ANTIBACTERIAL AND PHOTOCATALYTIC PROPERTIES OF THE ENGINEERED NANOPARTICLES AGAINST INFECTIOUS PATHOGENS

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Abstract
Engineered nano particles (ENPs) have been intensively studied within the past decade. Nano sized materials have been an important subject in basic and applied science. Nano particle with good antimicrobial activity have been used in field of medicine for treating certain infectious pathogens. In this paper the toxicity of engineered nanoparticles (ENPs) towards the infectious microorganisms was studied by antimicrobial activity (modified well diffusion method). The comparison of antimicrobial activity of six nano particles was studied, the results show that among those nano particles $AgTiO_2$(Silver doped titanium oxide) and $AgTiO_2SiO_2$have antibacterial effect against both Bacillus*sp*,(Gram +ve) *and E.coli sp*, (Gram -ve). Their toxicity is viewed by the zone of inhibition or clearance measurement using standard cm scale. The photocatalytic activity of nano particles against *E.coli* and *Bacillus* shows good reduction in the number of viable cells after the treatment process. $AgTiO_2$ and $AgTiO_2SiO_2$also act as goodphoto catalysts. These nanoparticles were further characterised by SEM and FT-IR analysis. This study helps to understand the bactericidal properties of doped nanoparticle

Key words: Engineered nano particles (ENPs), *Bacillus sp*, *E.colisp*, Antimicrobial activity, Photocatalysis, SEM, FT-IRSS

Introduction

Nanotechnology is an emerging field in which nanoparticles have wide range of application in health care, biomedical science, Drug gene delivery, water and wastewater treatment etc. Several authors (Fresta et al., 1995 and Hamouda, M et al., 1939 Valerie et al., 2018 ; Villalan 2018) reported that antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal material. Antibacterial agents are very important in medicine and food packing. Using of organic compound disinfectant may cause some side effect to the human body. Therefore, the anti bacterial efficiency of inorganic nano material against gram positive and gram negative bacteria has been studied. Antibacterial agents are the one which fight against the infectious pathogens either by killing or slow down the growth of bacteria. Due to evolutionary process some of specified bacteria developed resistance properties against antibacterial agent which could lead to various infectious diseases (writte, 2004). Nowadays nano materials have proven to act best antimicrobial agent to treat the bacterial infectious diseases (Huh and won 2011). The mechanism of nano particle toxicity depends on the bacterial sp, physico chemical properties, composition, surface modification and intrinsic properties of the nano particle. Nano particle disrupt the integrity of bacterial membrane by forming electrostatic interaction and thereby create oxidative stress in bacteria (Soenen et al. 2011; Nel et al 2009). In addition thephotokilling of bacteria by photocatalytic treatment has been practiced (Ahmadi et al.,2008 ; Wei et al., 1994 Caitlin et al., 2018). Nano particle as photocatalysts generate strong oxidizing power by producing holes (H^+), hydroxyl radicals (OH•), electrons and superoxide ions under illumination of UV light

(Jacoby et al., 1998). During the photocataytic reaction nanoparticles generate reactive oxygen species (ROS) which cause various damages to living organisms (Robert et al., 2013). In this study the antibacterial properties as well as the photocatalytic properties of six engineered nano particle such as TiO_2 R- Degussa Titanium oxide, $AgTiO_2SiO_2$- SiO_2 at silver doped Titanium oxide,, $AgTiO_2$-Silver doped titanium oxide, BVZ- Bismuth vanadate doped zinc oxide BOT- Bismuth oxide doped TiO2 and BV- Undoped Bismuth vanadate against *E.coli* and *Bacillus* were tested. Corresponding photocatlytic inactivation of two bacterial species by respective nanoparticles were examined. In addition the structure and morphology characteristics of six nanoparticles were analyzed using SEM and FT-IR.

MATERIALS AND METHODS

Preparation of nano materials and sample

The preparation and characterization of nanomaterials are described in our earlier papers (Thirumalai et al., 2017). The sample was prepared by dissolving 50 mg/ml of nanoparticles in distilled water. The sample is mixed gently before inoculating into well for proper dispersion of nanoparticles in the distilled water.

Culture inoculation

Luria agar plates were prepared and overnight culture of *Bacillus* and *E. coli* (100 µL) were swabbed on the surface of Luria-Bertani medium (Becton Dickinson). The plates were the kept to dry for 10 min before the well punch.

Antimicrobial activity by disc diffusion method

The discs are commercially prepared which have the capacity to impregnate with standard concentration of nanoparticle and slightly pressed the agar surface.

The plates were incubated at 37^0C for 24 hours for the diffusion of the sample into the agar medium. The diameter of the zone of inhibition as indicated by the clear area which was devoid of growth of microbes was measured (Zaidan et al., 2005). All the antimicrobial tests were carried out in triplicate and the average is reported.

Scanning electron microscopy

Morphology of engineered nano particle were analysed using Scanning Electron Microscopic (SEM) EVO18 (CARL ZEISS). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra samples was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Fourier transforms infra-red spectroscopy (FT-IR)

The properties and characterization of engineered nanoparticles were investigated by FTIR analysis. The dried powder of the synthesized engineered nanoparticles were analyzed using Perkin Elmer Spectrum 1000 spectrum in attenuated total reflection mode, and the spectral range of 4000–400 cm^{-1} with the resolution of 4 cm^{-1}.

Photocatalytic analysis

The photocatalytic disinfection was carried out by using a 300 W xenon lamp (PLS-SXE-300, Beijing Perfect Light Co. Ltd. Beijing) as a light source. Light was passed through a UV cutoff filter (λ g 400 nm), and then was focused onto a flask containing a suspension of bacterial cells and photocatalyst (Ahmadi et al.,2008). All glassware used in the experiments were autoclaved. The bacterial cells were incubated in nutrient broth solution at 30ºC for overnight with shaking. The nanoparticle 0.005g/10ml and bacterial suspension 5×10^7 or 2×10^8 colony forming units per milliliter (cfu mL-1), were added into the four flask, 1st two flask contain Np(10ml)+Dw (20ml) and NP (10ml)+ Dw (10ml)+*E.coli* suspension (10ml). Same procedure followed for *Bacillus* in other two flask. The reaction temperature was maintained at 25ºC throughout the experiment. The sample was collected before and after treatment, the aliquot was spread on nutrient agar and incubated at 30ºC for 24 h. The number of colonies formed was counted to determine the number of viable cells.

Result and Discussion

The antibacterial activity of the six nanoparticles against gram positive (*Bacillus*) and gram negative (*E. coli*) microorganism was tested using well diffusion method. Fig. 1 and 2 shows the zone of inhibition of respective nanoparticles (in different plate cultures) against gram positive (*Bacillus*) and gram negative (*E.coli*) bacteria respectively. All the values mentioned for zones of inhibition are in cm scale.

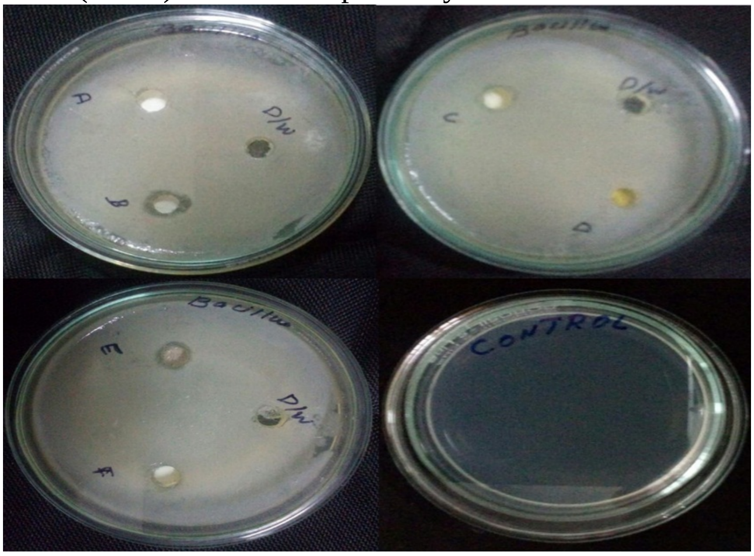

Fig 1 Antibacterial properties of gram positive *Bacillus* against nano particles. A-Degussa TiO_2; B- SiO_2@silverdoped TiO_2; C- Bismuth oxide doped TiO_2

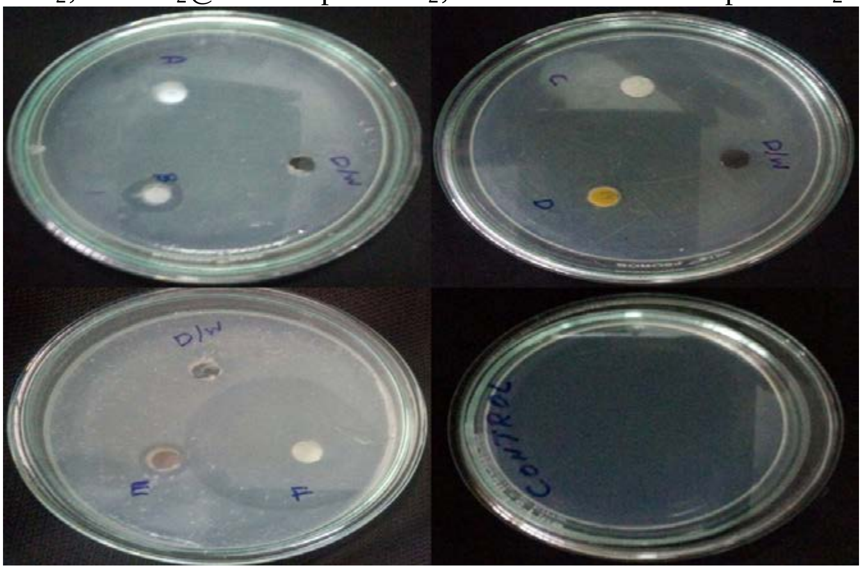

Fig 2 Antibacterial properties of gram negative *E.coli*against nano particle; D- Undoped Bismuth vanadate; E- Silver doped TiO_2; F- Bismuth vanadate doped zinc oxide

While comparing the six nano particles BOT shows antibacterial effect only against gram positive (0.4cm) and no effect against gram negative bacteria. According to Prabu and Anabarasan 2012 the FTIR spectra of Bismuth oxide doped TiO2, there is a presence of spectral range of 4000-400 (Ti-O-O) and 2489 (CH stretching of alkane group). These peak range were absent in BOT. This could be happed because of the difference in nano particle synthesised methods leads to the changes in the properties. Hence we think this could be the reason for not showing the bactericidal effect against gram negative bacteria (Abdulkadir et al., 2015). Bismuth oxide nano particle doesn't show any antibacterial effect against bacteria. But $AgTiO_2$ and $AgTiO_2SiO_2$ show antibacterial effect against both Gram positive and gram negative bacterial with the range of 0.4 cm; 0.2 cm and 0.4cm ;0.5cm respectively.Authors Tessy et al., 2012 and Liu et al., 2008 also demonstrate the excellent antibacterial properties of $AgTiO_2$. This can be due to the action of silver nanoparticle interact with the nucleic acid of bacterial and stops the DNA replication. This reaction depends up on the surface area available for interaction.Similarly, TiO2SiO2 shows efficient bactericidal effect due to its large surface area and fine crystal size which could be easily invaded the bacterial cell membrane.(Raffi et al., 2008). This results can be substantiating by Ahmadi et al 2018.

But BVZshows good anti-bacterial effect against gram +veupto 0.2cm and gram negative upto 0.8cm. According to Rishabh et al., 2016 andYanping et al., 2011 Bismuth vanadate and Zinc oxide itself shows antibacterial effect against E.coil respectively, As it get doped with one another, its show high antibacterial activity against *E.coli*.There is no antibacterial effect seen against gram positive and gram negative bacteria for both TiO_2R and BV. According to Pusit et al., 2013 FTIR spectra of bismuth vanadate the peak range at 474 and 700-900 codes for symmetric bending VO_4 and asymmetric $-VO_4$. But those peak range were absent in BV, this changes may be due to difference in the methodology while synthesis the nanoparticle. This may fails to establish the antibacterial properties.

Nanoparticle	Gram +ve	Gram -ve
BOT	0.4	-
AgTiO2	0.4	0.2
BV	-	-
AgTiO2SiO2	0.4	0.5
TiO2R	-	-
BVZ	-	0.8

Photo catalytic Activity

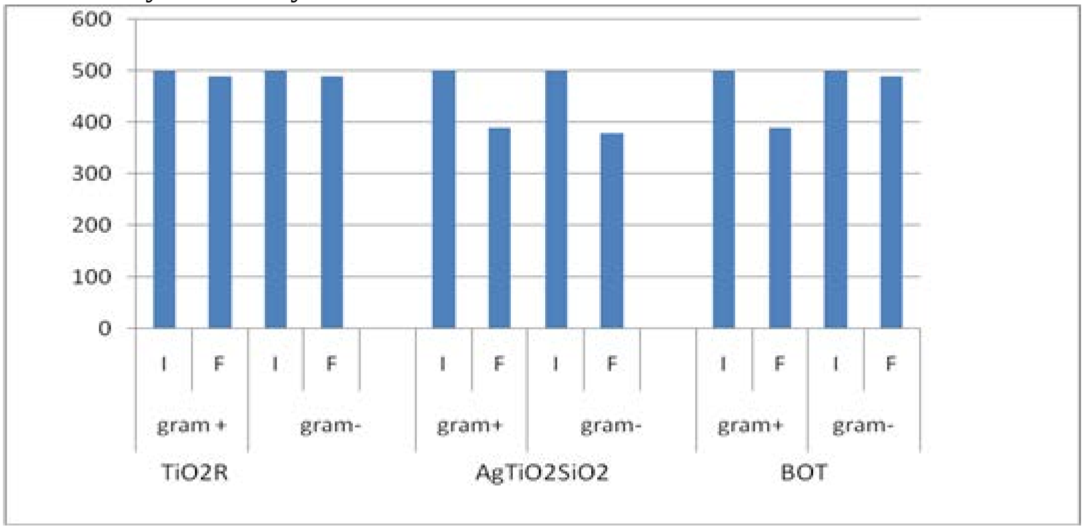

Fig 3 Bacterial growth against TiO_2R, $AgTiO_2SiO_2$ and BOT nanoparticles obtained before and after photocatalytic treatment

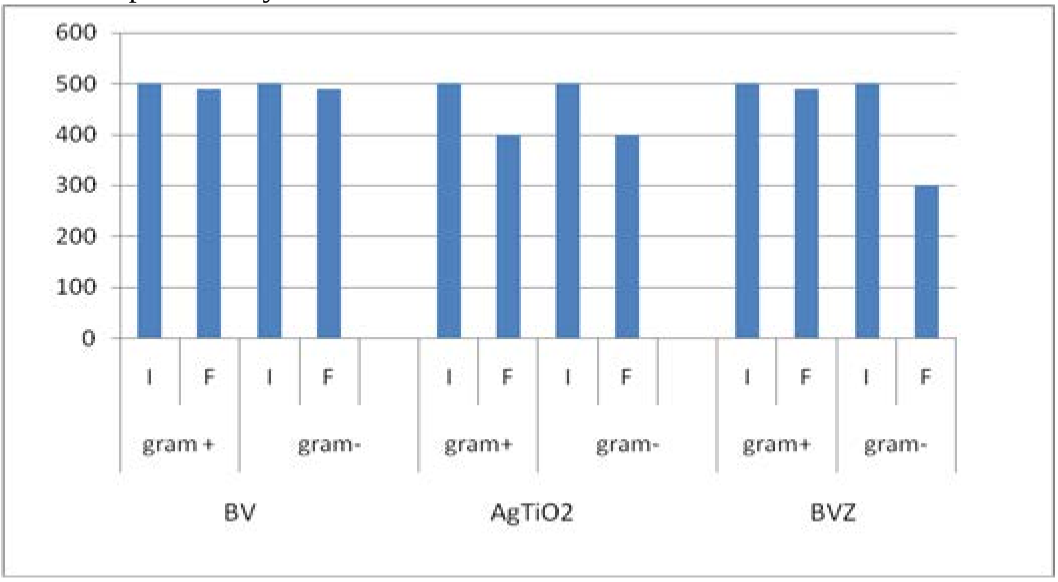

Fig 4 Bacterial growth against BV, $AgTiO_2$ and BVZ nanoparticles obtained before and after photocatalytic treatment

During photo catalytic experiment the growth of bacteria was determined before and after nano particle treatment. In Fig 3, the growth of bacteria gram+ve and gram –ve in the presence ofTiO_2R, $AgTiO_2SiO_2$ and BOT was represented. . In $AgTiO_2SiO_2$, the number viable colonies get decrease after the photocatalytic treatment. For gram +ve it is (500±0.05-38±0.05) and for gram-ve. (500±0.05-490±0.05. The photocatalytic properties of TiO_2SiO_2 were very well studied by Ahamid et al., 2008. During photocatalytic reaction OH radicals generated by the photoelectron leads to the inactivation of bacteria or oxidation of free radicals. Further Ag leads to the formation of band gap between the TiO_2(Blake et al, 1999).As the surface properties of the were changed, it bring about different photocatlytic activity. In BOT, there is slight decrease in number of viable colonies of gram+ve bacteria (500±0.05-385±0.05).There is no activity seen in both gram+ve and gram-ve in the presence $AgTiO_2$R nano particle. All the photocatalytic treatment get correlated with the antibacterial properties of the respective nanoparticles

Similarly, in Fig 4, . In the presence of $AgTiO_2$ number of viable colonies get decreased after the treatment process, gram +ve (500±0.05-420±0.05); gram-ve (500±0.05-465±0.05). Nanoparticle irradiated to produce ROS, which directly leads to the inactivation of bacterial strain (Robert et al., 2013). Kiran et al., 2013 study also proves the photocatalytic properties of AgTiO2. In BVZ, there is slight decrease in number of viable colonies in both gram +ve and gram -ve bacteria (500±0.05- 295±0.05;500±0.05-285±0.05) (Rishabh et al., 2016). There is no activity seen in both gram +ve and gram-ve in the presence BV nanoparticle. All this process is due to the interaction of nanoparticles with the intracellular substance of the bacterial cell and thereby destroyed the cells.

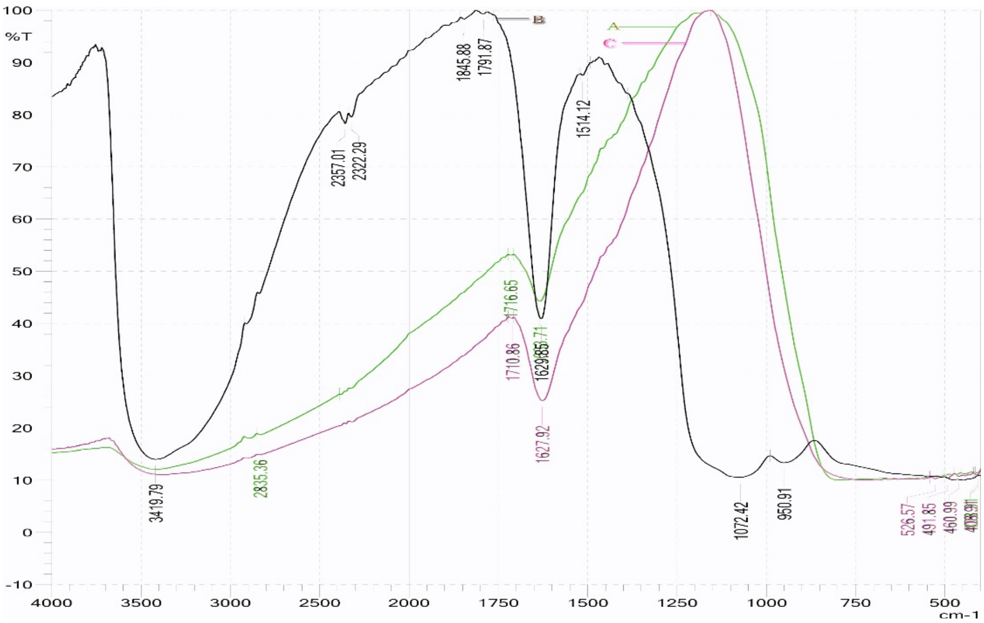

Fig 5 FT-IR spectra of nano particle TiO_2R (A), $AgTiO_2SiO_2$ (B) and BOT (C)

FTIR absorption spectra of synthesized six nano particle are shown in the Fig 5 and Fig 6 respectively. The spectra range of 3402 and 3394cm^{-1} indicates the presence of O-H vibration of free water molecule (Haw et al., 2011). The peaks at 1628 and 1627 cm−1 correspond to C=O stretching vibrations. The peaks at 1384 and 1400 cm−1 are due to C–C stretching. The peaks from 1233 to 975 cm^{-1} correspond to C–N stretching vibration. The peaks at 818 and 840 cm−1 correspond to C–H stretching vibration. (Seyed et al., 2013)

In Fig 5 FTIR spectra of A, B and C were seen. In TiO_2R (A), the spectra range of 408 cm^{-1}shoud be attributed to Ti-O bond in the TiO2 lattice (anatase titanium).Similar result was reported by Prabu and Anbarsan, 2012. In $AgTiO_2SiO_2$ (B), the spectra range of 1385 cm^{-1} revel the presence of $AgTiO_2$ (Prabu and Anbarsan, 2012). Further the peak at 1072cm^{-1}indicate the presence of

Si-O stretching (Emmanuel and John, 2016). The peak range of 580- 620 cm^{-1} corresponds to the presence of bismuth oxide doped TiO2 (Prabu and Anbarasan, 2012) Similarly in our study also the is the presence of peak range seen in BOT/37 (C) 526 cm^{-1}indicates Bismuth TiO_2

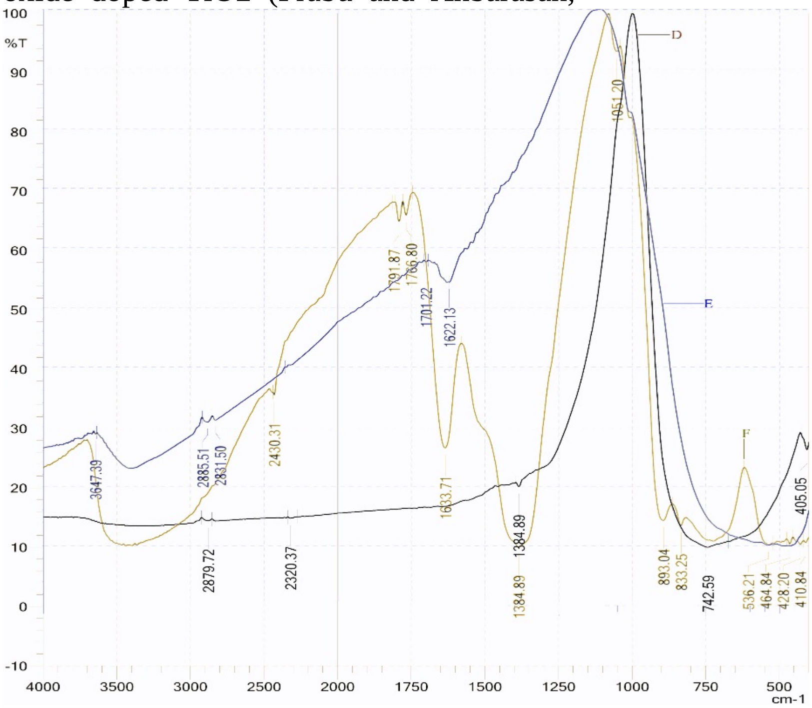

Fig 6 FT-IR spectra of nano particle BV (D), $AgTiO_2$ (E) and BVZ (F)

In Fig 6 the FTIR spectra of D, E and F were seen, In BV17 (D) the spectra range of, 405cm^{-1}indicates the presence of VO_4 vibration (Gotic et al., 2005). In $CPAgTiO_2$ (E), the spectra range of 1384 cm^{-1} indicates the presence of Ag doped TiO_2. In BVZ/33 (F) the spectra range of 1633 cm^{-1} indicates the presence of asymmetrical stretching of zinc carboxylate (Thangeeswari et al., 2016). And the spectra range of 1051, 893,833,536cm^{-1}indicates the presence of BiV (Gotic et al., 2005)

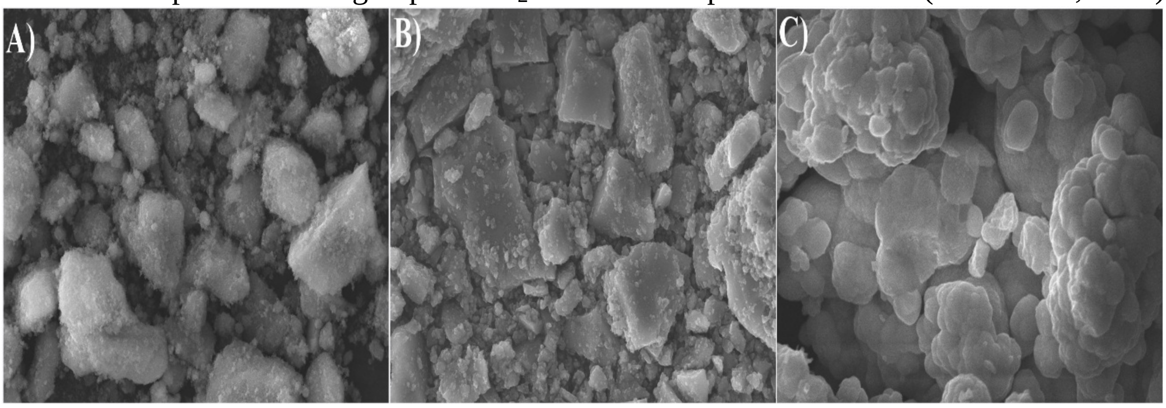

Fig 7 SEM image of TiO_2R (A), $AgTiO_2SiO_2$ (B) and BOT (C)nano particle

Fig 7 shows the SEM image of A, B and C. Degussa TiO_2 (A) nano particle are cuboid in nature which seen clearly under the magnification of 2 K at 10 µm in size (Parimaladevi et al.,2018). SiO_2@ silver doped TiO_2 (B) nano particle are square in nature which seen clearly under the magnification of 3 K at 3µm in size(Su and Andrea 2018). Bismuth oxide doped TiO_2 (C) nano particle has flower like structure in nature which seen clearly under the magnification of 10K at 1µm in size Ling et al., 2018.

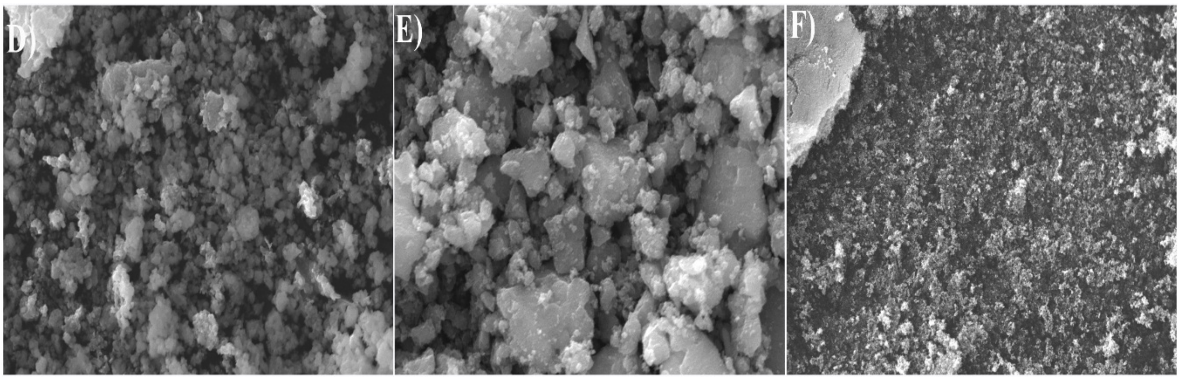

Fig 8 SEM image of BV (D), $AgTiO_2$ (E) and BVZ (F)nano particle

Fig 8 shows the SEM image of D, E and F. Undoped Bismuth Vanadate (D) nano particle are spherical in nature which seen clearly under the magnification of 10K at 1µm in size (Shanmugam et al., 2016). Silver doped TiO2 (E) nano particle are hexogonal in nature which seen clearly under the magnification of 10K at 1µm in sizeChunqiao et al., 2007. Bismuth vanadate doped Zinc oxide (F) nano particle are dispersive in nature which seen clearly under the magnification of 3K at 3µm in size.

Conclusion

During the treatment of infectious diseases many number of bacteriagot resistance against the antimicrobial agent. This could leads to serious problem. Therefore a novel antimicrobial substance could be developed against to prevent the multi resistance properties of bacterial strain. This study demonstrates the bactericidal effect of newly synthesis nanoparticle against gram+ve and gram-ve bacteria. Among those $AgTiO_2$ and $AgTiO_2SiO_2$ shows good antibacterial effect against Bacillus and E. coli by its capacity to interact with cellular metabolism and thereby causes death to the organism. Additionaly these two nano particle shows high photocatalytic activity due to the generation of free radicals.

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