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Synthesis of Hybrid ZnO@AG CORE-SHELL Nano Spheres and Its Anti-Bacterial Applications

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ABSTRACT

ZnO nanospheres were planned away hydrothermal approach the use of CTAB for protecting ligands. 3-aminopropyl-trimethoxysilane (APTMS) were used to functionalize the purified ZnO nanospheres also constructed into core-shell nanostructures for in-situ reduction coming from ag+ into silver nano clusters. Silver nanoclusters act as shell material and ZnO nanospheres act as the core while resulting in the formation of ZnO@Ag core-shell nanostructures.The anti-bacterial activity of silver on Zinc Oxide nanoparticles was tested on the two gram-positive bacterias (*Staphylococcus aureus* and *Bacillus subtilis*) and five gram-negative bacterias (*Escherichia-coli, Enterobacteraerogenes, Klebsiellapneumoniac, Proteus vulgaris and Pseudomonas fluorescens).* Gram staining was carried out to test the purity of cultures. Disc-diffusion and Agar-well diffusion methods were followed to qualify the antibacterial ability of the nanoparticles on the test organisms. These experiments were followed by growth kinetics to determine the Minimum inhibition concentration.
Keywords: *Nanomaterials; Metal Oxide nanoparticles; Core-shell nanoparticles; Anti-bacterial activity.*

1. INTRODUCTION

Nanotechnology includes the correct of materials appearing in nuclear level to succeed in unique properties, which can be appropriately supervised in the interest of the specified applications(Gleiter H, 2000). A few of the natural processes in conjunction with modify inside the nanometre rate tenure. So, a junction connected with nanotechnology as a consequence biology can compose numerous biomedical problems furthermore keep revolutionize the sphere consisting of health as well as medicine (Curtis A***et al.,*** 2001). Nanotechnology is up to now settled being a device to investigate striking darkest roads in regard to medical sciences in a few other ways like imaging (Waren C W***et al.,***1998), artificial implants(Sachlos E***et al.,***2006)directed medication conveyance(Langer R,2001), gene delivery systems(Roy K***et al.,*** 1999) and detecting(Vaseashta A***et al.,*** 1998).On that account, nanosized natural additionally in-organic particles are discovery expanding difficulty in therapeutic applications (Xu Z P et al., 2006) as a result of their manageability as far as organic functionalization.In response to altered effectiveness, the recent century drugs are nanoparticles connected with polymers, metals or ceramics, that could strive against conditions like cancer (Farokhzad O C ***et al.,*** 2006) along with protect human pathogens like microbes. (Stoimenov P K***et al.,*** 2002;Sondi I***et al.,*** 2004;Panacek A***et al.,*** 2006;Morones J R***et al.,*** 2005;Baker C***et al.,*** 2005).

Over the last several decades semiconductor nanocrystals consider pleased significant importance due to their special size dependable optical and magnetic properties. Many of the semiconductor nanomaterials by various structural morphology, properties and dimensionality experience a great potential in the interest of applications in the field of optoelectronics, sensors together with nanoscale devices (ZhiLia***et al.,*** 2005). Immediately upon preceding semiconductor nanocrystals combine for opposite nanocrystals ends up in the formation of hybrid nanomaterials and that possess enhanced also exclusive optical, electrical, magnetic moreover chemical properties (Ping Wu***et al.,*** 2009). Multifunctional nanomaterials which comprises of 2 about over components in with the core zone of research attributable to their property of integration plus combination of materials that are on the whole no longer attained in particular nanocrystals (X. Qun***et al.,*** 2010;Wei-Qing Zhang***et al.,*** 2008). The above-mentioned multifunctional heterostructures are principally of 2 types, the core-shell nanostructures along with the heterodimers. The core-shell structures include a nanocrystaline core too that one shells of different nanocrystaline material roughly several materials are coated. Considering that latest heterodimers two or more inorganic compounds are associated as a result a small interfaces. Some of the Metal

oxide semiconductors such as In_2O_3, TiO_2, ZnO, $SnO_2$and Fe_2O_3 have been widely used for optoelectrical applications and also for detecting specific gases in atmosphere.Above-mentioned semiconductor nanomaterials possess a large potential in the field of emission displays, gas sensors also solar cell, made from the particular nanomaterials ZnO was well known as one of the important oxide semiconductor materials because of its outstanding optical, electrical along with piezoelectrical properties (Djurisic A. B et al., 2006) which are enhanced by the quantum confinement effect. In consequence the hybrid nanostructures like core-shell nanomaterials fit in excellent influence due to their enhanced properties along with performances. Hybrid nanostructures of ZnO/metal nanocomposites ZnO/Au (Wei-Qing Zhang***et al.,*** 2008) and ZnO/Ag (X. Qun***et al.,*** 2010) and their sensing, optical properties have attracted great interest thesedays. In this paper we discuss the synthesis and characterization of ZnO@Ag core-shell nanoparticles and its anti-bacterial activity.

2. EXPERIMENTAL

2.1 Synthesis of ZnO@Ag core-shell nanoparticles:

50mL of deionized water was used to dissolved 0.004 M ZnCl2 under stirring condition then 20 mL of 0.2 M KOH was added. In addition 1 mL of 0.1M (Cetyl Trimethyl Ammonium Bromide) CTAB was added into the solution. Then mixed solution was taken in a Teflon-lined stainless autoclave andhydrothermal approach be carried out at 120^{o}C for 5 hrs. Formed white precipitatewas collectedthen washed with distilled water and ethanol. At long last, the ppt was dried at 50^{o}C for 5hrs (JiaqiangXua***et al.,*** 2000). As synthesised ZnO nanoparticles was functionalised using3-aminopropyl-trimethoxysilane (APTMS) before forming core-shell nanoparticles. 10mL of 7.5 Mof TEA (Triethyl Amine) solution was added to 2mL of 0.6 M of $AgNO_3$ solution dropwiseunderstirring. 0.2g of APTMS functionalized ZnO nanospheres were added to make the volume up to100mL and the solution was left undisturbed. After 3 hours of growth, the colour of the solutionchanges to dark brown, this reflects the formation of ZnO@Ag core-shell nanoparticles. Theparticles were centrifuged separately and ultrasonically washed several times using deionized waterand dried to obtain a powder of ZnO@Ag core-shell nanoparticles.

3. MATERIALS AND METHODS

3.1 Preparation of nanoparticle suspension and sonication:

A stock of nanoparticles was prepared by dissolving 20 mg nanoparticle powder in 5 ml Nutrient broth. The stock was sonicated and diluted to obtain concentrations of 0.6 mg/mL, 0.4 mg/mL, 0.2 mg/mL, 0.1 mg/mL, 0.05 mg/mL, 0.04 mg/mL and 0.004 mg/mL. Each dilution so obtained was sonicated. To achieve uniform dispersion of nanoparticles, several parameters were set, as Time-4 minutes, Intensity-65 and Output-6.

3.2 Disc-Diffusion method:

Autoclaved nutrient agar plates were inoculated with 100 µL of 18 hrs. broth culture of the desired organism by spread plate technique. 5 mm Whatmann filter paper discs were dipped in nanoparticle suspensions of required concentrations and placed in respective quadrants. A control plate (without nanoparticle) for each organism was maintained. The plates were incubated for 18 hrs at 37 ^{0}C and examined for the presence of zone of inhibition. The susceptibility of the test organisms were determined by measuring the diameter of the zone of inhibition.

3.3 Agar-well diffusion method:

Autoclaved nutrient agar plates were inoculated with 100 µL of 18 hrs. broth culture of the desired organism by spread plate technique. Well of diameter 0.8 cm were made using cork borer and 50 µL of nanoparticle suspensions of desired concentrations were added into respective quadrants. A control (without nanoparticle) was maintained. Plates were incubated for 18 hrs and examined for the presence of zone of inhibition. The susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition.

3.4 Minimum Inhibition Concentration of test organisms:

100 µL of 18 hrs culture of the desired organism was inoculated into each of the nanoparticle suspensions of concentrations 0.05 mg/mL, 0.1mg/mL, 0.2 mg/mL, 0.4 mg/mL and 0.6 mg/mL. The concentration at which the microorganism showed inhibition was taken for time kinetics experiment.

3.5 **Time Kinetics of test organisms:**

3.6 At time intervals (in hours) of 1.5, 3.0, 4.5, 6.0 and overnight, turbidity in each tube was determined by means of absorbance measurements at 600 nm in a colorimeter. MIC of nanoparticle for each test organism was determined.

4. RESULT AND DISCUSSION

Hydrothermal treatment was used to synthesize ZnO nanospheres and functionalised usingAPTMS (amino propyl trimethoxysilane). Silver nanoparticleswere formed as shell layer over the ZnO nanospheres using TEA as a reducing agent. It is well known that ZnO nanoparticles havemany single ionized oxygen vacancies (Vo^{+}) the side oron the tip of the nanospheres. The surfaceenergy of the "activated center" is higher than the non-polar planes and is effectively favored to Ag deposition (Dang Hyok Yoon***et al.,***1997).

The zone of inhibition observed against Ag NPs by Disc-diffusion method and is summarized in Table 1. The results indicate that 0.4 mg/mL exhibited almost similar antibacterial efficacy against Gram-Positive *Staphylococcusaureus, Bacillus subtilis*and Gram-negative *Escherichiacoli, Enterobacteraerogenes, Proteus vulgaris*bacterias. Compare to other bacterias, Gram-negative *Klebsiellapneumoniac* bacteria showed bit highzone of inhibition and zone of inhibition was not formed by *Pseudomonas fluorescens*bacteria even in 0.4, 0.04, 0.004 mg/mL dilutions. It shows*Pseudomonas fluorescens*resistant to nanoparticles and*Proteus vulgaris* shows higher susceptibility.

Table 1

Zone of inhibition of spherical Ag nanoparticles
By Disc-diffusion method (Disc size-0.5 cm)

Gram stain	Bacterial strains		Dilutions (mg/mL)	Zone of inhibition (cm)
gram positive organism	*Staphylococcus aureus*		0.4 0.04 0.004	1.2± 0.66 ne ne
	Bacillus subtilis		0.4 0.04 0.004	1.0± 0.33 ne ne
gram negative organism	*Escherichiacoli*		0.4 0.04 0.004	1.0± 0.51 0.8 ± 0.57 ne
	Enterobacteraerogenes		0.4 0.04 0.004	1.0± 0.52 ne ne
	Klebsiellapneumoniac		0.4 0.04 0.004	1.9 ± 0.79 0.7± 0.33 ne
	Proteus vulgaris		0.4 0.04 0.004	1.0± 0.88 0.7± 0.57 0.6± 0.66
	Pseudomonas fluorescens		0.4 0.04 0.004	ne ne ne

Values are mean inhibition zone (cm) ± S.D of three replicates
Note: 'ne' indicates no effect

The zone of inhibition observed against Ag NPs by Well-diffusion method and is summarized in Table 2. The results indicate that 0.4 mg/mL exhibited almost similar antibacterial efficacy against Gram-Positive *Staphylococcusaureus*and *Klebsiellapneumoniac.* Compare to other bacterias, Gram-negative*Escherichia coli* bacteria showed bit high zone of inhibition in 0.4 mg/mL dilution. There was no zone formed by all the three bacterias like *Staphylococcusaureus, Klebsiellapneumoniac, Escherichiacoli* in 0.04, 0.004 mg/mL dilutions.

Table 2
Zone of inhibition of spherical Ag nanoparticles
By well-diffusion method (Well size-0.8 cm)

Gram stain	Bacterial strains		Dilutions (mg/mL)	Zone of inhibition (cm)
gram positive organism	*Staphylococcus aureus*		0.4 0.04 0.004	1.3± 0.33 ne ne
gram negative organism	*Klebsiellapneumoniac*		0.4 0.04 0.004	1.2± 0.51 ne ne
	Escherichia coli		0.4 0.04 0.004	1.5± 0.66 ne ne

Values are mean inhibition zone (cm) ± S.D of three replicates
Note: 'ne' indicates no effect

Minimum inhibitory concentration(MIC) of pathogenic bacterias like*Staphylococcus aureus,Bacillus subtilis,Escherichia coli, Enterobacteraerogenes andKlebsiellapneumoniac*is summarized in Table 3.Figure 1 and 2 shows Minimum inhibition concentration of different test organisms.

Table 3
Minimum inhibitory concentration

Bacterial strains		MIC (mg/mL)
Staphylococcus aureus		0.1
Bacillus subtilis		0.1
Escherichiacoli		0.4
Enterobacteraerogenes		0.4
Klebsiellapneumoniac		0.2

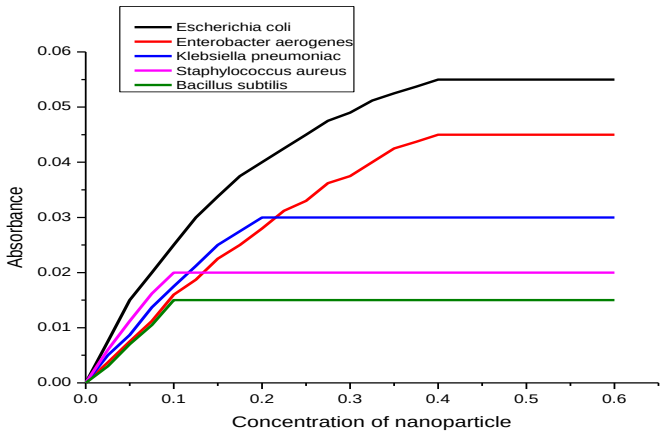

Figure 1

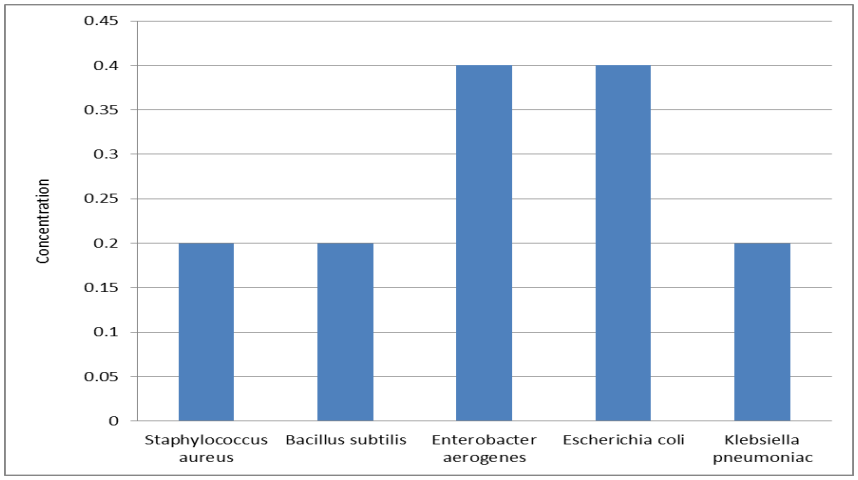

Figure 2

From Figure3 and 4 concluded that all the test organisms have shown the minimum inhibition time of 1.5 hrs.

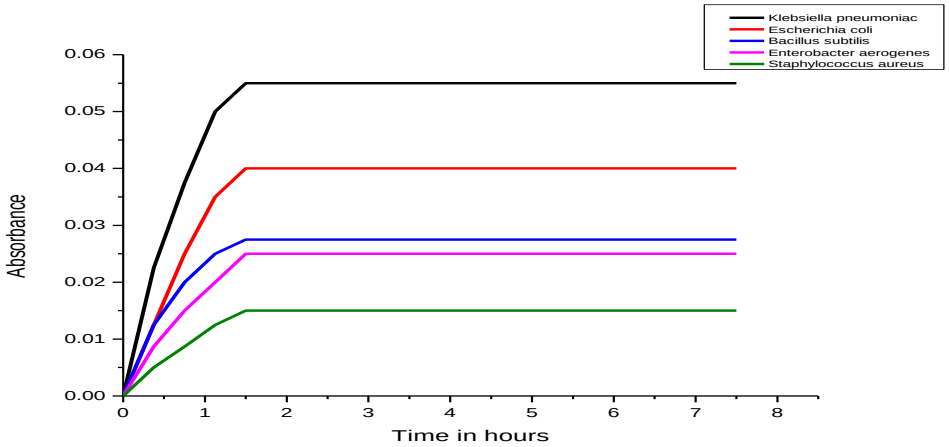

Figure 3

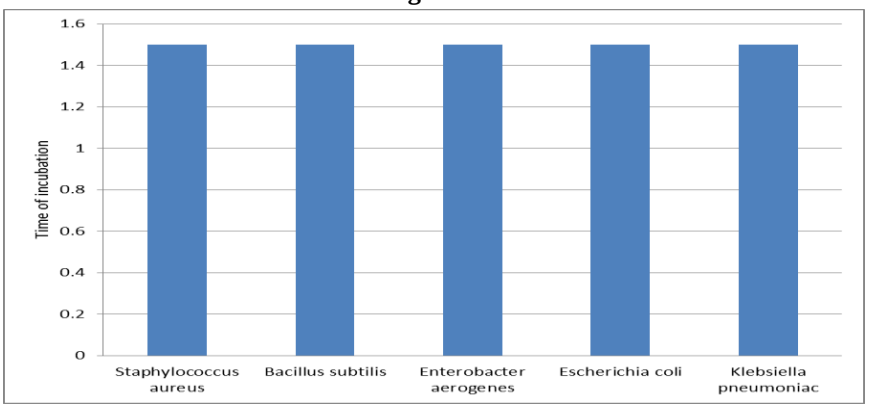

Figure 4

5. CONCLUSION

In summary, hydrothermal method was used to synthesize high quality functionalized ZnO@Ag core-shell nanoparticles and followed by *in-situ* reduction of Ag^{+} into Ag nanoclusters.Silver nanoclusters were attached as the shell material on the ZnO nanospheres acted as the core template to get ZnO@Ag core-shell

nanostructures. The higher surfacearea of these hybrid core-shell structures with isolated nanoclusters on ZnO surface pointed towardsthe possibility of having betteranti-bacterial activity against two gram-positive bacterias (*Staphylococcus aureus* and *Bacillus subtilis*) and five gram-negative bacterias (*Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniac, Proteus vulgaris and Pseudomonas fluorescens).*

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