



Research Article

Evaluation of In-vitro Biocompatibility and Antimicrobial activities of Titanium Dioxide (TiO₂) Nanoparticles by Hydrothermal Method

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Abstract

Titanium dioxide (TiO₂) nanoparticles (NPs) are one of the nanomaterials that have been widely used in cosmetics, pharmaceuticals and biomedical applications which include drug delivery, cancer treatment, biosensors, and genetic engineering. In this study, TiO₂ NPs have been synthesized via hydrothermal method. The samples were calcinated at different temperatures such as 450 and 500 °C. The structural, functional group, morphological and elemental composition analyses of the synthesized materials were conducted by various techniques such as X-ray diffraction (XRD), Fourier transform infrared (FTIR) analysis, Raman spectroscopy, field emission scanning electron microscopy (FESEM) and elemental dispersive X-ray (EDX) analysis. Various concentrations (25, 50, 75 and 100 µg/mL) of synthesized TiO₂ NPs were evaluated for their antimicrobial activity against the gram-positive and gram-negative bacteria. The maximum zone of inhibition was observed in the synthesized TiO₂ NPs (100 µg/mL) against *Staphylococcus aureus* (15 mm), *Bacillus Subtilis* (16 mm), *Escherichia coli* (18 mm) and *Pseudomonas aeruginosa* (17 mm), and the results indicated that the antimicrobial activities of the TiO₂ NPs were tested against pathogenic bacteria. Overall, the results indicated that TiO₂ nanoparticles could elicit the viability of lymphocytes at all investigated concentrations (50, 100, 250 and 500 µg/mL). Hence, we concluded that the in-vitro hemolytic and antibacterial activities of TiO₂ NPs, indicated the good potential biomedical applications.

Keywords: Titanium dioxide nanoparticles, Hydrothermal method, Antibacterial activity, Agar well diffusion method, Hemolytic activity

Introduction

Nanotechnology is the control and designing of new materials at the nanoscale level with a length of at least one dimension <100 nm [1]. With the improvement and enlarging research interest of nanotechnology in nanoparticles and nanomedicine have prompted a huge potential for novel methods for rapid disease diagnosis, treatment and enhanced

quality of life [2]. Nanoparticles have possessed unique size-dependent physical (optical, catalytic), chemical and electrochemical properties, which give helpful attributes for biomedical applications for faster and more reliable methods to assess their safety [3, 4]. It can be classified into carbon based materials (e.g. fullerenes, carbon nanotubes), inorganic NPs such as metal (e.g. silver, palladium and gold), and metal oxides (e.g. aluminum oxide, titanium dioxide, zinc

oxide, copper oxide, silicon oxide) and quantum dots [5].

Titanium dioxide, also known as titania (TiO_2) is a very useful semiconducting transition metal oxide nanoparticle [6]. TiO_2 -NPs account for over 70% of the total production volume of nanoparticles around the world [7]. TiO_2 is the promising material as semiconductor and exhibits unique characteristics such as easy handling, non-toxicity and low cost [8]. It has four different crystalline polymorphs, namely rutile (tetragonal, $a = b = 4.59 \text{ \AA}$, $c = 2.95 \text{ \AA}$), anatase (tetragonal, $a = b = 3.78 \text{ \AA}$, $c = 9.50 \text{ \AA}$), brookite (rhombohedral, $a = 5.43 \text{ \AA}$, $b = 9.16 \text{ \AA}$, $c = 5.13 \text{ \AA}$) and TiO_2 (B) [monoclinic, $a = 12.16 \text{ \AA}$, $b = 3.74 \text{ \AA}$, $c = 6.51 \text{ \AA}$] [9] and its band gap values of anatase (3.2 eV), rutile (3.02 eV), and brookite (2.96 eV) phases, respectively [10].

TiO_2 nanoparticles is a well-researched material due to excellent dielectric, gas-sensitive, physical, optical, electrical properties, high chemical stability, biocompatibility with great optical transparency, refractive index and wide band gap energy [11]. Titanium dioxide nanoparticles (TiO_2 NPs) are used for wide range of applications, such as catalysis, white pigment for paints, cosmetics, food colorants, pharmaceuticals and biomedical fields [12]. Its photocatalytic properties have been utilized in various environmental applications such as air purification and waste water treatment [13]. Recently, TiO_2 nanostructures with various morphologies prepared by various methods, such as, nanocrystals, nanorods, nanowires, nanotubes and mesoporous structures [14].

A significant area of research in nanotechnology deals with the synthesis of titanium dioxide nanoparticles (TiO_2 NPs) of different chemical compositions, dimensions and controlled monodispersity [15]. The preparation of ultrafine titanium dioxide nanoparticles has been investigated using various methods such as hydrothermal, solvothermal, chemical co-precipitation, chemical vapour deposition (CVD), sol-gel technique, sputtering, hydrolysis and micro emulsion method [16].

Hydrothermal method is one of the most common and effective synthetic routes to fabricate the TiO_2 NPs due to its simplicity and high yield [17]. In this method is based on the ability of water and aqueous solutions to dilute at high temperature (500 °C) and pressure (10-80 MPa, sometimes up to 300 MPa) [18]. Advantages of the hydrothermal synthetic route

include high purity, ease of large scale production, high-crystallized powders with narrow grain size-distribution, environmental friendliness and less hazardous [19]. Furthermore, the disadvantages of hydrothermal methods include high cost of equipment and long synthesis duration [20].

In spite of the antimicrobial activity of TiO_2 nanoparticles was demonstrated by the present concern for the experts due to the development of microbial resistance against metal ions, antibiotics and enhancement of resistant [21]. The Quantification of hemolytic activities of a nanoparticle is considered as one of the most fundamental tests in determining the safety of a blood contacting biomaterial. In this method, in-vitro studies of particle induced hemolysis evaluate the percentage of hemolysis by spectrophotometrically method to detecting plasma-free hemoglobin derivatives after incubating the particles with blood and then separating undamaged cells by centrifugation [22].

In the present work, we have synthesized TiO_2 nanoparticles via hydrothermal method and characterized by Powder X-ray Diffraction, Raman Spectroscopy, Fourier Transform Infrared Spectroscopy, Field Emission Scanning Electron Microscope, Energy Dispersive Spectroscopy, UV-visible spectroscopy and performing the antimicrobial and hemolytic activity of titanium dioxide nanoparticles [23].

Experimental

Materials

Different chemicals and reagents used during this experiment including titanium tetra isopropoxide [$\text{Ti}(\text{OCH}(\text{CH}_3)_2)_4$], isopropanol ($\text{CH}_3\text{CHOHCH}_3$), ethanol ($\text{C}_2\text{H}_5\text{OH}$), dimethyl sulfoxide (DMSO) ($\text{CH}_3)_2\text{SO}$, Muller Hinton agar were purchased from Sigma Aldrich Pvt Ltd., India. All glassware was washed with sterile distilled water and dried in hot air oven before use. The distilled water was used as solvent to prepare the solutions.

Collection of bacterial pathogens

Gram positive bacteria such as *Bacillus subtilis* (MTCC 1305) and *Staphylococcus aureus* (MTCC-3160), Gram negative bacteria (*Escherichia coli* (MTCC-1677) and *Pseudomonas aeruginosa* (MTCC-4030) were procured from Microbial Type Culture

Collection (MTCC), India. All the microbial cultures were stored and maintained at 4 °C.

Synthesis of titanium dioxide nanoparticles

Titanium dioxide nanoparticles were prepared via hydrothermal method using the titanium tetra isopropoxide, isopropanol and distilled water as the starting materials. Titanium tetra isopropoxide (TTIP) and isopropanol at 1:4 molar ratio was added to 10 mL distilled water to get white color solution formed, then stirred for 1 h. Clear solution formed after that, the solution was transferred into a Teflon vessel and placed in hot air oven and set temperature at 180 °C for 12 h. After 12 h, the autoclave was cooled down to room temperature, then removed the supernatant, washed the precipitates several times with distilled water and ethanol. TiO₂ nanoparticles were dried at 80 °C at 4 h. Finally, the powder was annealed at different temperature for 450 and 500 °C for 4 h.

Characterization of TiO₂ nanoparticles

The XRD pattern of TiO₂ nanoparticles was obtained by an X-ray diffractometer (XRD, Rigaku Ultima IV) with CuK β radiation at 25 °C and the structural assignments were made with reference to the JCPDS powder diffraction files. Functional group analysis of the compound was examined by using Fourier Transform infrared spectroscopy (BRUKER, TENSOR 27, INDIA). Chemical structure of the material was analyzed by Raman Spectroscopy (HORIBA, LabRAM HR). The surface morphologies of TiO₂ samples were studied using FE-SEM FEI Quanta 250. Chemical composition of the sample was analysis using energy dispersive spectrum (BRUKER INDIA). UV-visible absorption spectra of TiO₂ nanoparticles were recorded using a JASCO V-650 Spectrophotometer.

Antimicrobial activity of TiO₂ nanoparticles

The in-vitro antimicrobial activities of the TiO₂ nanoparticles were evaluated by agar well diffusion method against Gram + ve bacteria (*S. aureus* and *B. subtilis*) and gram -ve bacteria (*P. aeruginosa* and *E. coli*). In Brief, 20 mL of Muller Hinton Agar (MHA) was poured into petri dishes and allowed to solidify. Then, 6 mm thick sterile discs were placed appropriately on petridishes. Finally, different concentrations of TiO₂ NPs (25, 50, 75 and 100 μ g/mL) were dissolved in

DMSO and loaded on each disc. The standard drug streptomycin (10 μ g) was used as a positive control to determine the sensitivity of each microbial species tested. All the plates were incubated at 37 °C for 24 hours and the respective inhibition zones were measured in millimeters range. The results (mean value $n = 4$) were recorded by measuring the zones of growth inhibition surrounding the disc.

Hemolytic activity of Titanium dioxide nanoparticles

Isolation of erythrocytes from human blood

Heparinized fresh human blood was collected from healthy volunteer. The collected blood sample was then mixed with 0.2 M phosphate buffered saline (PBS) (pH 7.0) to prevent coagulation. The blood sample was centrifuged at 1500 rpm for 10 min at 4 °C in a clinical centrifuge, and the plasma and buffy coat were removed by aspiration. The erythrocyte pellet was washed thrice with phosphate buffered saline (PBS) and resuspended in PBS to give a 5% hematocrit.

Treatment of erythrocytes with TiO₂ NPs

The reaction mixture containing 200 μ L of erythrocyte suspension (ES), 0.1% Triton X-100, and 50, 100, 250 and 500 μ g/mL of TiO₂ nanoparticles were added to 96-well microplates. After that it was incubated for 3 hours at 37 °C under constant agitation. The release of hemoglobin was determined after centrifugation (6000 rpm for 15 min) by photometric analysis of the supernatant at 540 nm. Complete hemolysis was achieved using 0.1% Triton X-100 yielding the 100% (positive control) and PBS yielding the 0% (negative control) Hemolysis.

The % hemolysis was calculated as

$$\text{Hemolysis (\%)} = \frac{(\text{OD test sample} - \text{OD negative control})}{(\text{OD positive control} - \text{OD negative control})} \times 100. \quad (1)$$

Results and Discussion

Structural XRD analysis

The XRD patterns of the as-prepared sample and annealed TiO₂ nanoparticles synthesized by hydrothermal method are shown in Fig. 2(a), (b) and (c) respectively. The nanoparticles were calcinated between two different temperatures 450 and 500 °C / 4 h.

Fig. 1 shows the XRD pattern of TiO₂ nanoparticles with 2 θ peaks lying at 25.6° (101), 37.8° (004), 48.1°

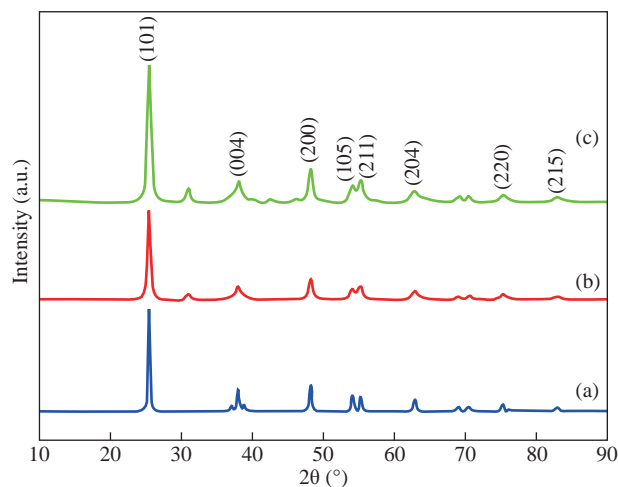


Fig. 1 XRD pattern of TiO₂ nanoparticles for (a) as prepared sample, and (b) and (c) annealed samples.

(200), 53.9° (105), 56.0° (211), 62.8° (204), 75.1° (220) & 83.2° (215) reflections, respectively and confirmed the nanocrystalline nature of the synthesized particles. The XRD sample showed a dominant peak at $2\theta = 25.6^\circ$ (101), which proved the crystallographic plane of anatase form of TiO₂, which are closely in agreement with the standard (JCPDS NO: 89-4921).

The particles size estimation was performed by the Scherrer's formula:

$$D = k\lambda / \beta \cos\theta, \quad (2)$$

where D is the mean diameter of the nanoparticles, K is the Scherrer's constant with the value 0.94, λ is the wavelength of the X-ray radiation source, β is the angular full-width at half-maximum of the XRD peak, and θ is the Bragg angle. From the powder XRD spectrum the average crystallite size was found to be 13 nm.

Morphological FE-SEM and elemental EDX analysis

The surface morphology of the TiO₂ nanostructure for as prepared and annealed samples was analyzed by FF-SEM and corresponding images are shown in Fig. 3. This image was observed within the magnification of 1 μm .

Fig. 2(a)-(c) depicts the FE-SEM images of TiO₂ nanoparticles which exhibited a spherical shape with little aggregations observed in the as-prepared and annealed sample. However, comparing the spherical structures of both samples it's seen that particles sizes and morphology changes with the increasing in annealing temperature. The grain size of the particles 500nm, with increasing in sintering temperature. All the three samples similar morphologies were observed, it conform the presence of TiO₂ nanoparticles.

Energy dispersive X-ray spectra of synthesized

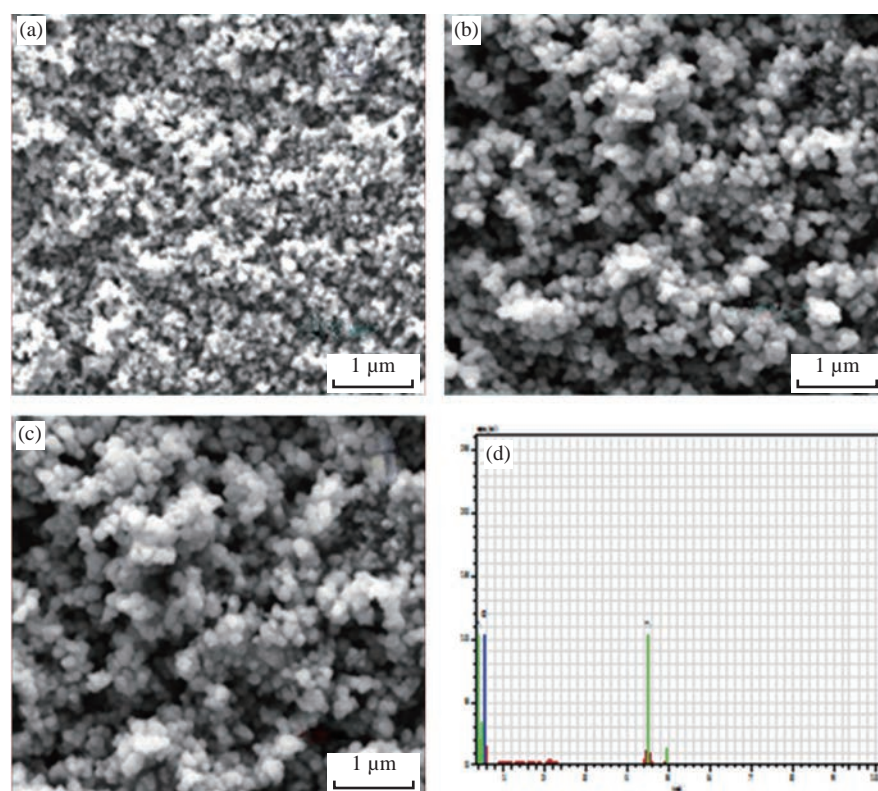


Fig. 2 FE-SEM images of TiO₂ nanoparticles for (a) as prepared sample, (b) and (c) annealed samples, and (d) EDX spectrum showing the chemical composition.

TiO₂ nanoparticles are represented in Fig. 2(d), which indicated the existence of oxygen and titanium. The energy dispersive X-ray analysis study (EDX) proves that the particles are crystalline in nature and indeed metallic TiO₂ NPs.

Raman spectra analysis

Fig. 3 shows the Raman spectra of TiO₂ nanoparticles for as prepared sample and annealed samples.

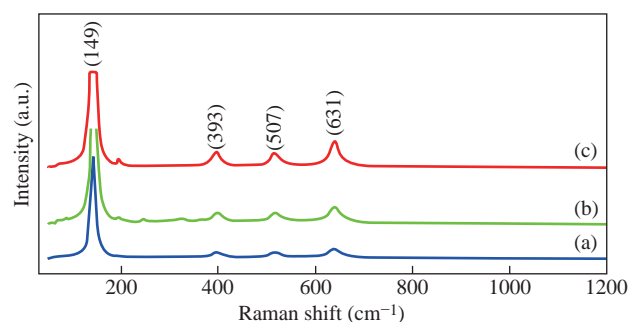


Fig. 3 Raman spectra of TiO₂ nanoparticles for (a) as prepared sample, and (b) and (c) annealed samples.

The scattered light was filtered by a dielectric edge Rayleigh rejection filter at 100 to 1200 cm⁻¹ line/mm diffraction grating. Fig. 4 shows the Raman spectra of pure TiO₂ NPs which exhibited the anatase vibration modes centered at 149, 393, 507, and 631 cm⁻¹. All of these bands are assigned to the anatase strong bands phase, and they can be attributed to the five Raman-active modes of anatase phase with the symmetries of E_g, B_{1g}, A_{1g}, and E_g, respectively. Both position of 149 cm⁻¹ showed and confirmed the strongest anatase raman mode of TiO₂ nanoparticles.

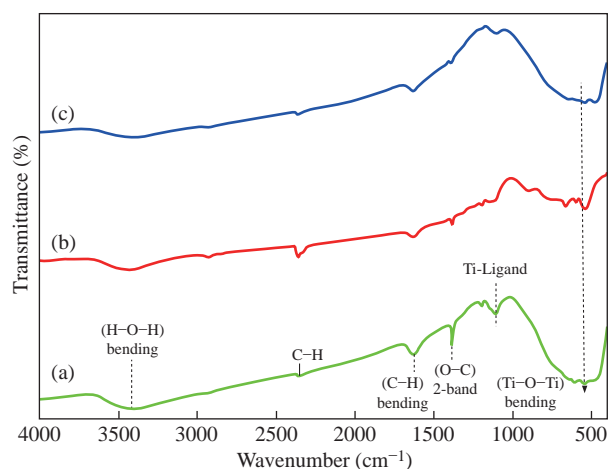


Fig. 4 FTIR spectra of TiO₂ nanoparticles for (a) as prepared sample, (b) and (c) annealed samples.

FTIR functional group analysis

FTIR analysis was used to determine the functional

groups of titanium dioxide (TiO₂) nanoparticles. The FTIR spectra of as prepared and annealed TiO₂ nanoparticles were analyzed in the range of 500 – 4000 cm⁻¹ as shown in Fig. 4.

Fig. 4 represents the FTIR absorption spectrum of the synthesized TiO₂ nanoparticles which showed an intense peak at 3425 and 1631 cm⁻¹ confirmed due to OH (hydroxyl) stretching and bending vibration mode. The bands at 1625 cm⁻¹ are related to the presence of C-H bending and vibration frequency of O-H bond in water. Peaks below 800 cm⁻¹ correspond to Ti-O (750-500 cm⁻¹) and Ti-O-Ti (metal-oxygen) (530 cm⁻¹) bending vibrations. The band located at 137 cm⁻¹ corresponds to a vibration of Ti-ligand bond. It is known that the existence of hydrogen bonds between the hydroxyl results in the broad IR absorption band and the shift to higher wave number.

Antimicrobial activity

The agar well diffusion method was performed against the *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*, and the maximum zone of inhibition was observed in the TiO₂ NPs against 4 different pathogens (Fig. 5).

Table 1 shows the synthesized TiO₂ NPs which displayed antibacterial activity of pathogenic strains of *S. aureus* (15 mm), *B. subtilis* (16 mm), *E. coli* (18 mm) and *P. aeruginosa* (17 mm) at 100 µg/mL, respectively. In the case of *E. coli* (gram -ve bacteria) shows high inhibition zone than the other *B. subtilis* (gram +ve), *S. aureus* (gram +ve) and *P. aeruginosa* (gram -ve) species in the zone inhibition range between 15-22 mm. The results were compared with the standard drug streptomycin, which suggests that the TiO₂ NPs showed better antibacterial activity.

Table 1 Zone of inhibition of TiO₂ NPs

Organisms	Zone inhibition (mm in diameter)				
	25 µg/ mL	50 µg/mL	75 µg/mL	100 µg/mL	PC
<i>P. aeruginosa</i>	06	09	14	17	18
<i>E. coli</i>	04	07	11	18	16
<i>S. aureus</i>	05	08	12	15	22
<i>B. subtilis</i>	05	09	13	16	17

The possible mechanisms are involved in the nano metal oxide (TiO₂ NPs) carrying a positive charge and microorganisms carrying a negative charge. Hence, electromagnetic interactions between metal oxide and microorganisms lead to oxidation. Titanium nanoparticles are capable of dissolving the outer membranes of bacteria due to the presence of hydroxyl

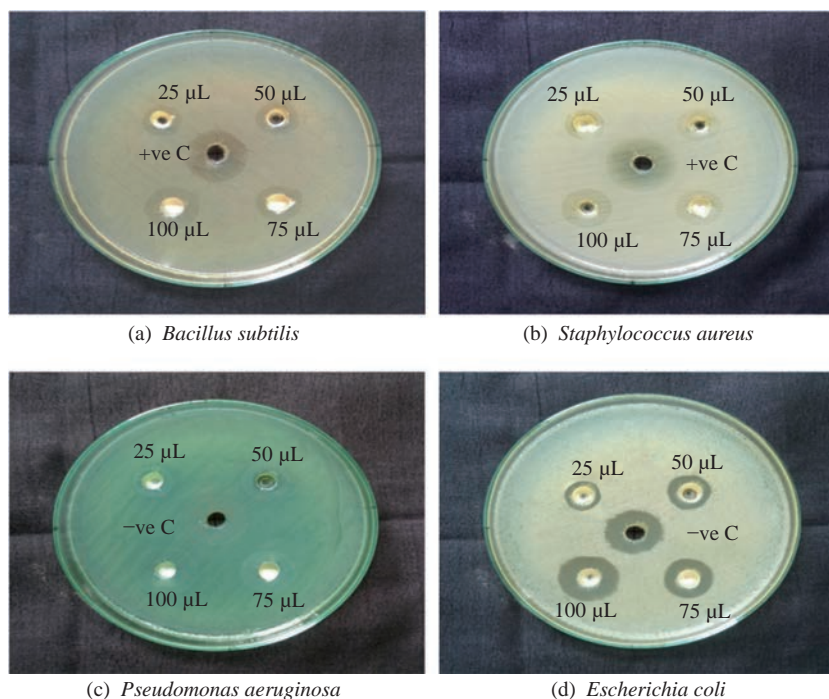


Fig. 5 The antimicrobial activity of TiO₂ nanoparticles.

groups leading to the death of the organisms.

In vitro hemolytic activity of TiO₂ nanoparticles

The photograph (Fig. 6) shows the in-vitro biocompatibility of different concentrations of 50, 100, 250 and 500 µg/mL TiO₂ nanoparticles showed the different percentage ranges such as 28.2, 32.5, 39.2 and 50.1% (Table 2). All nanomaterials enter into the blood get in contact with red blood cells (RBC). To assess the impact of TiO₂ NPs on erythrocyte, hemolysis test was

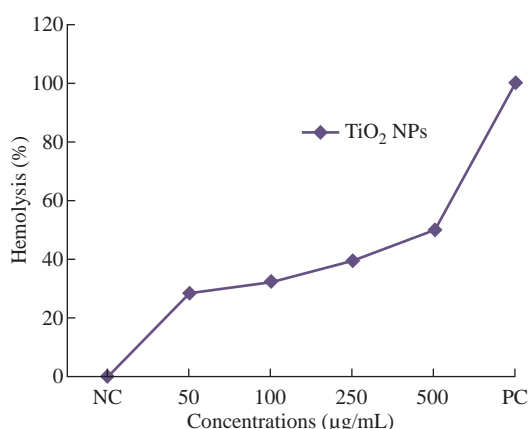


Fig. 6 The hemolytic activity of TiO₂ nanoparticles.

Table 2 The hemolytic percentage of TiO₂ nanoparticles

Sample concentrations (µg/mL)	Hemolysis (%)
50	28.2
100	32.5
250	39.2
500	50.1

performed by spectrophotometric method.

It was observed that the interaction of TiO₂ nanoparticles with RBC revealed a hemolysis percentage of 500 µg/mL caused a significant range of higher level of hemolysis, exceeding 50.1%. The performance of hemolysis assay was tested by the negative control phosphate buffered saline (PBS) and positive control (Triton-X-100). The observed hemolysis properties of these synthesized TiO₂ nanoparticles could be essentially attributed to their size, shape, surface chemistry and physiochemical properties. Hemolysis process involves the denaturation of cells through the physiochemical interaction between NPs and the cell surface. The results stated that TiO₂ NPs showed lower hemolytic activity compared with positive control. The mechanism of the hemolytic activity of the NPs depends on the increasing permeability to complete lysis of the cell. The cell lysis caused free radical formation and cell death and resulted in harmless RBC cell count.

Conclusions

The present study has demonstrated that the TiO₂ NPs were successfully synthesized by hydrothermal method using titanium tetra isopropoxide and isopropanol. They were calcinated between 450 °C and 500 °C. The calcinated TiO₂ nanopowders were characterized by powder XRD, FTIR, FE-SEM,

EDX and Raman analysis. The powder XRD spectra revealed that the main phase of TiO₂ nanoparticles were anatase phase and crystalline in nature. FTIR spectra of TiO₂ nanoparticles showed an intense peak at 3425 and 1631 cm⁻¹ confirmed due to OH (hydroxyl) stretching and bending vibration mode. Also, FTIR spectra showed the vibrational mode of TiO₂ around 530 cm⁻¹. The spherical shaped particles were studied by the FE-SEM analysis. Elemental analysis of the samples was investigated by EDX spectroscopy in order to confirm the presence of titanium and oxygen. The in-vitro antibacterial activity of TiO₂ NPs was observed against both gram-positive and gram negative bacteria and the results showed that TiO₂ NPs had potential inhibitory zone of inhibitions against *E. coli* and *P. aeruginosa* while there was less action against *S. aureus* and *B. subtilis*. Moreover, the TiO₂ nanoparticles exhibited considerable antimicrobial activity against pathogenic bacteria, which is comparable with that of standard antibiotic. Nevertheless, a rare study has been conducted in this area that can provide a clear understanding of the toxic effect and mechanism of TiO₂ NPs exposure to RBCs. The present study compared the adsorption, uptake and hemolytic potentials of different sizes of TiO₂ NPs in the RBC cells. Based on these results, we conclude that the TiO₂ nanoparticles may have potential biomedical applications due to its enhanced dispersibility, stability and surface coatings.

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Conflict of Interests

All author(s) declare that they have no conflict of interest regarding the publication of this paper.

References

[1] R. Allen, The cytotoxic and genotoxic potential of

- titanium dioxide (TiO₂) nanoparticles on human SH-SY5Y neuronal cells in vitro. *The Plymouth Student Scientist*, 2016, 9(2): 5-28.
- [2] H. Shi, R. Magaye, V. Castranova, et al., Titanium dioxide nanoparticles: A review of current toxicological data. *Particle and Fibre Toxicology*, 2013, 10: 15.
- [3] B.F. John, M. Sharfudeen, A.F.A. Latheef, et al., Synthesis and characterization of TiO₂ nanoparticles and investigation of antimicrobial activities against human pathogens. *J. Pharm. Sci. & Res.*, 2017, 9(9): 1604-1608.
- [4] S. Di Bucchianico, F. Cappellini, F. Le Bihanic, et al., Genotoxicity of TiO₂ nanoparticles assessed by mini-gel comet assay and micronucleus scoring with flow cytometry. *Mutagenesis*, 2017, 32: 127-137.
- [5] A.A. Bakare, A.J. Udoakang, A.T. Anifowoshe, et al., Genotoxicity of titanium dioxide nanoparticles using the mouse bone marrow micronucleus and sperm morphology assays. *Journal of Pollution Effects & Control*, 2016, 4: 2.
- [6] P. Pookmanee, S. Phanichphant, Titanium dioxide powder prepared by a sol-gel method. *Journal of Ceramic Processing Research*, 2019, 10(2): 167-170.
- [7] T. Chen, J. Yan, and Y. Li, Genotoxicity of titanium dioxide nanoparticles. *Journal of Food and Drug Analysis*, 2014, 22: 95-104.
- [8] R. Sharmila Devi, R. Venkatesh, and R. Sivaraj, Synthesis of titanium dioxide nanoparticles by sol-gel technique, international journal of innovative research in science. *Engineering and Technology*, 2014, 3(8).
- [9] Z.F. Yin, L. Wu, H.G. Yang, et al., Recent progress in biomedical applications of titanium dioxide. *Phys. Chem. Chem. Phys.*, 2013, 15: 4844-4858.
- [10] L.U. Okoli, J.O. Ozuomba, A.J. Ekpunobi, et al., Anthocyanin-dyed TiO₂ electrode and its performance on dye-sensitized solar cell. *Research Journal of Recent Sciences*, 2012, 1(5): 22-27.
- [11] F. Kamil, K.A. Hubiter, T.K. Abed, et al., Synthesis of aluminum and titanium oxides nanoparticles via sol-gel method: optimization for the minimum size. *Journal of Nanoscience and Technology*, 2016, 2(1): 37-39.
- [12] M. Malekshahi Byranvand, A. Nemati Kharat, L. Fatholahi, et al., A review on synthesis of nano-TiO₂ via different methods. *Journal of Nanostructures*, 2013, 3: 1-9.
- [13] P.S.M. Kumar, A.P. Francis, and T. Devasena, Comparative studies on green synthesized and chemically synthesized titanium oxide nanoparticles. a validation for green synthesis protocol using hibiscus flower. *Journal of Environmental Nanotechnology*, 2014, 3(4): 78-85.
- [14] N. Syuhada, B. Yulianto, and Nugraha, Synthesis and characterization hierarchical three-dimensional TiO₂ structure via hydrothermal method. *Materials Science and Engineering*, 2018, 367.
- [15] T. Santhoshkumar, A.A. Rahuman, C. Jayaseelan, et al., Green synthesis of titanium dioxide nanoparticles using Psidium guajava extract and its antibacterial and antioxidant properties. *Asian Pacific Journal of Tropical Medicine*, 2014, 7(12): 968-976.
- [16] M. Hudlikar, S. Joglekar, M. Dhaygude, et al., Green synthesis of TiO₂ nanoparticles by using aqueous extract of *Jatropha curcas* L. latex. *Materials Letters*, 2012, 75: 196-199.
- [17] W.A. Abbas, I.H. Abdullah, B.A. Ali, et al., Recent advances in the use of TiO₂ nanotube powder in biological, environmental, and energy applications. *Nanoscale Adv.*, 2019, 1: 2801-2816.
- [18] H. Hayashi, Y. Hakuta, Hydrothermal synthesis of metal oxide nanoparticles in supercritical water. *Materials*, 2010, 3: 3794-3817.
- [19] P.M. Aneesh, K.A. Vanaja, and M.K. Jayaraj, Synthesis of ZnO nanoparticles by hydrothermal method.

- Nanophotonic Materials*, 2007, 6639.
- [20] P. Nyamukamba, O. Okoh, H. Mungondori, et al., Synthetic methods for titanium dioxide nanoparticles: A review. *Materials for a Sustainable Environment*, 2018.
- [21] J. Upadhyay, A. Kumar, B. Gogoi, et al., Antibacterial and hemolysis activity of polypyrrole nanotubes decorated with silver nanoparticles by an in-situ reduction process. *Materials Science and Engineering C*, 2015, 54: 8-13.
- [22] M.A. Dobrovolskaia, J.D. Clogston, B.W. Neun, et al., Method for analysis of nanoparticle hemolytic properties in vitro. *Nano Letters*, 2008, 8(8): 2180-2187.
- [23] O.Y. Golubevaa, O.V. Shamovab, D.S. Orlovb, et al., Study of antimicrobial and hemolytic activities of silver nanoparticles prepared by chemical reduction. *Glass Physics and Chemistry*, 2010, 36(5): 628-634.
- [24] H.K. Al-Hakeim, K.M. Jasem, High ionic strength enhances the anti-pepsin activity of titanium dioxide nanoparticles. *Nano Biomed. Eng.*, 2016, 8(3): 136-143.

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