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Biological of TiO_2 and study its biological property

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Introduction

The word Nanotechnology can be defined as the idea that covers the design, construction, and utilization of the functional structures with at least one characteristic dimension measured in nanometers (Kelsall*et al*., 2005). These kinds of materials are designed to carry out the novel and significant chemical, physical and biological activity and processes due to the limited size of their constituent particles (Kelsall*et al*., 2005). Mostly the metal nanoparticles are synthesized by all three physical, chemical, and biological methods with some modifications for the different metal sources (Soomro*et al*., 2014 and Salam *et al*., 2012).

Applications and properties of NPs are different and dependent on their morphology, size, and distribution (Perez *et al*., 2005) and also on their mode of synthesis. In recent times, Titanium dioxide has confirmed to be I of the hopeful nn-type semiconductors because of its wide bandgap of 3.2 eV under UV light exposure (Zaleska, 2008). In addition to this, it has high corporeal and living stability as fine as high refractile catalogue types it a solid to be highly explored (Xie*et al*., 2012). It can be used in several fields including photocatalyst, solar cells, sensors, self-cleaning and bactericidal actions due to its optical and electronic properties (Gupta *et al*., 2008). Titanium dioxide being an n-type semiconductor shows a good photocatalytic and photoconductive activity (Lv*et al*., 2011).

In recent years several other applications of it have been studied like they are secondhand as a photocatalyst, solar cells, in preparation of UV-shielding materials and electric devices (Lee *et al*., 2009). Much important application of TiO_2has been reported in the field of environment clean up (Sakai *et al*., 1998).Numerous studies have reported the belongings of titanium dioxide besides its use for the degradationof substances in an aqueous solution and the reduction of inorganic ions (Motta *et al*., 2013) and TiO2 has been considered the maximum widely secondhand oxide in photocatalysis. The unique surface chemistry and smaller size act as two major characteristic features of these nanostructures to be exploited well in the field of medicine, nutrition, and energy (Chandran*et al*., 2006). Due to its unique surface chemistry, titanium dioxide nanoparticles are used in the union of tints, textiles, papers, plastic, and cosmetics (Muhd*et al*., 2014). Filth of toxic elements from water is achieved by the application of a colloidal solution of TiO_2NPs (Pirkanniemi*et al*., 2002).

Material and Methodology

Fusion of Titanium Nanoparticles

Titanium dioxide nanoparticles was done by three different methods, that is green synthesis using *Curcuma longa* (Turmeric), biological synthesis using *Bacillus subtilis*, and chemical synthesis using Titanium isopropoxide.

Green Synthesis

For the green synthesis of nanoparticle, 10gm of fine grounded powder of C. longa was extracted with 200 ml of distilled water by the soxhlet extraction method at 40ºC for 3-4 hours. The obtained extract was filtered through Whatman no. 1 filter and the filtrate was used for synthesis as soon as it was obtained. For synthesis 50 ml of the filtrate was mixed with 2.5ml of Titanium dioxide bulk particle (50mg/ml) and the mixture was placed on a magnetic hot plate stirrer at 50ºC and 1000rpm for 5 hours. After this solution was permissible to unruffled at room infection and then centrifugated at 1500rpm for 10 minutes. The pellet obtained on centrifugation is the synthesized titanium dioxide nanoparticle that was dried at RT for 24 hours.

Biological Synthesis

For the biological synthesis, the suspension culture of Bacillus subtilis was grown in 100ml of sterile nutrient broth medium for 36 hours, and this culture was treated as the source culture. From the source culture working culture was prepared by diluting 25ml of culture with 75 ml of sterile nutrient broth and this culture was allowed to grow for 24 hours. After incubation, 20 ml of 0.0025 M titanium tetraisopropoxide was mixed with this culture and kept in a water bath at 60ºC for 20 minutes. The appearance of the white deposition at the bottommost of the flask indicates the initiation of transformation and now the culture was incubated at RT. After 12-18 hours of incubation, a remarkable coalescent white cluster deposit was observed that confirms the synthesized TiO_2NPs, the culture was decanted and the deposit was air-dried.

Chemical Synthesis

For the chemical synthesis, 5ml of titanium tetrachloride was added in 50ml of ethanol in a glass besides it was stirred for 30minutes at RT, and it turned to form a yellow sol phase. To this mixture 200ml of refined water

was added and this solution was again stirred at RT for 30 minutes and this led to the development of a gel which stood dried at 50ºC for 24 hours to obtain the final titanium dioxide nanoparticle.

Isolation of the Pathogenic bacteria

Pathogenic bacterial species were isolated on the selective media using the sewage water as inoculum. For isolation of *Pseudomonas aerugenosa*Cetrimide agar media and for *Vibrio cholerae* and *Vibrio parahaemolyticus* TCBS agar media was used. The media was prepared, sterilized and then was inoculated with the inoculum by spread plate technique followed by incubation at 37ºC for 24 hours. The isolated colonies of these pathogenic bacterial species were obtained in pure culture form.

Characterization of bacteria

The pathogenic bacterial species were characterized by certain biochemical tests selected according to Bergey's manual. The Biochemical test was performed with the specific media composition and the test reagent required for the detection based on the standard protocol. These tests were performed for all three organisms and the test includes, Indole test, MR-VP test, Catalase test, and Citrate utilization test.

Analysis of Antimicrobial activity

The antimicrobic motion of all trio made titanium dioxide nanoparticles counter to the isolated morbific type was ended by Agar glowing dissemination method. Nanoparticles were dissolved in DMSO as 20mg/ml concentration. The bacterial culture (24h) was inoculated on the sterile Muller Hinton agar plates and after 10 minutes wells were punctured. Three wells each for the different NPs and two for negative (DMSO) and positive (Ciprofloxacin) control. The plates after sample loading were kept stable for 30 minutes to allow the samples to diffuse properly and then incubated at 37ºC for 24hrs. Next day the plates were observed and the ZoI(ZOI) was measured in mm.

Result and Discussion

The pathogenic bacterial species were isolated on their selective media, *V. cholerae* and *V. parahaemolyticus* gave yellow and bluish green colonies respectively on the TCBS gar media while *P. aerugenosa* gave distinctive white-colored circular colonies on the Cetrimide agar media. These isolated pathogenic bacterial species were characterized biochemically by the IMViC test and the results obtained for the same are depicted in table no.1. These isolated were cultured in the liquid media for the antibacterial assay.

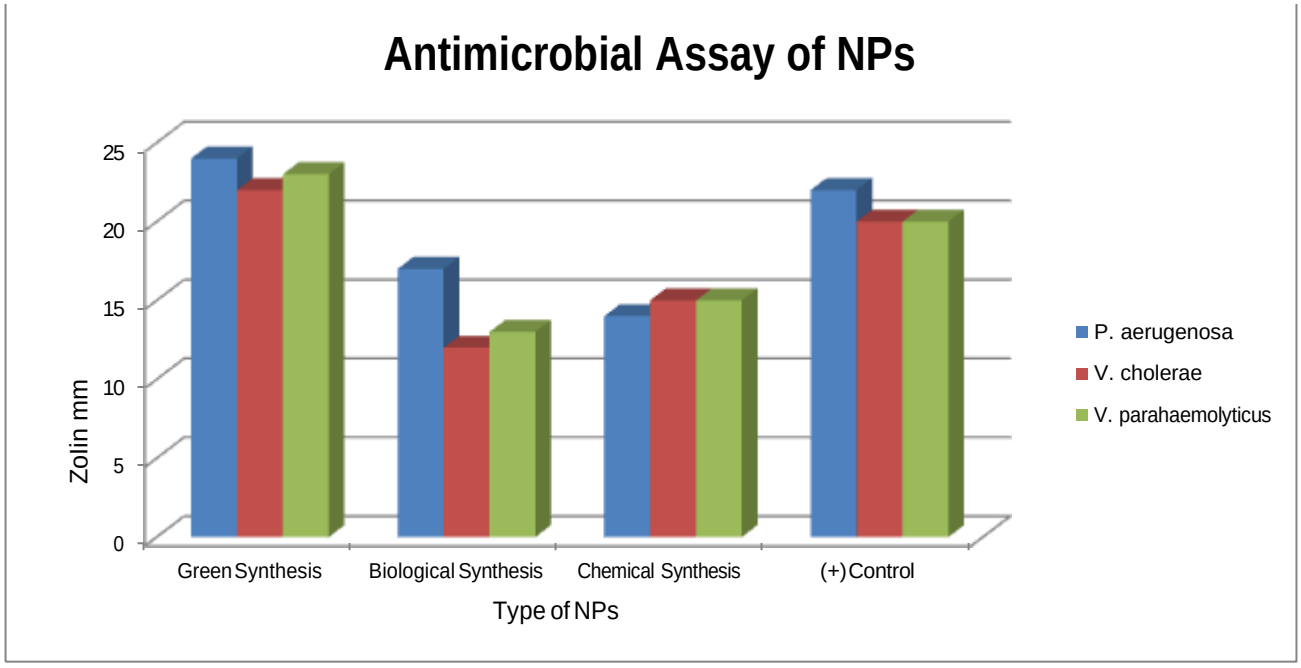
All the synthesized nanoparticles were obtained in the powder form at the end of their synthesis process and they were stored at 4ºC. For the antibacterial analysis solution of NPs with a concentration of 20mg/ml was prepared in DMSO. The antibacterial analysis of all three NPs revealed their potential to act as a potent antibacterial agent as all the NPS exhibited moral inhibition activity against all three pathogenic species undertaken in the studies. Comparatively the Tio_2 NPs obtained from green synthesis using *Curcuma longa* were the best at the inhibition property. The results for the antibacterial assay are summarized in table no. 2 and graph no. 1.

Tabel no. 1: showing the results for the biochemical test of the isolated pathogenic bacterial spp.

S. No.	Biochemical Test	Results		
		P. aerugenosa	***V. cholerae***	***V. parahaemolyticus***
1	Indole test	-	+	+
2	Methyl red test	-	-	-
3	Voges-Proskauer test	-	-	-
4	Citrate utilization test	+	+	+
5	Catalase test	+	+	+

Tabel no. 1: showing the results for antimicrobial assay of Titanium dioxide NPs against the isolated pathogenic bacterial spp.

S. No.	Type of TiO_2 NPs	ZoI(mm)		
		P. aerugenosa	***V. cholerae***	***V. parahaemolyticus***
1	Green Synthesis	24	22	23
2	Biological Synthesis	17	12	13
3	Chemical Synthesis	14	15	15
4	(+) Control	22	20	20



Graph no. 1: showing the results for antimicrobial assay of TiO_2 NPs against pathogenic bacterial spp.

In the current study, three different sources including plants, microorganisms, and chemicals were used intended for the fusion of titanium nanoparticles. All three sources have their importance and characteristic properties. The use of plants for synthesis comes with certain advantages like they are easily available, posses wide variability of metabolites or the phytochemical compounds, and are safe to handle and process. There are several plants under study to determine their character for the nanoparticle synthesis (Torresday*et al.*, 2002).

Conclusion

In conclusion, the present three different biotechnological methods are capable of producing TiO2 nanoparticles with significant antimicrobial activity. We have used an efficient and eco-friendly approach for the rapid TiO2 nanoparticles. The obtained nanoparticles have shown good antimicrobial activity against the pathogenic bacterial species hence makes it a possible and potent source to be used in the pharma and cosmetic industries aimed at the training of antibacterial gels and ointments. These nanoparticles can be further used for the coating purposes in medical devices (e.g. catheters) to control the concerned bacterial infections.

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