Biological, Chemical and Green synthesis of TiO_2 NPs and study its biological property

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Abstract

Nanotechnology is a new star in the science horizon with many valuable applications and promisesto offer. It includes the synthesis and utilization of nanostructure materials ranging from 1 to 100 nm. Nano-sized materials have been an important tool in basic and applied sciences. Titanium oxide (TiO2) nanoparticles have been mostly exploited for their photocatalytic, antimicrobial, and antiparasitic applications. A diverse set of biological entities is used to reduce the precursor metal salt into respective nanoparticles. In the present study, titanium dioxide nanoparticles were synthesized by using three different methods that are chemically by titanium tetrachloride, green synthesis by *Curcuma longa* extract, and biological synthesis by *Bacillus subtilis.* The titanium dioxide nanoparticle obtained by all three methods was analyzed for its antibacterial potential against the pathogenic bacterial species that is *P. aerugenosa, V. cholerae,* and *V. parahaemolyticus.* The results revealed the potential of titanium dioxide nanoparticles, obtained from all three methods, as a good antibacterial agent as it showed good efficacy against all three pathogenic spp. In the conclusion this study derives some potent and easy methods for obtaining the Titanium dioxide (TiO_2) nanoparticles that also possess good antimicrobial property hence finds its application in pharma and cosmetic industries apart from its other potential uses.

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 proven to be one of the promising 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 physical and chemical stability as well as high refractive index makes it a material to be highly researched (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 used 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 properties of titanium dioxide and 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 most widely used 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 synthesis of tints, textiles, papers, plastic, and cosmetics (Muhd*et al*., 2014). Degradation of toxic chemicals from water is achieved by the application of a colloidal solution of TiO_2NPs (Pirkanniemi*et al*., 2002).

Material and Methodology

Synthesis of Titanium dioxide Nanoparticles

Synthesis of the 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 allowed to cool at room temperature and then centrifuged 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 bottom 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 beaker and it was stirred for 30minutes at RT, and it turned to form a yellow sol phase. To this mixture 200ml of distilled water was added and this solution was again stirred at RT for 30 minutes and this led to the formation of a gel which was 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 antimicrobial activity of all three synthesized titanium dioxide nanoparticles against the isolated pathogenic species was done by Agar well diffusion 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 Zone of inhibition (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.

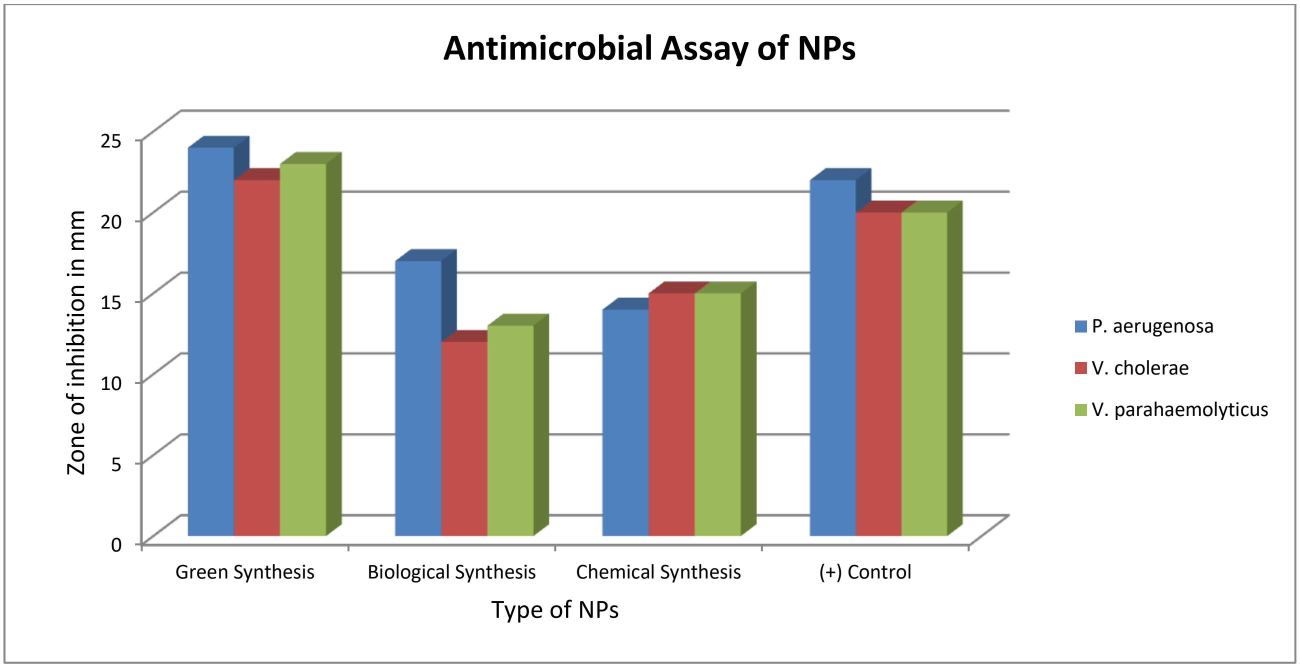
All the synthesized nanoparticles were obtained in the powder form at the end of their synthesis process and they were stored at 4ºC. For the antibacterial analysis solution of NPs with a concentration of 20mg/ml was prepared in DMSO. The antibacterial analysis of all three NPs revealed their potential to act as a potent antibacterial agent as all the NPS showed good 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	Zone of inhibition (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 for the synthesis of titanium dioxide 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 role for the nanoparticle synthesis (Torresday*et al*., 2002). Studies have reported that the plant extracts are known to act as reducing and stabilizing agents for nanoparticle synthesis (Mukunthan and Balaji, 2012). The kind of nanoparticles synthesized is affected by the nature of plant used and most importantly the source of plant extract and variability in the concentration of biochemical reducing agents affects the morphology of synthesized nanoparticles (Li *et al*., 2011). In the current study, Titanium dioxide nanoparticles were synthesized successfully by extract of *Curcuma longa*and its ability to do so comes from the presence of bioactive compounds like terpenoids, flavonoids, and proteins as they are considered to be responsible for the formation and stabilization TiO_2Nanoparticles (Krishnasamyet*et al*., 2015). The concentration of *Curcuma longa* extract may be responsible for the size of synthesized NPs as reported in a study (Jalill*et al*., 2016). Synthesis of metallic NPs has been achieved by many microbial species (Nadeem *et al*., 2017).

TiO_2NPs have been synthesized in various shapes and sizes using the microbial source as the bacterial extracts containing their metabolites play a crucial role in the bioreduction and stabilization of NPs. Synthesis of Titanium dioxide NPs of spherical shape with 40-60nm size have been reported using *Bacillus mycoides*(Ordenes, *et al*., 2014) and in the present study other *Bacillus spp*. was used. Titanium dioxide NPs are known to possess the eco-friendly biocidal properties and this attribute of there is due to the strong oxidizing property they carry. These NPs have been assessed against a wide range of infectious microbes that includes various bacterial strains, endospores, fungi, algae, microbial toxins, and prions (Visai*et al*., 2011). When confronted with the microbial cells the TiO_2triggers the onset of reactive oxygen species (Jayaseelan*et al*., 2013) and the ROS generated kills the microbes by disruption of their cell wall mainly by phospholipid oxidation that leads to reduced adherence and imbalance of ionic exchange across the membrane**.** Inside the cytosol, it inhibits the respiratorycytosolic enzymes and modifying macromolecules structures,producing substantial effects on cellular integrityand gene expression (Kubacka*et al*., 2014). The same fashion of killing is attributed by both green and chemically derived TiO_2 but the biologically synthesized one shows better antibacterial activity (Kumar *et al*., 2014). As the present study has also shown that all three differently synthesized NPs have shown good antibacterial activity against the pathogenic bacterial species.

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 synthesis of 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 for the preparation 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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