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Synthesis of TiO_2 nanoparticles by electrochemical method and their antibacterial application

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Abstract

Titanium dioxide nanoparticles were successfully prepared by electrochemical method. The tetra propyl ammonium bromide salt was used as stabilizing agent in an organic medium *viz*. tetra hydro furan (THF) and acetonitrile (ACN) in 4:1 ratio by optimizing current density. The parameters such as current density, solvent polarity, distance between electrodes and concentration of stabilizers were used to control the size of nanoparticles. The synthesized titanium dioxide nanoparticles were characterized by using UV–Visible spectroscopy, X-ray diffraction, scanning electron microscopy (SEM), energy dispersive spectrophotometer (EDS) and transmission electron microscopy (TEM) analysis techniques. TEM analysis proved a nearly tetragonal structure with size of 25–30nm which was in agreement with the result calculated from the XRD analysis. EDS analysis revealed the presence of Ti and O element. The nanoparticles were screened for their in vitro antibacterial activity against human pathogens such as gram negative *Escherichia coli* (*E. coli*), and gram positive *Staphylococcus aureus* strains and which proved excellent results.

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Keywords

Titania nanoparticles; Tetra propyl ammonium bromide salt; Electrochemical reduction method; Human pathogens; Antimicrobial activity

1. Introduction

Nanomaterials, especially metal nanoparticles, have received much attention over the past decades because of their many technological applications that they have to offer (Cheng et al., 2012, Porod et al., 2003, Rothenberg et al., 2002, Cooper et al., 2014). Studying these applications has always been of great interest to many scientists. In fact, nanoparticles display completely unique properties in comparison with their bulk size counterparts. Nanoparticles are currently known as antibacterial materials. The antibacterial effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to attach closely with microbial membranes and is not merely due to the release of metal ions in solution (Chwalibog et al., 2012). Among the inorganic antibacterial agents, metals and photocatalysts are most commonly used. Most metallic ions exhibit antimicrobial effect. For efficiency and safety reason, silver, copper and zinc are the most widely available metallic antibacterial agents (Gajbhiye et al., 2009, Akhavan, 2009, Xie et al., 2011).

TiO_2 nanoparticles, approximately less than 100 nm in diameter, have become a new generation of advanced materials and are of great interest due to its different environmental applications, such as gas sensor (Obida et al., 2005, Tian et al., 2013) photocatalytic degradation of various contaminants in waste water treatment (Chong et al., 2010), degradation of carcinogenic dyes (Bumajdad et al., 2014), solar cell (Baraton, 2011). TiO_2 nanoparticles are used in cosmetics and filters that exhibit strong germicidal properties and remove odors. The TiO_2 photocatalysts have been investigated extensively for the killing or growth inhibition of bacteria due to its powerful oxidation strength, good chemical stability and nontoxicity (Kim et al., 2003, Liou and Chang, 2012, Foster et al., 2011). Being strong oxidant, the reactive oxygen species generated by the TiO_2 photocatalytic reactions cause various damages to microorganisms ensuring their rapid inactivation.

Generally, the most common approach in the literature for synthesizing and stabilizing TiO_2 nanoparticles is sol–gel method based on the hydrolysis of titanium alkoxide.

However, this method encounters some problem, such as weak anatase crystallinity and poor monodispersity. Therefore this study was designed to synthesize pure anatase TiO_2 nanoparticles by electrochemical method; a controlled current electrolysis is used throughout the synthesis process. The tetra propyl ammonium bromide salt used was highly soluble and dissociated in the solvent to play the role of electrolyte. It was absorbed on the metal oxide surface to stabilize the synthesized nanoparticles. The effect of change in current density on particle size was also studied. A bioevaluation assay of the inhibitory activity of the synthesized TiO_2 nanoparticles on bacterial strain was performed to establish the potential of these nanoparticles as antibacterial agents at different concentrations with well known antibiotics like Gentamicin.

2. Experimental methods

2.1. Materials

All chemicals (up to 98.99% purity) were purchased from Aldrich and Rankem chemical suppliers and used as received. *Staphylococcus aureus* (ATCC No. 25923) and *Escherichia coli* (ATCC No. 25922), provided by Govt. Institute of Science, Aurangabad 431,004. Antibiotics Gentamicin was subjected to analysis. Agar medium was used for screening the antibacterial activity.

2.1.1. Synthesis of titanium dioxide nanoparticles

The synthesis of titanium dioxide nanoparticles was done by electrochemical method (Reetz and Helbig, 1994, Reetz et al., 1995), for narrow size distributed metal nanoparticles. In the overall process the bulk metal is oxidized at the anode, the metal cations migrate to the cathode and reduction takes place with formation of metal or metal oxide in the zero oxidation state. Agglomeration with formation of undesired metal powder is prevented by the presence of the ammonium stabilizer.

As shown in Fig 1, in the initial experiment we have used a Titanium metal sheet (1×1 cm) as anode and a platinum sheet (1×1 cm) as the cathode. The two electrodes were 1 cm apart. Tetra Propyl Ammonium Bromide (TPAB-0.01 M) in acetonitrile/tetrahydrofuran (4:1) served as the supporting electrolyte. Upon applying current density we obtained >95% of titanium dioxide clusters stabilized by tetra propyl ammonium bromide (TPAB). Electrolysis was carried out in nitrogen atmosphere. Titanium dioxide nanoparticles were found to be white in color. Since the material was insoluble in the solvent mixture used, the work up turned out to be simple decantation, decanted solid product was washed with dry THF for three to four times to remove excess TPAB and dried under vacuum desiccators. This dried sample was calcinated at 550 °C and stored in closed glass vials under ambient conditions. In order to study the

effect of current density on particle size, the current in the electrolysis process was varied from at 10 mA/cm^2 to 14 mA/cm^2. It was found that variation in current density affects the particle size of nanocluster in the electrolysis process.

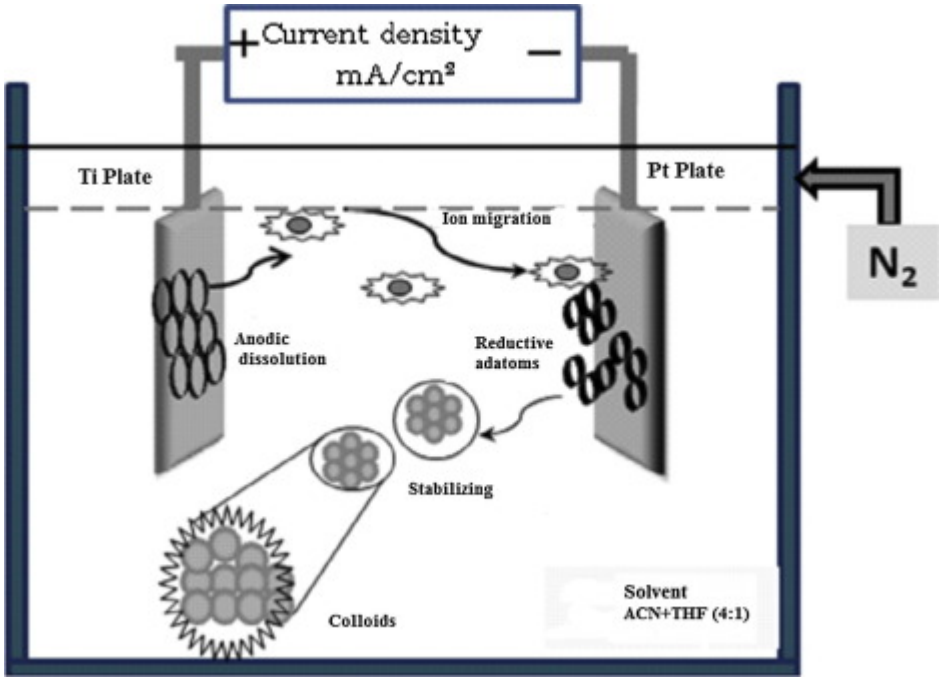

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Figure 1. Diagram representing the synthesis process of TiO_2 nanoparticles.

Reaction mechanism for electrochemical synthesis of metal clusters:

M^0 *anode* → $M^{n+} + ne^-$

$M^{n+} + ne^- +$ stabiliser → M^0 *(nanoparticle)*

M^0 *anode* + stabiliser → M^0 *(nanoparticle)*

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2.1.2. Characterization of titanium dioxide nanoparticles

The prepared titanium dioxide nanoparticles were characterized by UV–Visible spectrophotometry, XRD, TEM, SEM–EDS techniques. The wavelength of absorbance was determined by UV–Visible spectrophotometer [JASCO 503] using a quartz cuvette and acetonitrile/tetrahydrofuran as reference. The X-ray powder diffraction patterns of the TiO_2 nanoparticles were recorded on Bruker 8D advance X-ray diffractometer using Cu Kα radiation of wavelength=1.54056Å. To study the morphology of TiO_2 nanoparticles, the SEM analysis was carried out with JEOL; JSM-6330 LA operated at 20.0kV and

1.0000 nA. The presence and elemental composition in TiO_2 nanoparticles were examined using energy dispersive spectrophotometer (EDS). The TEM analysis was carried out with Philips model CM200 operated at 20–200 kV.

2.1.3. Antibacterial activity experiment

Antibacterial activity of the TiO_2 nanoparticles was determined, using the agar well diffusion assay method (Thornberry, 1950). Approximately, 25 ml of molten and cooled nutrient agar media were poured in the sterilized petri dishes. The plates were left over night at room temperature to check for any contamination to appear. The bacterial test organism *S. aureus* and *E. coli* were grown in nutrient broth for 24 h. A 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 100 µl of different concentration of TiO_2 nanoparticles. Antibiotic gentamicin used as positive control for each bacterium to compare the inhibition of growth of bacteria with TiO_2 nanoparticles. The plates containing the bacteria and solutions of TiO_2 nanoparticles were incubated at 37 °C. All the tests were repeated in triplicates. The antibacterial activity was taken on the basis of diameter of Zone of Inhibition which was measured at cross-angles after 24 h of incubation and the mean of three readings is shown in Table 1. The inhibition of the bacterial growth on the agar plates by using different concentration of TiO_2 nanoparticles synthesized at two different current densities is shown in Fig. 6.

Table 1. In vitro antibacterial screening of synthesized titanium dioxide nanoparticles.

Human pathogenic bacteria	**TiO_2 nanoparticles**				**ACN+THF (4:1)**		**Gentamicin**	
	10 mA/cm^2		**14 mA/cm^2**		**50 µl**	**100 µl**	**50 µl**	**100 µl**
	50 µl	**100 µl**	**50 µl**	**100 µl**				
Zone of inhibition (mm)								
Staphylococcus aureus	13.2	17.2	16.0	19.1	00	00	21.3	24.4
E. coli	10.5	14.3	15.2	17.0	00	00	19.2	22.8

3. Results and discussion

3.1. UV–Visible spectrophotometry

As the phenomenon of Surface Plasmon occurs only in the case of nanoparticles and not in case of bulk metallic particles, hence unique optical properties of nanoparticles can be

studied using UV–Visible spectroscopy (Burda et al., 2000). In present synthesis method as the electrochemical reduction proceeds, the color of reaction mixture changes from white to opaque milky white indicating formation of TiO_2 nanoparticles. About 4ml of colloidal reaction mixture was withdrawn after 2h to record UV–Visible spectrum. Prepared sample of TiO_2 was capped with TPAB at current densities 10mA/cm^2 and 14mA/cm^2 showed sharp peaks at 362nm and 346nm respectively indicating the formation of anatase TiO_2 in nanoform (Fig. 2a and b). The size and shape of nanoparticles could be qualitatively described by the peak position and shape of the absorption spectrum. General trend is that the absorption at long wavelengths was due to the scattering of light by big particles (Bian et al., 2008).

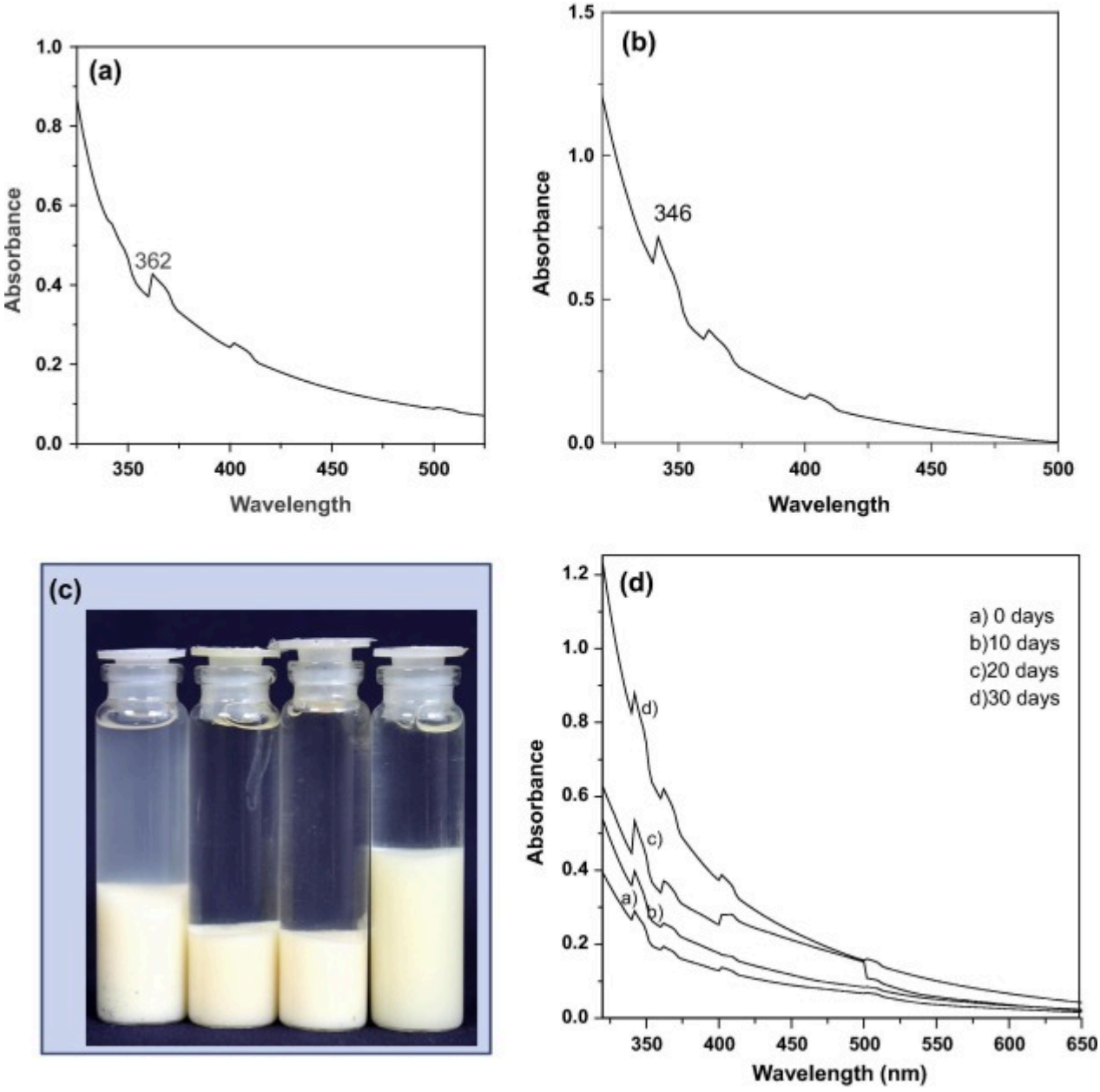

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Figure 2. UV–Visible Spectra of TiO_2 nanoparticles synthesized at different current densities (a) at 10mA/cm^2 and (b) at 14mA/cm^2. (c) Shows photograph of colloidal TiO_2 nanoparticles stored in glass bottles. (d) Time dependence of the absorption spectrum of a TiO_2 nanoparticles colloid.

In the electrochemical method for the preparation of the metal nanoparticles, the size of the nanoparticles decreased with the increase in the current density. It can thus be said that the colloidal TiO_2 nanoparticles prepared at a current density of 10mA/cm^2 were bigger in size as compared to the colloidal TiO_2 nanoparticles prepared at a current density of 14mA/cm^2. As an evident from (Fig. 2d) the particles showed hardly any change in the absorption spectra even after a month of aging time, consistent with the highly stable nature of nanoparticles.

3.2. X-ray diffraction

The particle size of nanomaterials is related to the diffraction peak broadening, so X-ray diffraction spectra of synthesised TiO_2 nanoparticles were taken and particle size and phase composition were determined. Fig. 3 shows XRD pattern for TiO_2 nanoparticles. The lattice parameter observed $a=b=3.780$, $c=9.513$. The nanocrystalline anatase structure was confirmed by sharp peaks obtained corresponding to the planes (101), (004), (200), (105) (204), (220) and (215) indicates the tetragonal structure of TiO_2 nanoparticles. All peaks obtained were in good agreement with the JCPDS card no. 21-1272.

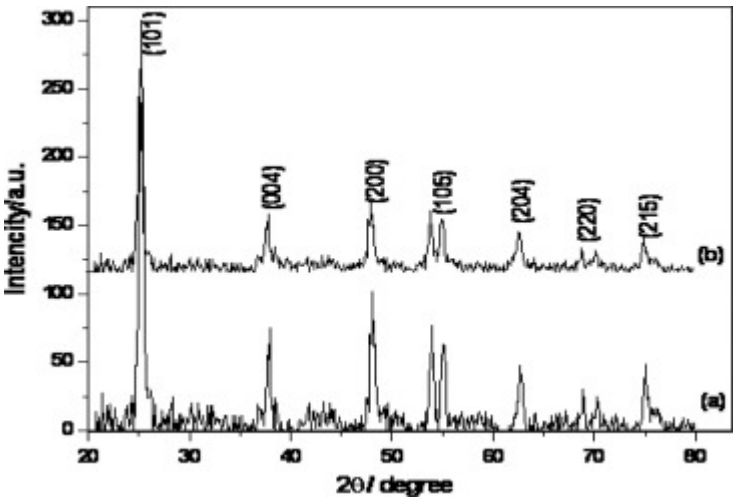

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Figure 3. XRD pattern of TiO_2 nanoparticles (a) corresponding to current density 10mA/cm^2 and (b) corresponding to current density 14mA/cm^2.

The average particle size calculated by using Debye–Scherrer Eq. (1) indicated high surface area.

$$d = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where K known as Scherrer's constant (shape factor), ranging from 0.9 to 1.0, λ is the wavelength of the X-ray radiation source which is 1.54056, β is the width of the XRD peak at half height and θ is Bragg angle. XRD plot of TiO_2 nanoparticles synthesized at 10 and 14 mA/cm^2 showed intense peak at (101) plane. The corresponding 2θ and FWHM values are 25.316° (0.3626°) and 25.306° (0.4392°) respectively and average crystalline size found to be 25 nm and 20 nm respectively.

3.3. SEM–EDS analysis

The SEM micrograph of TiO_2 nanoparticles synthesized at 10 mA/cm^2 (Fig. 4a) and 14 mA/cm^2 (Fig. 4b) showed there is dense agglomeration of particles and was irregular in shape. This was probably due to the partial solubility of the surfactant in the solvent under the given experimental conditions. TiO_2 nanoparticles synthesized with capping agent TPAB at both current densities were analyzed by EDS and are shown in Fig. 4a and b. The Ti and O peaks can be obviously found in both EDS spectra without any other peak like Br indicates that the pure TiO_2 particles are successfully prepared and there was complete removal of capping agent.

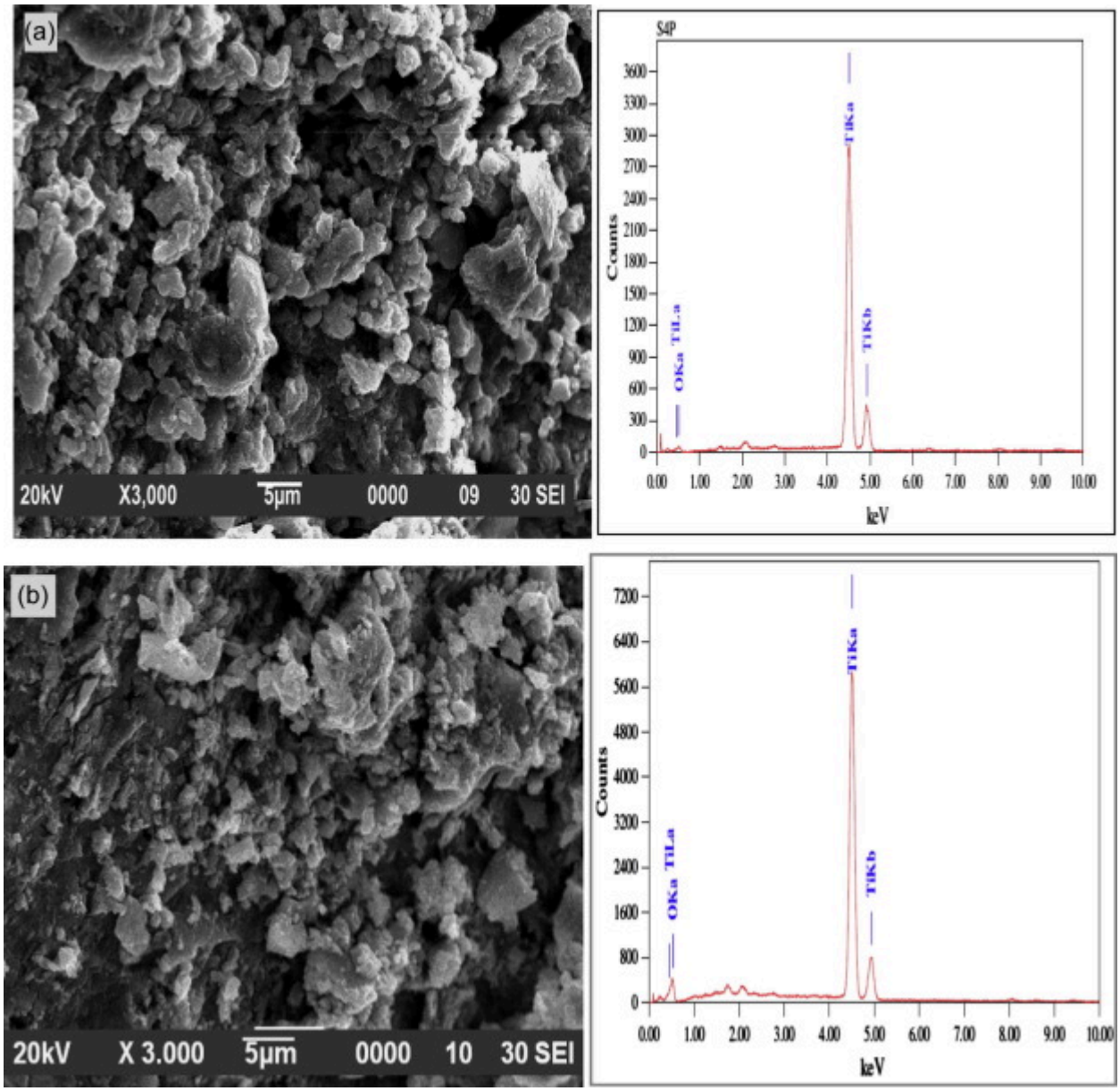

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Figure 4. SEM image of TiO_2 nanoparticles. (a) Corresponding to current density 10 mA/cm^2 with EDS spectrum and (b) corresponding to current density 14 mA/cm^2 with EDS spectrum.

3.4. TEM analysis

The size, shape and phase composition of particles were studied by TEM. The sample for TEM analysis was obtained by evaporation of very dilute alcoholic suspensions onto carbon-coated copper grids. A TEM image (Fig. 5a and b) along with histogram of particle size distribution of the typical product showed that TiO_2 nanoparticles were dispersed and no aggregation was observed. The magnified image showed that these TiO_2 nanoparticles were entirely tetragonal in shape. The corresponding particle size distribution of TiO_2 nanoparticles was in agreement with the results calculated from the

XRD analysis. The electron diffraction studies indicated that TiO_2 nanoparticles were highly crystalline, as the pattern could be indexed to the anatase phase only (right inset of Fig. 5a and b). The *d* spacing calculated from the electron diffraction pattern matches the values obtained from XRD patterns. The crystalline nature of the electrochemically-synthesized TiO_2 nanoparticles is evident from the absence of the diffuse halo normally associated with the presence of amorphous phases.

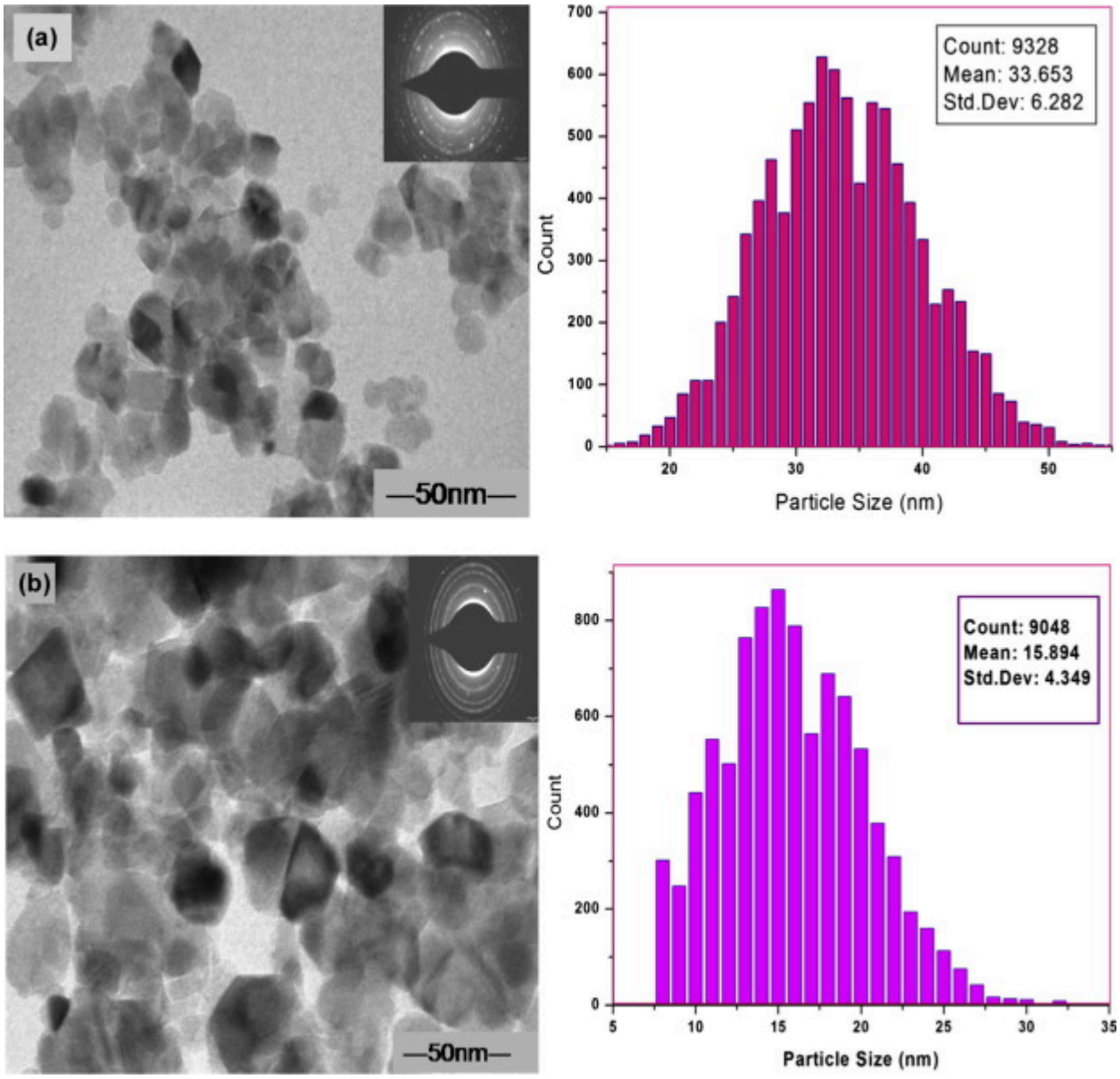

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Figure 5. TEM images of TiO_2 nanoparticles at two different current densities. The upper right inset in (a) shows the related SAED pattern, and the lower right inset is an enlarged TEM image of TiO_2 nanoparticles prepared at 10 mA/cm^2 along with histogram on right side and in (b) upper right inset shows the related SAED pattern, and the lower right inset is an enlarged TEM image of TiO_2 nanoparticles prepared at 14 mA/cm^2 along with histogram.

3.5. Antibacterial activity study

According to several studies, it is believed that the metal oxides carry the positive charge while the microorganisms carry negative charges; this causes electromagnetic attraction between microorganisms and the metal oxides which leads to oxidization and finally death of microorganisms (Zhang and Chen, 2009). They cause pits or holes of bacterial cell wall could be associated with internalized particles, leading to increased permeability and cell death (Ravishankar and Jamuna, 2011, Holt and Bard, 2005). TiO_2 nanoparticles due to their small size and high surface to volume ratio undergo a higher level of interaction with the bacterial cells surface than the larger particles, resulting in a high antibacterial activity. It was observed that TiO_2 nanoparticles synthesized at two different current densities showed good antibacterial activity.

The antibacterial activity of TiO_2 nanoparticles (synthesized at two different current densities) was carried out by agar well diffusion method against *S. aureus* and *E. coli* for two different concentrations of TiO_2 nanoparticles 50 µl and 100 µl and gentamicin. The antibacterial circle photos of all samples are shown in Fig. 6. It is well known that if the diameter of antibacterial circle of one sample is larger than 7 mm, it means that the sample has better antibacterial activity, however, if the diameter of antibacterial circle is equal to or less than 7 mm, it means that the sample has poorer antibacterial activity. From the results in Table 1 it can be seen that all samples have better antibacterial activity because their antibacterial circle diameter is much larger than 7 mm as well as the ACN/THF control did not show any antimicrobial activity against the tested bacterial strains. In addition, 100 µl concentration of TiO_2 nanoparticles is better as compared to 50 µl for inhibiting growth of bacterial test organism.

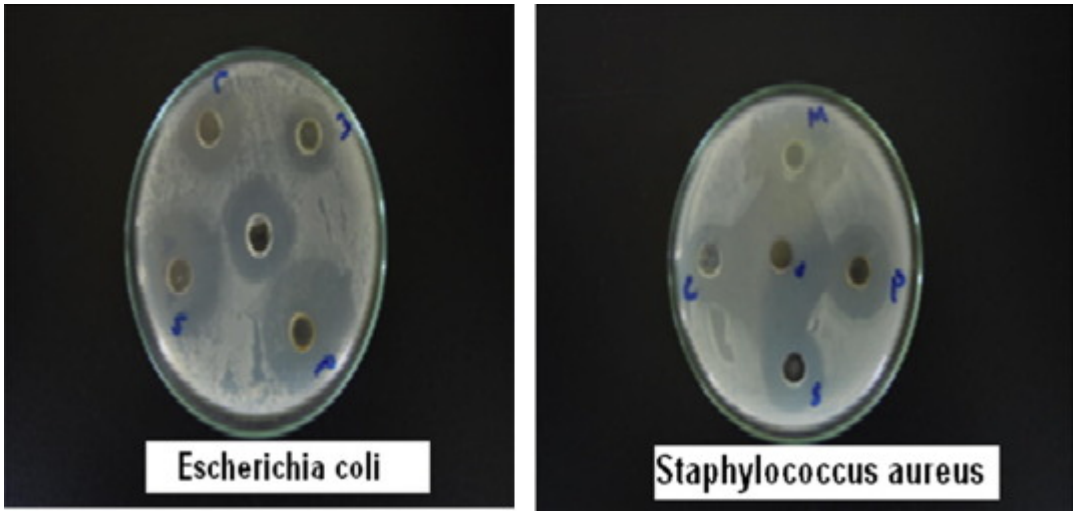

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Figure 6. Photographs of agar plate containing Gentamicin as standard antibiotics and different concentration of TiO_2 nanoparticles i.e. 50 µl and 100 µl of two different current

densities.

The TiO_2 nanoparticles proved to be very active on the tested Gram-positive strains, this differential sensitivity of Gram-negative and Gram-positive bacteria toward nanoparticles could be explained by the fact that the liquid medium is probably favoring the close interaction between the suspended nanoparticles and the Gram-positive microbial cells, which could better attach and anchor to the surface of the microbial cell, causing structural changes and damages leading to cell death (Chwalibog et al., 2012). The Gram-positive bacteria have a relatively thick wall composed of many layers of peptidoglycan polymer, and only one membrane (plasma membrane). The Gram-negative bacteria have only a thin layer of peptidoglycan and a more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane. The addition of the outer membrane of the Gram-negative bacteria cells influences the permeability of many molecules. Under certain conditions, the Gram-negative bacteria are more resistant to many chemical agents than Gram-positive cells. In addition, the cell walls of Gram-negative bacteria are more prone to mechanical breakage because of the low amount of peptidoglycan (Tortora et al., 2001).

It appears that the antibacterial activity of the nanomaterials increased with increase in surface-to-volume ratio due to the decrease in size of nanoparticles. It is clear from the XRD and TEM results that TiO_2 nanoparticles synthesized at 14 mA/cm^2 are smaller in size compared to TiO_2 nanoparticles synthesized at 10 mA/cm^2. Table 1 shows TiO_2 nanoparticles synthesized at 14 mA/cm^2 (15.99 nm) nanoparticles exhibited maximum (19.1 mm) bacterial growth inhibition against *S. aureus* and (17.0 mm) against *E. coli* in the form of zone-of-inhibition studies. In contrast, TiO_2 nanoparticles synthesized at 10 mA/cm^2 showed zones of inhibition of 17.2 mm against *S. aureus* and 14.3 mm against *E. coli* respectively.

4. Conclusions

The efficiency of electrochemical method for the synthesis of titanium dioxide nanoparticles at two different current densities is demonstrated. The procedure offers several advantages including control of particles size, excellent yields, operational simplicity and minimum environmental effects. UV–Vis spectra showed an absorption band at 362 nm and 346 nm which is blueshifted compared to the bulk anatase TiO_2, indicating the formation of nanoparticles solution. From XRD analysis it is clear that particle size decreased with increase in current density as the current density is proportional to the rate of formation of Ti ion reduction to Ti adatoms. TEM and SEM analysis proved a nearly tetragonal morphology of particles. EDX analysis revealed the presence of Ti, O element without any other peak like Br indicates that the pure TiO_2 particles are successfully prepared and there was complete removal of capping agent.

The broadest range of antibacterial activity showed by TiO_2 nanoparticles against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacterial reference may be due to those particles' small size, large surface area and more active sites for carrying out catalytic reactions. Obtained results recommend the use of TiO_2 as effective antibacterial agent in practical application.

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