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Preparation and characterization of titanium dioxide nanoparticles and *in vitro* investigation of their cytotoxicity and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) are photo-active metallic nanoparticles becoming promising agents in modern biomedical applications. Herein, a novel process for the synthesis of TiO_2 NPs with high stability was developed by a sol–gel process and to investigate their cytotoxicity and antibacterial activity. Numerous experiments have been performed to confirm the morphologies, compositions, and physicochemical properties of prepared TiO_2 NPs, such as field emission scanning electron microscopy, dynamic light scattering, Zeta potential, Fourier transform infrared spectroscopy and X-ray diffraction. MTT assay was applied to assess the cytotoxicity of the prepared nanoparticles. The results indicate that the synthesized nanoparticles' diameter is about 68 nm and contains the anatase phase, in the range of 2θ from 25 to 80 °C. The hydrodynamic radius of nanoparticles is about 140.4 nm, and the zeta potential of nanoparticles is about −44.6 mV. The MTT results have not shown any toxicity; the antibacterial inhibitory effect of TiO_2 NPs at 200 mg/mL concentrations exhibited superior antibacterial activity at 15.9 ± 0.1, 14.0 ± 0.1 against *Staphylococcus aureus* and *Escherichia coli*, respectively. In conclusion, colloidal solutions with high stability were successfully synthesized, contributing to decreased dimensions and increased antibacterial properties.

KEYWORDS

Titanium dioxide; nanoparticles; sol–gel method; cytotoxicity; antibacterial activity

The prevalence of microbial infections poses a significant threat to global public health. Antibiotics are a common treatment for treating bacterial infections due to their cost-efficacy and positive efficacy; however, numerous studies have shown clearly that extensive antibiotic use has contributed to the development of multidrug-resistant bacterial strains.[1,2] More recently, metallic nanomaterials have become the most attractive and rapidly emerging material in the scientific area.[3] The significant interest of nanomaterial research and development, particularly nanoparticles (NPs), is due to their attractive characteristics, revealed by their small size, remarkable high surface area activity offering excellent catalytic, optical, and electrical properties.[4] Therefore, Metallic NPs have been active in comprehensive research, methods, and advanced micro- and nanotechnology applications. Due to their unique physicochemical properties in biological applications, metal oxide nanoparticles such as zinc oxide (ZnO), manganese oxide (MgO), titanium dioxide (TiO_2), and superparamagnetic iron oxide (Fe3O4) have been widely applicable in recent years.[5–8] TiO_2 nanoparticles have a large surface region, superior surface morphology, and naturally, non-toxicity and their photocatalytic antimicrobial activities have been documented, Implementing excellent biological action against microbial contamination.[9] TiO_2 nanoparticles exhibit broadband UV absorption, sunscreen applications.[10] The sol–gel route is a low-cost method for the preparation of titanium dioxide.[11] The current study is designed to prepare and characterize TiO_2 NPs synthesized by the sol–gel technique and in vitro investigation of their cytotoxicity and antibacterial activity. Field emission scanning electron microscopy (FESEM), DLS, zeta potential, FTIR, and X-ray powder diffraction (XRPD) were used to characterize samples. Furthermore, potential cytotoxic and antibacterial testing were performed successively to verify the effectiveness in the removal of

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bacterial agents of specific colonies of *Staphylococcus aureus* and *Escherichia coli.*

Materials and methods

All chemicals were obtained from and used as such without further purification from (Sigma-Aldrich, USA). Titanium tetrachloride (TiCl4), ≥99%; titanium isopropoxide (C12H28O4Ti), 99%; glacial acetic acid, ≥99%; and isopropanol ≥99%.

TiO_2 NPs were produced using sol–gel technique according to the procedure previously mentioned.[12] A beaker with 80 mL of isopropanol was mixed with 20 ml of titanium isopropoxide at room temperature to make a homogenized solution with a magnetic stirrer for 5 min. A 20 mL of deionized water was mixed in a second beaker with 20 mL of isopropanol with a magnetic stirrer for 5 min for homogenized time.

The contents of the second beaker were then added to the first beaker. At this stage, the solution obtained was milky in color. Hydrochloric acid was added drop-wise to the suspension above to achieve the appropriate pH = 2–4 (pH). During this stage, the resulting suspension was applied for 2 h on a 500 rpm magnetic stirrer and then sonicated for 32 min to complete the homogenization reaction. Then, sol–gel is formed about 48 h. The solution is converted to gel at this point, and its viscosity increased. The resulting gel was then heated to 110° C for 2 h to make dry. Finally, the dry gel is heat in a muffle furnace at 500° C for 5 h to produce a fine powder.

Characterization of TIO_2 NPs

XRD test

Applications of Cu – Kα-wavelength ($T_{\lambda} = 1.5405$ Å) were produced using a Phillips diffractometer in powder samples of TiO_2 NPs. The voltage used was 40 kV, and the current intensity was 25 mA.

Field emission (SEM)

The morphological features and size of TiO_2 NPs were studied by FESEM, MIRA3 TESCAN-XMU, under a 20-kV electron acceleration.

DLS measurement

DLS of the prepared TiO_2 was carried out using (Horiba SZ-100 nanoparticle analyzer). An appropriate powder concentration of 0.01 g/100 mL is distributed in DMSO. The same medium has been used to estimate the cytotoxicity effect on the distribution of DMSO particles.

Zeta analysis

We were performed zeta potential measurement using (Horiba SZ-100 nanoparticle analyzer) to investigate particles' surface charge of prepared TiO_2 NPs. The particle's electrostatic potential was performed in ultrasonic dispersion of 0.01 g/100 mL in DMSO at room temperature.

FTIR analysis

The prepared samples were analyzed over a wavelength range of 400–4000 cm^{-1} in an FTIR machine (Shimadzu Corporation, Japan) to determine the specimens' functional groups.

MTT assay

For the measurement of the cytotoxic effect and biocompatibility of prepared TiO_2 NPs, (MTT) assay was used following instruction from the manufacturer. Vero cultures were incubated at a density of 1×10^5 cells per well at 37 °C in 5% CO2. TiO_2 NPs were added in four variable concentrations (12.5, 25, 50, and 100 µg/mL), and after 24 h, the cells were washed twice with phosphate buffer saline PBS) before adding fresh 100 µL of culture medium and 0.5 mg/mL of MTT reagent to each well. The control group served as the non-labeled cells. Subsequently, the labeled cells were incubated at 37° C in 5% CO2 for 4 h. The medium was kindly aspired and replaced by 100 µg/mL of fresh DMSO. Dissolved formazan product absorbance was inspected at a wavelength of 570 nm.

Antibacterial activity

Kirby- Bauer used the disk diffusion method to test antibacterial activity[13] was considered a suitable method for evaluating the antibacterial activity of newly synthesized material. Briefly, A sterile inoculating loop was collected from four or five bacterial colonies and suspended in 2 ml of the saline buffer of sterilized phosphate (PBS), the studied bacteria, including *S. aureus* (ATCC 29213) and *E. coli* (ATCC 35218). The bacteria suspension turbidity has been modified to 0.5 McFarland level by diluting with sterile PBS. Sterile swabs have been inserted into the inoculum channels. Muller–Hinton agar plates inoculated with bacteria by streaking swabs. Dissolve 0.1 mg TiO powder in 1 ml of distilled water to disperse TiO_2 suspension. The suspension has been soniced 10 min before use. A 35 µl of TiO_2 suspension and distilled water (as negative control) and AgNO3 (as positive control) has been impregnated into the standard

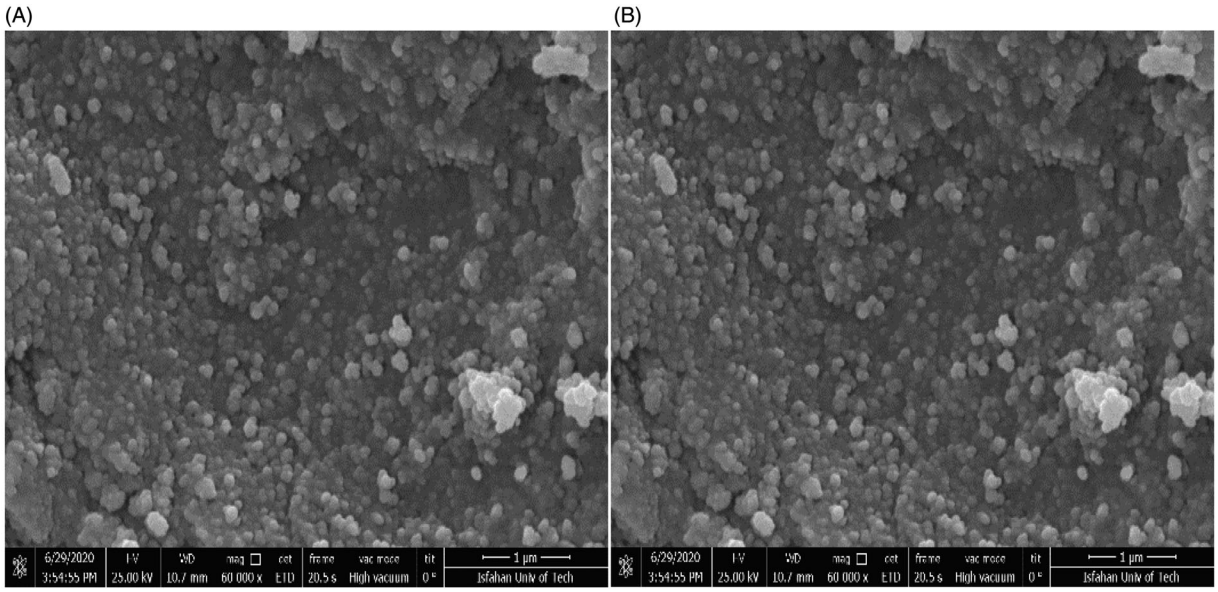

Figure 1. FESEM images of prepared TiO_2 NPs with different magnification (A and B). Abbreviations: FESEM, Field emission scanning electron microscope; TiO_2, titanium dioxide. NPs, Nanoparticles.

antibiotic disk. Lastly, all disks were used to evaluate antibacterial activity in Mueller-Hinton (Merck, Germany) against two bacterial strains. Impregnated disks were placed with sterile forceps on the surface of the agar. Plates were incubated for 24 h at 37° C to control antibacterial activity. This activity (inhibition zone) was examined in millimeters. The tests were done three times.

Statistical analysis

The mean ± standard division (SD) was used for the statistical analysis of our results. Excel was also used to evaluate statistical significance. Results $p < 0.05$ and $p < 0.01$ are defined as the criteria for statistical significance.

Results and discussion

Characterization

FESEM

The synthesized TiO_2 nanoparticles (Fig. 1), with an average size of 68 nm, were visualized using an FESEM technique for displaying the size and shape of relatively spherical nanoparticles

XRD

The diffraction peak appeared at 2θ with 25.3°, 38.3°, 48°, 54°, 62°, corresponds to the crystal planes of (101), (004), (200), (105), and (204), respectively, indicating the formation of anatase phase of TiO_2 (Fig. 2).

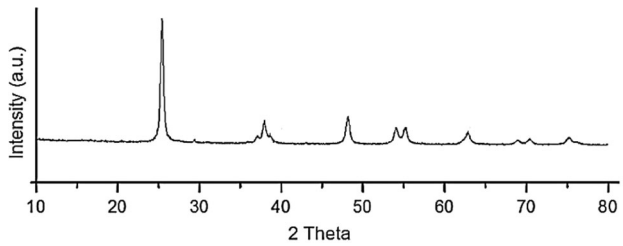

Figure 2. XRD pattern of prepared TiO_2 NPs by the sol-gel method. Abbreviations: XRD, X-ray diffraction; TiO_2, titanium dioxide; NPs, Nanoparticles

The peaks of the graph are in good agreement with the previously reported studies.[14–15]

Dynamic light scattering and zeta analysis

The DLS and zeta potential analysis of prepared TiO_2 nanoparticles are shown in Figs 3 and 4, respectively. The nature of hydrodynamic size (diameter) in DMSO of prepared nanoparticles of TiO_2 studied. DLS showed quite a small body with a hydrodynamic diameter of an average of 140.4 nm.

For the TiO_2 Nanopart suspense in DMSO, a clear indicator for stability without particle settlement was found the Zeta potential (A) with an adverse value of −44,6 mV with electrophoretic movement (mean): −0,000 346 cm^2/Vs. The study on prepared suspension also confirms the general zeta potential criteria negatively for improved stability.[16]

Zhang et al. (2000) confirmed that the zeta potential of the material for understanding the

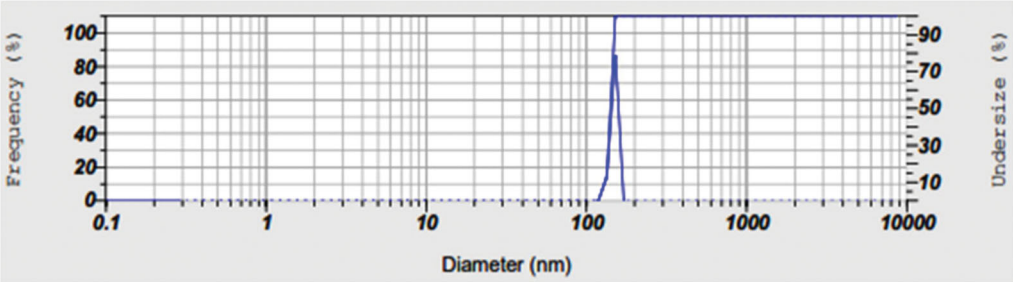

Figure 3. Dynamic light scattering (DLS) image of titanium nanoparticles size TiO_2 NPs distribution by volume of particles (DLS) using HORIBA Scientific Nanoparticle analyzer SZ-100.

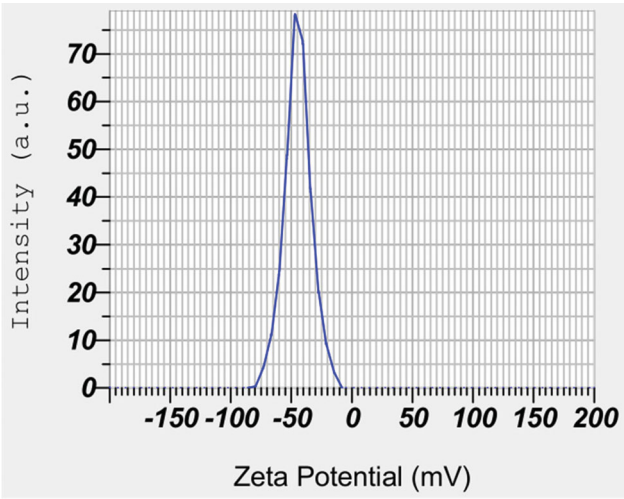

Figure 4. Zeta potential analysis of prepared TiO_2 nanoparticles.

nature of cell interactions, cell diagnosis, and normal and cancer-cell effect therapeutics had been measured.[17–19]

FTIR analysis

FTIR analysis was used to determine the functional groups of titanium dioxide nanoparticles (Fig. 5).

MTT results

The MTT assay used to evaluate the viability/proliferation of TiO_2 at various concentrations, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL on Vero cell lines for 24 h (Fig. 6). Compared to the control group (without TiO_2 NPs), the samples' cell viability/proliferation did not show any potential cytotoxicity at concentrations of 1, 25 µg/mL, 50 µg/mL, and 100 µg/mL for 24 h.

Titanium Nanomaterial induced endothelial leakiness (NanoEL) may be viewed as an emerging strategy to improve nanomedicine's biodistribution to target sites.[20–22] Several studies show that exposure of endothelial cells to TiO_2 nanomaterials causes endothelial cell leakiness. This effect is caused by the physical interaction between TiO_2 nanomaterials and endothelial cells' adherens junction protein vascular endothelial cadherin (VE-cadherin).[23–25] Overall, determination of this size–charge–mass density window will equip nanobiotechnologists with rules to engineer NM that either avoid or capitalize on this NanoEL effect.

A study by Setyawati et al. suggested and supported a novel mechanism that TiO_2–NM being small enough to migrate into the inter-endothelial adherens junction niche, binds directly VE-cadherin and disrupts these cell-cell interactions.[26] VE-cadherin's fate appears to follow the canonical ligand–receptor-mediated pathway where VE-cadherin may be internalized and degraded. Setyawati et al. demonstrated that TiO_2–NPs might cause NanoEL effects resulting in enhanced circulating melanoma metastasis to the lungs. Endothelial leakiness could be attributed to the toxic effects of inorganic NPs in biological systems. The size of nanomaterials, such as TiO_2 NPs and endothelial cell type sensitivity then helps the nanotechnologists to design future nanoparticles that either exploit NanoEL as nanotechnology driven strategy to avoid NanoEL as a nanotoxicity side effect.[27,28]

Antibacterial activity

The synthesized TiO_2 NPs were used for antibiotic susceptibility at three concentrations (50–100–20 mg/ml) at three different temperatures, as shown in Fig. 7, that exhibited the inhibition zone of TiO_2 NPs against tested bacteria. Fig. 8 shows a well diffusion assay that TiO_2 nanoparticles were more efficient as antibacterial agents on *S. aureus* and *E. coli*. The reduction of antimicrobial potency may be due to the calcination of TiO_2 NPs, which in turn caused an increase of particle size. TiO_2 NPs exhibited interesting antimicrobial reduction due to the enhancement of the specific surface area. The nature of titanium dioxide can explain this phenomenon, and one of the main mechanisms of its action is the production of reactive oxygen species (ROS) on its surface during the photocatalysis process when it is exposed to light

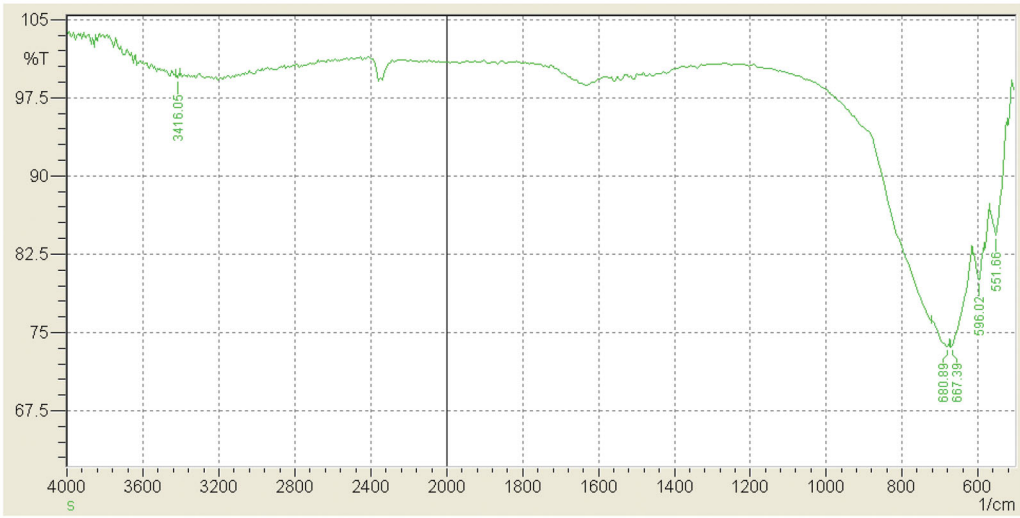

Figure 5. FTIR spectrum of anatase TiO_2 nanoparticles indicating relative functional groups.

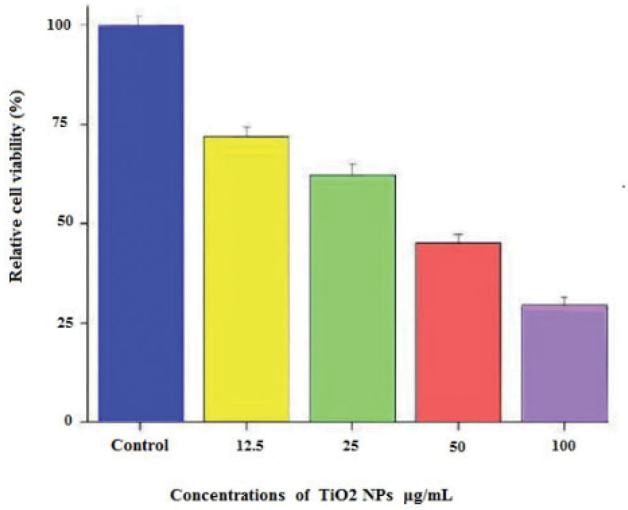

Figure 6. Vero cell lines' cellular viability treated with different concentrations of TiO_2 nanoparticles for 24-h study period. Data are expressed as mean ± standard error from three independent experiments.

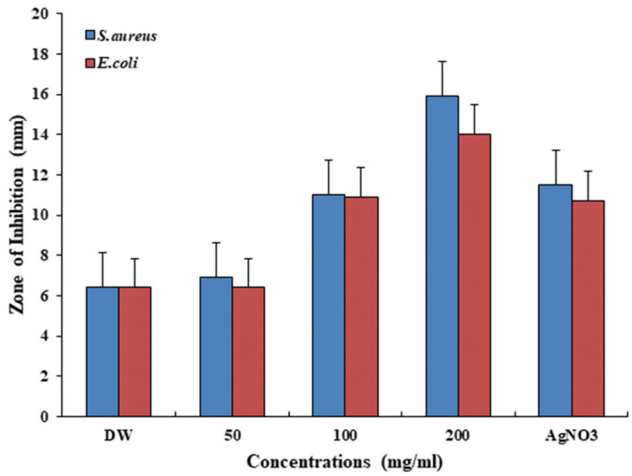

Figure 7. Antibacterial activity of sol–gel generated TiO_2 NPs against *Staphylococcus aureus* and *Escherichia coli*. Data are presented as mean ± SD. Compared to *S. aureus* at the same concentration of $AgTiO_2$ NPs, $p \# 0.05$, $p \# 0.01$; $n = 3$.

at a suitable wavelength, which leads to phospholipid peroxidation and finally cells death.[14,15,29,30]

This research was carried out to strengthen further the physical-chemical properties of TiO_2 NPs by antibacterial activity. With a specific surface area, the crystalline category of our TiO_2 sol–gel synthesized, the negative zeta potential being −44.6 mV, besides, the average diameter in DMSO 140,4 NM for the nanomedicine assessment of the antibacterial activity of the TiO_2 NP.

In recent decades, many studies have been made to understand biomaterials' biocompatibility effect on cells to produce good osteointegration. The prevailing environment, like adhesion, cell viability, and proliferation, increases cell viability. The physicochemical properties such as particle size, shape, surface area, phase purity, crystallite composition, and surface topography like surface charging properties and particle concentration play an essential role in the prepared material's biological activity. However, Shi et al.[31] have reported the potential side effects and beneficial activity of the conventional TiO_2. A profuse and overdoses, form, size, composition, crystalline properties, added functional groups, solubility, and the manufacture of impurities of such a heterogeneous nature was applied on different cell lines such as lung cells, nerve cells, cardiovascular cells, dermal and mucosal cells, and reproductive and renal cells to confirm the effect of titania.[17,32]

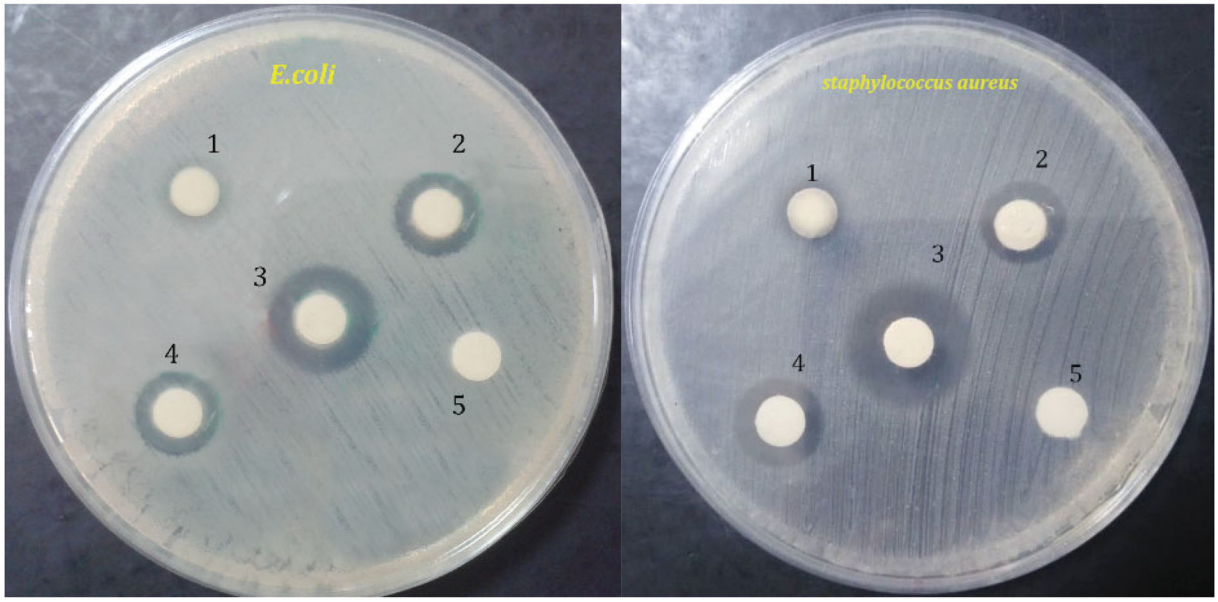

Figure 8. Well diffusion assay exhibiting the impact of TiO_2 nanoparticles as antibacterial agents against *Staphylococcus aureus* and *Escherichia coli*.

TF-TiO_2 NPs stuck in a suspension on bacterial surfaces, resulting in TiO_2 particles being adsorbed on the surface of bacteria,[33] that could lead in combination with a photocatalytic oxidation reaction to inactivating bacteria.[34] There are several possible mechanisms to explain bacteria's impact on TiO_2 particles.[35]

Not all antibiotics have synergistic antimicrobial effects when used with TiO_2 since one study showed the lack of increased antibacterial activity using CEF antibiotics in combination with TiO_2. Compared to the CEF antibiotic combined with TiO_2, the CEZ antibiotic showed an excellent synergistic impact.[36]

Conclusion

Sol–gel is one of the most powerful methods for the synthesis of TiO_2 nanopowder, based on the findings and analyses carried out. The scale of the oxide is 68 nm.

Acknowledgments

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Disclosure statement

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