulfur atom in the mercapto group.

Particle Size Measurement Using TEM:

Silver nanoparticles that produced the spectrum in Figure 3 were examined using transmission electron microscopy. A sample of silver nanoparticles from a freshly synthesized clear yellow sol was prepared by drying a small drop on a carbon-coated 200-mesh copper grid. The TEM image of one region of the sample is shown in Figure 6. The TEM image shows the silver particles are spherical with sizes of 40 ± 2 nm.

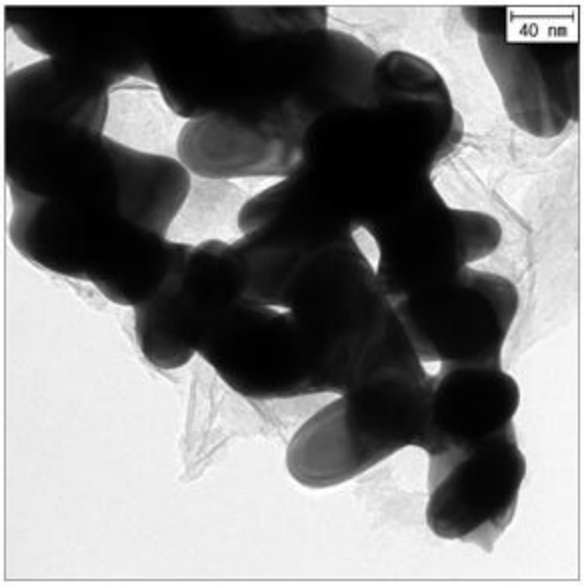


Figure 6: TEM Image of Capped Ag-Np

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

Antimicrobial activity of 5-Methyl 2-mercaptobenzimidazole capped Silver nano particles.

The antimicrobial activity of 5-Methyl 2-mercaptobenzimidazole capped metal NPs and metal ion as $AgNO_3$ in disperse solution was evaluated against Bacteria. For Minimum Inhibitory Concentrations (MICs) evaluation the microorganisms were grown overnight in TSB at 37 ^{0}C. Washed cells were re-suspended in Dulbecco's PBS and optical density (OD) was adjusted to 0.1 at 655 nm wavelength, corresponding approximately to 1 - 108 Colony Forming Units/mL (CFU/mL). MIC values of capped NPs and ionic metals were determined by the standard broth macro-dilution method in ISB with an average inoculums of 107 CFU/mL as described by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). MIC was defined as the lowest concentration that completely inhibited bacterial growth after an incubation of 24 h at the temperature of 37^{0}C.

Agar well diffusion assay:

The modified agar well diffusion method of Perez *et al*., was employed.[22,23] Each selective medium was inoculated with the microorganism suspended in Muller Hinton broth. Once the agar was solidified, it was punched with a six millimetres diameter wells and filled with required concentration of compounds and Ciprofloxacin (antibiotic) used as standard for positive control while Pure Solvents were used as negative control. Results were determined based on size of the inhibitory zone surrounding the wells containing the extract comparing with standard and blank. The zones of inhibition was measured in mm.

Antimicrobial activity studies of synthesized nanoparticals:

The compounds were tested for antibacterial activity against bacteria: viz, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* at concentrations of 50, 100, 200 µg /ml. The cultures of organisms were grown overnight at 37^{0}C and used for testing the antibacterial activity which was checked employing cup plate method. Nutrient agar medium (Himedia, India) was dissolved in water and pH was adjusted to 7.0. This was then distributed in 20 ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50 µl) was then added aseptically to the agar medium maintained at 45^{0}C, mixed well and poured immediately in sterilized petriplates. Test solutions of different concentrations of compounds were prepared in DMSO. After hardening, cups of 8 mm diameter each were cut into agar and 50 µl test solutions of varying concentration (50, 100, 150 and 200 µg/ml) were placed in these cups. The plates were incubated at 37 ^{0}C for 24 hours and the diameter of inhibition zone was measured in mm's. Solvent DMSO was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotics Ciprofloxacin. Antimicrobial activities inhibition zones of the compounds are presented in Table-1.

Results & Discussions:

The synthesized Nanomaterials were tested for *in vitro* antimicrobial activity. The activity was reported by measuring the diameter of inhibition zone in mm, against Gram-positive and Gram-negative bacteria, which were presented in Table 1. The tested compounds have shown moderate activity against tested bacteria. However, the compounds showed good activity (nearly equal to the inhibition zone value of ciprofloxacin) against to the *E.coli* bacteria. In general the antimicrobial activity of the silver nanomaterials has shown good activities. In conclusion, the Ag-NP's increases the activity against *S. aureus*, *B.subtilis*, *E. coli and P. aeruginosa*. Thus from the above discussion it may be conjectured that Ag-NP's are playing their role in enhancing the antibacterial activity.

Table 1: Antibacterial *in vitro* activity expressed as diameter of growth inhibitory zone
(GIZ, mm) for tested compounds (applied 400 µg per disc)

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

Compound	**Conc.of Compound**	***E.coli***	***P.aeruginosa***	***S.aureus***	***B.subtilis***
Ciprofloxacin	A	30	28	26	25
5-Methyl 2-Mercapto benzimidazole	A	23	24	22	20
	B	19	16	16	15
	C	12	13	10	9
Capped Ag-NP	A	27	25	23	20
	B	18	13	11	14
	C	11	9	-	9
DMSO	A	27	25	20	22
	B	17	15	12	15
	C	11	10	9	9

A, 200µg/ml; **B**, 100µg/ml; **C**, 50µg/ml

Conclusions:

Uniform size and shaped 5-Methyl 2-mercapto benzimidazole capped Silver Nanoparticles are prepared via chemical reduction method and their morphology photo physical, biological activity was studied. The microbial activity of these compounds showed good activity (nearly equal to the inhibition zone value of ciprofloxacin) against to the *E.coli* bacteria, *S. aureus*, *B.subtilis, and P. Aeruginosa* compared to 5-Methyl 2-Mercapto benzimidazole and also the activity is increased with the concentration. Thus Ag-NP's are playing their role in enhancing the antibacterial activity.

References:

1. D.V. Leff, *Journal of Physical Chemistry*, 1995, **99,** 7036.
2. J.L offler, J. Weissm uller, *Physical Review B,*1995, **52,** 7076.
3. L. Kundakovic, M.F. Stephanopoulos, *Applied Catalysis A*, 1999, **183**, 35.
4. P. Knauth, J. Schoonman, Nanostructured Materials Selected Synthesis Methods, Properties and Applications, Kluwer Academic Publishers, Boston, 2002.
5. H. Shengtai, Y. Jiannian, J. Peng, S. Dongxia, Z. Haoxu, G.H. Jun, *Langmuir*, 2001, **17**, 1571.
6. J.B. Lambert, H.F. Shurvell, D.A. Lightner, R.G. Cooks, Organic Structural Spectroscopy, Prentice Hall, New Jersey, 1998.
7. A. Miller, D.W. Mayo, R.W. Hannah, Course Notes on the Interpretation of Infrared and Raman Spectra: Deducing Structures of Complex Molecules, Wiley-IEEE, 2004.
8. B.A. Korgel, S. Fullam, S. Connolly, D. Fitzmaurice, *Journal of Physical Chemistry B*, 1998, **102**, 8379.
9. S. He, J. Yao, S. Xie, H. Gao, S. Pang, *Journal of Physics D: Applied Physics*, 2001, **34**, 3425.
10. A. Curtis, C. Wilkinson, *Trends Biotechnol.* 2001, **19**, 97.
11. C. W. Waren, S. Nie, *Science*, 1998, **281,** 2016.
12. A. Vaseashta, D. Dimova-Malinovska, *Sci. Technol. Adv. Mater*, 2005, **6,** 312.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE
B.Syama Sundar et al, The Experiment, 2016., Vol. 35(1), 2162-2170

13. R. Langer, *Science*, 2001, **293,** 58.
14. K. Roy, H. Q. Mao, S. K. Huang, K. W. Leong, *Nat. Med*., 1999, **5,** 387.
15. E. Sachlos, D. Gotora, J. T. Czernuszka, *Tissue Eng*, 2006, **12,** 2479.
16. O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, R. Langer, *Proc. Natl Acad. Sci. USA*, 2006, **103,** 6315.
17. P. K. Stoimenov, R. L. Klinger, G. L. Marchin, K. J. Klabunde, *Langmuir*, 2002, **18,** 6679.
18. I. Sondi, B. Salopek-Sondi, *J. Colloids Interface Sci*, 2004, **275,** 177.
19. A. Panacek, L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V. K. Sharma, T. Nevecna, R. Zboril, *J. Phys. Chem. B*, 2006, **110,** 16248.
20. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez, M. J. Yacaman, *Nanotechnology*, 2005, **16,** 2346.
21. C. Baker, A. Pradhan, L. Pakstis, D. J. Pochan, S. I. Shah, *J. Nanosci. Technol*, 2005, **5,** 244.
22. Clinical Laboratory Standards Institute Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically ; Approved Standard – Ninth Edition. January 2012, M07-A9, **32(2),** 12.
23. F. Osaki, T. Kanamori, S. Sando, T. Sera, Y. A. Aoyagi, *J. Am. Chem. Soc*., 2004, **126**, 6520.

R.Sarada [1], V. Jagannadharao[1], M.Padma[2], B.Syama Sundar*

[1]Department Of Chemistry, Anil Neerukonda Institute of Technology and sciences. Sangivalasa, Visakhapatnam Dt., India-531162.
[2]Department Of Chemistry, Andhra University, Visakhapatnama
[*] Vice Chancellor, Yogi Vemana University, Kadapa, A.P.,India.