Formulation, characterization and applications of titanium dioxide, polyoxometalates and silver nanocomposites

Hemant Kumar Daima[1,2*], Vinay Kumar P[2], Rashmi S[2], Sarwagya Raj Pandey[2], Navya PN[2], Devendra Jain[3], Sixberth Mlowe[4] and Neerish Revaprasadu[4]

[1]Amity Institute of Biotechnology, Amity University Rajasthan,
Kant Kalwar, NH-11C, Jaipur Delhi Highway, Jaipur-303007, Rajasthan, India,
hkdaima@jpr.amity.edu, hkdaima@gmail.com
[2]Nano-Bio Interfacial Research Laboratory (NBIRL), Department of Biotechnology,
Siddaganga Institute of Technology, Tumkur – 572103, Karnataka, India
[3]Department of Molecular Biology and Biotechnology,
Maharana Pratap University of Agriculture and Technology, Udaipur – 313001, Rajasthan, India
[4]Department of Chemistry, University of Zululand,
Private Bag X1001, Kwadlangezwa – 3886, South Africa

ABSTRACT

Titanium dioxide (TiO_2) possesses excellent photocatalytic activity; polyoxometalates (POMs) are keggin ions with exceptional biomedical potentials; and silver (Ag) is best known for its antimicrobial behaviour. In present work, we combine all these three materials in a single system at nanoscale level, which may provide better prospects towards industrial and biological contaminants. Composites of TiO_2 and two types of POMs (phosphotungstic acid / PTA or phosphomolybdic acid / PMA) were prepared separately followed by their functionalization with tyrosine reduced Ag nanoparticles. After in-depth physicochemical characterization using TEM, DLS and Zeta potential analyser, all these materials were subjected to *in-vitro* Congo red dye degradation and antibacterial studies against *Pseudomonas aeruginosa* and *Escherichia coli*. The composites have shown significant impact on dye degradation and antibacterial potentials.

Keywords: titanium dioxide, polyoxometalates, silver, dye degradation, antibacterial

1 INTRODUCTION

Nanotechnology is the most promising research field to generate new industrial and biological applications [1]. In current scenario, independently TiO_2, POMs and Ag are employed for various applications due to their well-known properties. It is believed that presence of TiO_2, POMs (PTA or PMA) and Ag in a single composite system at nanoscale level may provide better prospects for biomedical applications along with industrial or biological contaminants [2-13]. However, tri-nanocomposites of TiO_2, POMs and Ag have not yet be explored for their potential applications.

Therefore, in current research, we have strategically synthesized series of nanocomposite materials such as TiO_2-Ag, TiO_2-POM, TiO_2-POM-Ag, TiO_2-NH_2-Ag, TiO_2-NH_2-POM and TiO_2-NH_2-POM-Ag as shown in Figure 1. After formulations, all these composites were subjected to extensive characterization prior to their *in-vitro* dye degradation and biological applications. Dye degradation studies were performed on Congo red, whereas biological potential of fabricated nanocomposites were evaluated against medically important bacteria *P. aeruginosa* and *E. coli*.

2 EXPERIMENTAL SECTION

2.1 Reagents and Materials

Titanium dioxide, silver nitrate, phosphotungstic acid, phosphomolybdic acid, L-tyrosine, potassium hydroxide and Congo red were purchased from Sigma-Aldrich and used as received. Deionized MilliQ water employed to prepare various solutions, Ag nanoparticles and nanocomposites of TiO_2, PMOs and Ag. 12 kDa cellulose membrane dialysis tubing was also purchased from Sigma-Aldrich and used after boiling twice for 15 min in deionized MilliQ water followed by efficient washing with detergent.

2.2 Synthesis of amino acid-conjugated Ag nanoparticles

To produce amino acid-conjugated Ag nanoparticles, 100 mL aqueous solution with 1 mM KOH containing 0.1 mM tyrosine was allowed to boil under strong stirring experimental condition. In the vigorous stirring boiling solution, Ag^+ ions were added to obtain Ag nanoparticles. The manufactured nanoparticles solution was further allowed to stir vigorously overnight at room temperature. After the synthesis, the nanoparticles were dialyzed for removing unbound tyrosine molecules and unreduced ions. The dialysis was carried out in 12 kDa membrane placed with deionized MilliQ water for 3 hours and subsequent

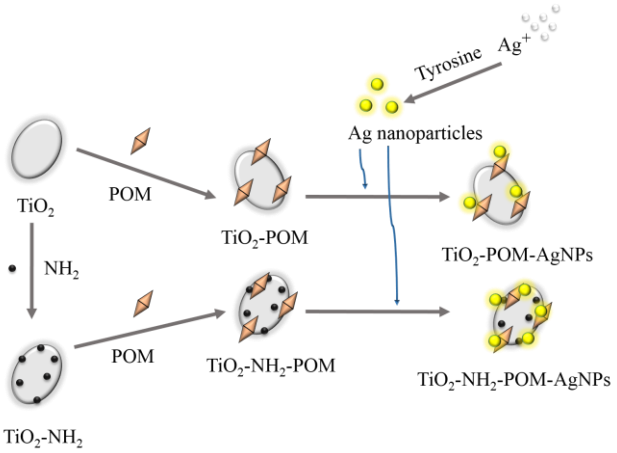

Figure 1: Schematic representation of synthesis of silver nanoparticles (AgNPs) by employing tyrosine amino acid and their functionalization on TiO_2-POM and TiO_2-NH_2-POM surfaces

change of deionized MilliQ water for overnight at room temperature under stirring condition.

2.3 Synthesis of TiO_2-POMs composites

Separately, in a typical experiment, 50 mg of anatase-TiO_2 powder was dispersed in 50 ml of 10^{-2} M PMA or 10^{-2} M PTA solutions and left overnight under mechanical stirring condition. These TiO_2-PMA or TiO_2-PTA solutions were centrifuged three times with deionized MilliQ water to remove unbound PMA or PTA molecules and dispersed in 25 ml of deionized MilliQ water.

2.4 Amine modification of TiO_2

Amine (-NH_2) functionalization was done to control the surface charge of TiO_2. In a distinctive experiment, 100 mg of anatase-TiO_2 powder was added to a solution containing 20 ml of ethanol, 1 ml of 28% ammonia and 4 ml of (3-Aminopropyl)triethoxysilane [APTES]. The suspension was allowed to blend overnight under mechanical stirring to avoid the settling of TiO_2 powder. The suspension was centrifuged, the supernatant was discarded and the pellet was washed three times with deionized MilliQ water. At the end, the amine modified TiO_2 was redispersed in 25 ml deionized MilliQ water.

2.5 Synthesis of TiO_2-NH_2-POM composites

In an experiment, 12.5 ml of TiO_2-NH_2 was added to 50 ml of 10^{-2} M PMA or 10^{-2} M PTA solutions which corresponds to 50 mg in its powder form. The solutions were left overnight under vigorous mechanical stirring. The solutions were centrifuged three times with deionized MilliQ water to facilitate the removal of unbound PMA or PTA molecules. At the end, both the composites were independently dispersed in 25 ml of deionized MilliQ water.

2.6 Synthesis of TiO_2-POM-AgNPs and TiO_2-NH_2-POM-AgNPs

In 5 ml of TiO_2-PTA, TiO_2-PMA, TiO_2-NH_2-PTA or TiO_2-NH_2-PMA solutions, 10 ml of Ag nanoparticles were added and incubated overnight in vigorous stirring settings. After overnight incubation, all the solutions were stored at room temperature for characterization and further investigations.

2.7 Photocatalytic degradation of an organic dye Congo red

To perform Congo red dye degradation, aqueous solution of Congo red dye (10 µM) was prepared using deionized MilliQ water. 10 ml of the Congo red dye solution was incubated with 12 mg equivalent of particles for 30 minutes under stimulated solar light. The stimulated solar light was generated by using 150W Xe arc lamp. The intensity of absorption spectrum at 500 nm was recorded to determine % dye degradation. Please note that after 30 minutes of irradiation, the samples were centrifuged to remove the composite material, and the remaining solutions were examined by UV-vis spectroscopy.

2.8 Antibacterial assays on human pathogenic strains

The antibacterial assays were done on human pathogenic *E. coli* (ATCC-25922) and *P. aeruginosa* (ATCC-7853) by standard disc diffusion method. Briefly Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (100 µl) of each culture were spread on to LB agar plates. Sterile paper discs of 5 mm diameter (containing composite materials) along with standard antibiotic containing discs were placed in each plate.

3 RESULTS AND DISCUSSION

In the current research work, we have demonstrated a generalized approach towards creating a class of tri-composites containing TiO_2, POMs (PMA or PTA) and Ag. In drive to synthesize nanocomposites which could provide a synergy of individual properties of selected materials, the tri-conjugates were prepared on a TiO_2 microparticle as demonstrated in Figure 1. TiO_2 microparticles were chosen as core material because major focus of the research was to utilize engineered composites toward the treatment of industrial or biological contaminants; wherein TiO_2 can provide a platform for POMs and Ag nanoparticles functionalization, and it has very prominent photocatalytic potential, which may be further explored for application point of view. Moreover, it was important to use synthesis methods that didn't involve any chemical reducing/stabilizing agents, organic solvents or other chemical substances so that less chemically hazardous

materials may provide direct opportunity to employ such designer made materials for various biological applications including waste water treatment by synergistic manner.

First of all, Ag nanoparticles were synthesised by a protocol developed by us, wherein tyrosine amino acids was employed as reducing agents for Ag^+ ions. Tyrosine reduced Ag^+ ions to Ag nanoparticles due to the presence of phenol group [2-4, 14]. In another part of synthesis, TiO_2 was employed and surface functionalized with POMs (PTA/PMA) or $–NH_2$ as displayed in Figure 1 [15, 16]. NH_2 was employed for surface modification to alter the surface charge of TiO_2 microparticle, whereas parallel to this separately PTA or PMA molecules were also directly incubated with TiO_2 as discussed in materials and method section to acquire TiO_2-PTA or TiO_2-PMA. Furthermore, alternation in the surface charge of TiO_2 after their modification with $-NH_2$ molecules was confirmed by zeta potential measurements as it became +12.0 mV for TiO_2-NH_2 from -22.1 mV of TiO_2 as shown in table 1, illustrating strong binding between negatively charged microparticles of TiO_2 and $–NH_2$ groups. TiO_2-NH_2 particles were also incubated with PTA or PMA molecules as discussed earlier to develop TiO_2-NH_2-PTA or TiO_2-NH_2 -PMA.

All the composites were then capped with tyrosine reduced Ag nanoparticles. It was interesting to observe that while modifying the TiO_2 surface with amine functional groups increased the number and size of Ag nanoparticles on the surface of the TiO_2 based composites, largely due to a higher affinity for the POM molecules due to a more positive surface charge of TiO_2-NH_2. Notably, the photocatalytic activity of the metal nanoparticle decorated nanocomposites studies seemed to increase with increasing nobility of the metal employed, this has significant ramifications as the choice of an appropriate metal-decorated TiO_2-POM photococatalytic system can be particularly important for industrial photocatalysis applications considering the significant difference in the cost of different noble metals.

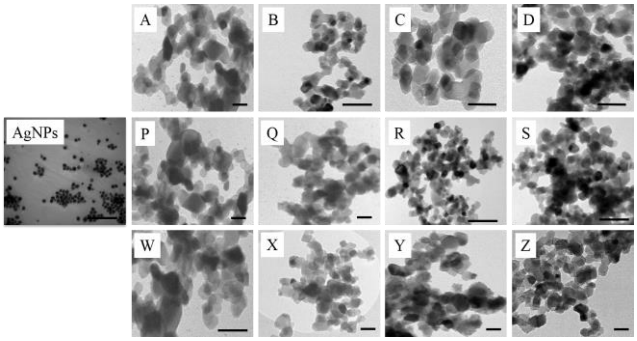

Figure 2: Representative TEM images of fabricated multifunctional TiO_2-POM-Ag nanocomposites. Where: [A] TiO_2, [B] TiO_2-NH_2, [C] TiO_2-AgNP, [D] TiO_2-NH_2-AgNP, [P] TiO_2-PTA, [Q] TiO_2-NH_2-PTA, [R] TiO_2-PTA-AgNP, [S] TiO_2-NH_2-PMA-AgNP, [W] TiO_2-PMA, [X] TiO_2-NH_2-PMA, [Y] TiO_2-PMA-AgNP, [Z] TiO_2-NH_2-PMA-AgNP (Scale Bar: 50 nm)

Sample name	Average size (nm)	ζ potential (milliVolts)
TiO_2	2317	-22.7
TiO_2-NH_2	4121	12.0
TiO_2-PTA	253.2	-30.2
TiO_2-PMA	241.4	-25.0
TiO_2-NH_2-PTA	309.4	-24.3
TiO_2-NH_2-PMA	262.9	-32.6
TiO_2-AgNPs	1603.0	-17.0
TiO_2-NH_2-AgNPs	3066.0	-9.12
TiO_2-PTA-AgNPs	331.6	-23.9
TiO_2-PMA-AgNPs	351.0	-20.4
TiO_2-NH_2-PTA-AgNPs	280.7	-28.4
TiO_2-NH_2-PMA-AgNPs	299.0	-23.8

Table 1: Average size and surface charge of composites measured by DLS and Zeta potential analyser

Figure 2 shows representative TEM micrographs of Ag nanoparticles and fabricated multifunctional composites. Ag nanoparticles are spherical, whereas all the synthesised composites are quasi-spherical in shape. It is interesting to observe that after functionalization with Ag nanoparticles, the decoration of Ag nanoparticles was observed on TiO_2 surface for instance image Y and Z, in Figure 2. Average diameter of these composites particles varied significantly based on the surface modification and it has been illustrated in table 1 along with zeta (ζ) potential values. The characterization of the composite using DLS showed average diameter of TiO_2 and TiO_2-NH_2 complexes were found to be around 247nm and 285nm after their surface modification with PTA or PMA molecules. Average diameter of these materials increased after their functionalization of Ag nanoparticles, indicating decoration of TiO_2–POMs and TiO_2-NH_2–POMs with Ag nanoparticles. Zeta potential showed that POM and Ag nanoparticles are effectively functionalizing on TiO_2 surface as the surface charge varied based on the surface modification as expected and shown in table 1.

After in-depth physicochemical characterization, all these materials were subjected to *in-vitro* dye degradation using Congo red and antibacterial studies against medically important bacterial strains *P. aeruginosa* and *E. coli*. *In-vitro* Congo red dye degradation experiments were performed under stimulated solar light as discussed. After 30 minutes of irradiation, the samples were centrifuged to remove composite materials, and the remaining solutions were studied by UV-vis spectroscopy at 500 nm. As illustrated in table 2, TiO_2 and TiO_2-NH_2 showed 18.7% and 20.3% Congo red dye degradation, respectively. This potential of TiO_2 increased sequentially based on the functionalization and reached to the highest level of degradation 68.5% for tri-composite TiO_2-NH_2-PTA-AgNPs. Furthermore, it was interesting to observe that in case of PTA modification, with respect to PMA, the

Sample name	% Degradation
TiO_2	18.72
TiO_2-NH_2	20.37
TiO_2-PTA	25.61
TiO_2-PMA	19.85
TiO_2-NH_2-PTA	29.58
TiO_2-NH_2-PMA	25.24
TiO_2-AgNPs	44.19
TiO_2-NH_2-AgNPs	45.31
TiO_2-PTA-AgNPs	58.80
TiO_2-PMA-AgNPs	43.82
TiO_2-NH_2-PTA-AgNPs	68.46
TiO_2-NH_2-PMA-AgNPs	48.31

Table 2: *In-vitro* photocatalytic degradation of Congo red

composite materials showed enhanced % dye degradation (Table 2), which may be contributed due to the inherent nature of PTA.

Furthermore, antibacterial activities of these materials have also been explored against medically important bacterial strains *P. aeruginosa* and *E. coli*. All these materials have shown different level of antibacterial activities against tested bacteria at very low concentation by showing clear inhibition zone like standard antibiotics kanamycin and ampicillin, which suggest potential of these materials to be used as antimicrobial agent.

4 CONCLUSIONS

The research work presented here is mainly aimed at understanding the correlation between TiO_2, POMs and tyrosine reduced Ag nanoparticles to employ these tri-composites for *in-vitro* dye degradation and antibacterial activities. The POMs are capable of adhering to the surface of TiO_2 and at the same time capable of allowing other molecules like Ag nanoparticles to adhere to its surface due to charge switching property of amino acids present on Ag nanoparticles. These designer made composite materials have potential to work in synergistic manner and presence of TiO_2, POMs and Ag in a single composite system at nanoscale level may provide better prospects for biomedical applications along with industrial/biological contaminants.

5 ACKNOWLEDGEMENTS

VKP, SRP, RS and HKD gratefully acknowledge Karnataka State Council for Science and Technology (KSCST) for 38th Series: Year 2014-15 SPP award (7.1.03/SPP/1018). Authors also acknowledge support of Malvern Instruments Aimil Ltd, Bangalore for Zeta potential and DLS studies.

REFERENCES

1. Daima, H.K. and P.N. Navya, *Rational engineering of physicochemical properties of nanomaterials for biomedical applications with nanotoxicological perspectives.* Nano Convergence, 2016. **3**(1): p. 1-14.
2. H.K. Daima, P.S., S.K. Bhargava, S.K. Shastry, V. Bansal. *Amino acids-conjugated gold, silver and their alloy nanoparticles: Role of surface chemistry and metal composition on peroxidase like activity*. in *Technical Proceedings of Nanotech 2014 TechConnect World Conference and Expo* 2014. Washington, USA: NSTI
3. Daima, H.K., et al., *Synergistic influence of polyoxometalate surface corona towards enhancing the antibacterial performance of tyrosine-capped Ag nanoparticles.* Nanoscale, 2014. **6**(2): p. 758-765.
4. Daima, H.K., et al. *Tyrosine mediated gold, silver and their alloy nanoparticles synthesis: Antibacterial activity toward gram positive and gram negative bacterial strains*. in *2011 International Conference on Nanoscience, Technology and Societal Implications, NSTSI11*. 2011.
5. Jain, D., et al., *Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti microbial activities.* Digest Journal of Nanomaterials and Biostructures, 2009. **4**(3): p. 557-563.
6. Hasenknopf, B., *Polyoxometalates: Introduction to a class of inorganic compounds and their biomedical applications.* Frontiers in Bioscience, 2005. **10**(1): p. 275-287.
7. Casan-Pastor, N. and P. Gomez-Romero, *Polyoxometalates: From inorganic chemistry to materials science.* Frontiers in Bioscience, 2004. **9**: p. 1759-1770.
8. Rhule, J.T., et al., *Polyoxometalates in medicine.* Chemical Reviews, 1998. **98**(1): p. 327-357.
9. Yamase, T., H. Fujita, and K. Fukushima, *Medical chemistry of polyoxometalates. Part 1. Potent antitumor activity of polyoxomolybdates on animal transplantable tumors and human cancer xenograft.* Inorganica Chimica Acta, 1988. **151**(1): p. 15-18.
10. Kubacka, A., et al., *Understanding the antimicrobial mechanism of TiO2-based nanocomposite films in a pathogenic bacterium.* Scientific reports, 2014. **4**.
11. Chung, C.J., et al., *An antimicrobial TiO2 coating for reducing hospital-acquired infection.* Journal of biomedical materials research Part B: Applied biomaterials, 2008. **85**(1): p. 220-224.
12. Fu, G., P.S. Vary, and C.T. Lin, *Anatase TiO2 nanocomposites for antimicrobial coatings.* Journal of Physical Chemistry B, 2005. **109**(18): p. 8889-8898.
13. Linsebigler, A.L., G. Lu, and J.T. Yates Jr, *Photocatalysis on TiO2 surfaces: Principles, mechanisms, and selected results.* Chemical Reviews, 1995. **95**(3): p. 735-758.
14. Dubey, K., et al., *Tyrosine- and tryptophan-coated gold nanoparticles inhibit amyloid aggregation of insulin.* Amino Acids, 2015: p. 1-10.
15. Pearson, A., et al., *Gold nanoparticle-decorated keggin ions/TiO2 photococatalyst for improved solar light photocatalysis.* Langmuir, 2011. **27**(11): p. 6661-6667.
16. Pearson, A., S.K. Bhargava, and V. Bansal, *UV-switchable polyoxometalate sandwiched between TiO2 and metal nanoparticles for enhanced visible and solar light photococatalysis.* Langmuir, 2011. **27**(15): p. 9245-9252.