Piperine Loaded Titanium Dioxide Nanoparticles: Development, Characterisation and Biomedical Application

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Abstract

TiO₂ nanoparticles were prepared by using electrochemical anodization method. UV-Vis absorption spectrum of TiO₂ and TiO₂: piperine nanoparticles have an absorption edge in the range of (340 -355) nm, suggesting that the TiO₂ colloidal obtained is anatase phase. The band gap energy (E_g) for TiO₂ nanoparticles (3.46 eV) is higher than the value of (3.20 eV) for bulk TiO₂. X-Ray diffraction results for TiO₂ nanoparticles with a wavelength of 1.54 Å were investigation in this work. Planes (101), (200), (111), (220), (210), (211), (105), (220), (310), (221), and (220) crystal planes all had peaks with the lattice constants a = 3.755 Å and c = 9.5114 Å confirms the anatase phases of the TiO₂ nanoparticles according to the JCPDS file 21-127231. Scanning Electron Microscope images of TiO₂ samples in revealed the prevalence spherical nanosized crystallities, where clear nanostructures with a grain size of 15 nm. In TEM image, the shape of the nanoparticles was sphericial, with small size variance and found to be 15 nm. The EDX study of the nanoparticle reflects the atomic percentage of elements such as Ti and O with a ratio of 83:17 and no other peaks are observed this confirm the presence of TiO₂ nanoparticles.

Keywords: TiO₂, TiO₂: piperine, SEM, Optical properties

Introduction

Piperine, a dietary polyphenol isolated from black and long peppers, distinguished with its intrinsic features, does not only improve curcumin’s existing anti-cancer activity, but also its extremely poor bioavailability (Teiten et al., 2010; Patial et al., 2015; Tang et al., 2017). Besides, piperine alone possesses anti-mutagenic and anti-tumor influences (Srinivasan, 2007; Chinta et al., 2015). Piperine has been widely reported to inhibit the growth of colon cancer cell lines by G1 arrest in cell cycle and by triggering apoptosis (Yaffe et al., 2015), and to enhance exhibition of antitumor activities in prostate cancer (Ouyang et al., 2013). Despite the proven antitumor activities of curcumin and piperine, the low solubility and the poor chemical stability of the compounds in water, largely limit their clinical applications. Targeted and triggered drug delivery systems accompanied by nanoparticle technology have been investigated as a prominent strategy to address these limitations (Bisht and Maitra, 2009; Ucisik et al., 2013b; Yallapu et al., 2013; Purpura et al., 2018; Wong et al., 2019). Polymeric nanoparticles (Anand et al., 2010; Bisht
lipid nanoparticles (Ucisik et al., 2012; Li et al., 2014; Mahran and piperine nanoformulations (Naksuriya et al., 2016) are among the mostly applied curcumin and copolymeric micelles (Gou et al., 2015), cyclodextrin nanoparticles (Ndong Ntoutoume et al., 2015; Dutta and Bhattacharjee, 2017), mixed and lipid nanoparticles (Ucisik et al., 2013b; Jourghanian et al., 2016) are among the mostly applied curcumin and piperine nanoformulations (Naksuriya et al., 2014; Mahran et al., 2017). As a lipid-based, safe and surfactant-free system, emulsomes emerge as a promising drug delivery system among the alternatives [1]. TiO2 exists in three different crystalline habits: rutile (tetragonal), anatase (tetragonal) and brookite (orthorhombic). Both anatase and rutile have tetragonal crystal structure but belong to different phase groups. Anatase has the space group I41/amd [4] with four formula units in one unit cell and rutile has the space group P42/mnm [2] with two TiO2 formula units in one unit cell [3]. The low-density solid phases are less stable and undergo transition rutile in the solid state. Rutile TiO2 has some advantages over anatase phase, such as higher refractive index, higher dielectric constant, higher electric resistance and higher chemical stability. The transformation is accelerated by heat treatment and occurs at temperature degrees in the range of (450-1200) °C [4]. This transformation is dependent on several parameters such as initial particle size, initial phase, dopant concentration, reaction atmosphere and annealing temperature [5, 6]. Among several metal oxide nanoparticles, titanium dioxide nanoparticles (TiO2 NPs) are non-toxic with oxidation potency and elevated stability to light resulting into their broad applications in environmental remediation [7, 8]. In addition, TiO2 NPs possess fascinating dielectric, optical, antimicrobial, chemical and catalytic properties which lead to industrial applications such as cosmetics, pigment, fillers, whitening and brightening of foods, in personal care products like toothpaste, and photocatalyst [9-14]. The vital applications of nano TiO2 are photocatalytic degradation and splitting; PV cells, electrochromic and electronic devices, and sensing instruments have heartened huge interest and widespread advancement for synthesis of TiO2 NPs [15-18]. TiO2 NPs have been widely used in daily life and can be synthesized through various physical, chemical, and green methods [19]. Various chemical synthesis techniques are largely employed for the synthesis of TiO2 nanoparticles such as sol-gel method [20], solvo-thermal method [21] co precipitation method [22], and hydrothermal method [23]. Green TiO2 NPs have good points rather than chemically synthesized NPs as they have a good antifungal as well as antimicrobial activity [24-26].

The purpose of the present manuscript is the determination of an improved chemical synthesis of TiO2 NPs using piperine with conditions that could be easily reproducible in industry, both in terms of energy saving and cost reduction.

Experimental

Experimental Section

Titanium Tetrachloride TiCl4 99.99% and absolute ethanol CH3CH2OH 99.99% for producing TiO2 nanoparticles by adding drop wise from TiCl4 in ethanol with 1:10 ratio under centrifuge with 8 hour. The reaction was performed at room temperature while stirring under fume hood due to the large amount of Cl2 and HCl at 30 min. The solution was left to rest and cool back at room temperature for 2 hour, then after that measured the pH of the solution in the range of (1-2). Before electrochemical anodization, titanium (Ti) foils (250 μm thick, purity 97%) with a size of 1 cm×2.5 cm were degreased by ultra-sonication in a mixture (15 mL) for each one of acetone, methanol, and methylene chloride for 30 min, followed by washing with a large amount of distilled water and drying with N2. Electrochemical anodization was carried out in a two-electrode cell using a power source PS-3030, where the Ti foil was used as the anode and cathod electrode after annealing at 800 °C for 4 hour. Anodization electrolytes were fabricated by using above solution TiO2 with 30 volt. Each potential static anodization was performed under the room temperature of ~23 °C, after a certain period of anodization, 2 hour; The final solution was dried at 80 °C until powder was formed. The obtained TiO2 powder was calcined for two hours in the box furnace at 500 °C in an ambient atmosphere in this temperature getting TiO2 nanoparticles in anatase phase when increasing the temperature degree to 900 °C the phase transformation from anatase to rutile in TiO2 powder [27]. TiO2: piperine nanostructure construction using (2.5 gm in 50 mL distilled water) of TiO2 nanoparticles dropped in (2.5 gm in 50 mL distilled water) Piperine ratio 50:50 mL.

Measurements and characterization

X-Ray diffraction (XRD) pattern

In order to explain the structural properties, the nature and the crystal growth of TiO2 nanoparticles,
X-ray diffraction measurement was carried out done according to the ASTM (American Society of Testing Materials) cards, using (Philips pw 1050 X-ray diffractometer of 1.54 Å from Cu-ka, Japan). Cu radiation source, at a scanning speed of 2 min⁻¹, 40 kV tube voltage, and 30 mA tube current.

The crystallinity of synthesized material, the average particle (green) size (Dg) of TiO₂ NPs has mostly calculated by using the Debye–Scherrer’s equation after the successful synthesis [28, 29]. The Scherrer’s equation is considered as the most fundamental and widely used equation to calculate the particle size by the combination of 2θ and FWHM values from the XRD data.

\[ D = \frac{K\lambda\beta\cos\theta}{4\tan\theta} \]  

In this equation, D represents the Particle size (Diameter), K is Scherrer’s constant (0.9), \( \lambda \) = 0.15406 nm (Wavelength of X-ray source), \( \beta \) represents Full width at Half-maximum intensity (FWHM) in radians and \( \theta \) is used to denote the Peak positions in (Radians), and \( \theta \) is the Bragg’s diffraction angle of the respective XRD peak.

Scanning electron microscopy and energy dispersive spectroscopy (EDSX)

The scanning electron microscope (SEM) is a type of electron microscope using an electron beam energy of 15 keV and a beam current of 2.62 A. The SEM study has been carried out by Hitachi (S-4160) scanning electron microscope the magnification power continuous form 6x to 100,000x. The setting in thin film laboratory at University of Tehran. Scanning electron microscopy (SEM) images of the same samples were recorded with a LED-1430VP microscope using an electron beam energy of 15 keV and a beam current of 2.62 A.

Transmission electron microscopy

Transmission Electron Microscope ((TEM) JEOL JEM 14000, Japan) was used for investigating the size and shape of nanoparticles.

Optical properties measurements

Optical transition measurement: A double-beam (UIR-210A spectrophotometer from Shimadzu, Japan) was used in order to record the optical transmission and absorption spectra of the TiO₂ nanoparticles within the wavelength range (300-900 nm). It is generally used for characterizing various metal nanoparticles in the size range of 2-100 nm and more [30, 31]. The optical band gap was estimated graphically by applying the Tauc model; the band gap of the prepared material with sharp fall off can be deduced from a plot of the squared absorption coefficient (\( \alpha \)hν)² versus photon energy (hν) by extrapolating the straight line of the plot to intersect the energy axis. UV-vis absorption measurements were recorded on UV-vis Spectrophotometer-UVD-3200 (LABOMED, Los Angeles, CA, USA). Furthermore, UV-vis spectrum of TiO₂ is used to calculate the band gap energy (Eg) using well known Tauc’s equation [32, 33].

\[ \alpha h\nu = A(h\nu - E_g) \]  

Where \( \alpha \): absorption coefficient, h: Planks constant (6.626 × 10⁻³⁴ J s), \( \nu \): light frequency, A: constant, E₉: optical band gap energy value of the semiconductor TiO₂.

FTIR measurement

Fourier Transform-Infrared Spectroscopy (FTIR) probes the molecular vibrations of molecules. Light of different energies (or frequency, represented by wavenumbers in the spectrum above) is directed through a sample. When a particular energy (or frequency) of light matches a vibrational frequency of the molecule, the molecule absorbs the light and vibrates. Peaks in an infrared spectrum are upside-down compared to other forms of spectroscopy to convey that the peak is a decreased intensity, or absorbance of light. The (SHIMADZU- 8400S, Koyoto, Japan) Scan of the FTIR measurements are performed over the range between (400-4000) cm⁻¹ for the prepared sample.

Results and Discussions

X-Ray diffraction results for TiO₂ nanoparticles

Figure 1 shows the X-Ray diffraction results for TiO₂ nanoparticles using X-rays with a wavelength of 1.54 Å. Planes (101), (200), (111), (220), (211), (105), (220), (310), (221), and (220) crystal planes all had peaks with the lattice constants a = 3.755 Å and c = 9.5114 Å confirms the anatase phases of the TiO₂ nanoparticles according to the JCPDS file 21-1272 31. X-ray diffraction profiles are usually influenced by crystallite size and lattice strain. The fact that the crystalline sizes of the samples are very small causes the peaks to broaden. Moreover, the intensities diffraction peaks decreases and becomes broader for TiO₂: piperine nanoparticles indicating smaller particle size [34], which is important for quality and decreasing
TiO₂ toxicity.

LD₅₀ measurement of nanoparticles toxicity for 60 days was measured after observing the number of dead mice. Changes in the weight of the mice and its organs including Brain, Heart, liver and kidneys were evaluated due to the administration of various doses of the TiO₂: Piperine nanostructure according to the organ index formula:

\[
\text{Organ Index} = \frac{\text{Wt. of experimental organ}}{\text{Wt. of the experimental mice}} \div \frac{\text{Wt. of control organ}}{\text{Wt. of the control mice}}
\]

### Scanning Electron Microscope (SEM) images of TiO₂ samples

Scanning Electron Microscope (SEM) images of TiO₂ nanoparticles are shown in Fig. 2(a) and 2(b). Clear nanostructures with a grain size of 15 nm can be observed. The TiO₂ nanoparticles seen in the SEM picture clearly contain a number of crystallites (see Fig. 2(a)). The grain size of TiO₂ nanoparticles characterized in XRD-Diffractometer by Debye-Sherrer equation is smaller than the results observed by SEM as compared to XRD results. As the solution stringed in a short time, the TiO₂ nanoparticles agglomerated, as shown by scanning electron microscopy (SEM) images. All scattered particles with a size less than 17 nm were seen in these images. By using SEM analysis, TiO₂: piperine NPs are in a spherical shape with small pores and normally found in the cluster that forms the bunch type surface (Fig. 2(b)).

### Transmission Electron Microscope (TEM) images of TiO₂ samples

TEM images were used to assess the morphology, crystallinity, and size of synthesized TiO₂ and TiO₂: piperine nanoparticles. The shape of the nanoparticles was spherical and small size variance (see Fig. 3). The scale was between 10 and 20 nanometers. The nanoparticles were found to be 15 nm in size on average. Synthesized TiO₂ and TiO₂: piperine nanoparticles.

### Table 1 Organ index of Brain, Heart, liver and kidneys of mice exposed to TiO₂: Piperine for 60 days compared with normal organs obtained from the control mice

<table>
<thead>
<tr>
<th>groups</th>
<th>Brain</th>
<th>P value</th>
<th>Heart</th>
<th>P value</th>
<th>Liver</th>
<th>P value</th>
<th>kidney</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.93 ± 0.058</td>
<td>p &gt; 0.05</td>
<td>1.01 ± 0.064</td>
<td>p &gt; 0.05</td>
<td>1.26 ± 0.048</td>
<td>p &gt; 0.05</td>
<td>1.04 ± 0.049</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>2 mg/mL</td>
<td>0.95 ± 0.053</td>
<td>p &gt; 0.05</td>
<td>1.20 ± 0.055</td>
<td>p &gt; 0.05</td>
<td>1.11 ± 0.057</td>
<td>p &gt; 0.05</td>
<td>1.13 ± 0.059</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>4 mg/mL</td>
<td>1.12 ± 0.065</td>
<td>p &gt; 0.05</td>
<td>0.95 ± 0.049</td>
<td>p &gt; 0.05</td>
<td>1.13 ± 0.054</td>
<td>p &gt; 0.05</td>
<td>0.94 ± 0.066</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>6 mg/mL</td>
<td>1.12 ± 0.058</td>
<td>0.96 ± 0.050</td>
<td>1.15 ± 0.057</td>
<td>1.10 ± 0.063</td>
<td></td>
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Figure 1 X-Ray diffraction results for TiO₂ and TiO₂: piperine nanoparticles.

Figure 2 (a) SEM results for TiO₂ nanoparticles; (b) SEM results for TiO₂: piperine nanoparticles.
nanoparticles are in anatase process with strong crystallinity and dotted concentric circles, which can be assigned to the spherical form of TiO$_2$, as determined by XRD analysis. All of this is depicted in Fig. 3. The diameter of the TiO$_2$ particles was noticed to be smaller for piperine modified TiO$_2$ nanoparticles (8-10 nm) (Fig. 3(b)) as compared to chemically prepared TiO$_2$. The TEM results were found to be in good agreement with the XRD measurements.

**EDXS results of TiO$_2$ samples**

EDX analysis was used to determine the elemental composition of the TiO$_2$ nanostructure, as shown in the Fig. 4. The EDX study of the nanoparticle (installed in SEM) reflects the atomic percentage (percentage) of elements such as Ti and O, which are clearly visible in the images with a ratio of 83:17 and no other peaks are observed this confirm the presence of TiO$_2$ nanoparticles.

**Absorption**

The effect of growing conditions on the optical properties of prepared films has been thoroughly investigated. Figure 5(a) and 5(b) shows that the UV-Vis absorption spectrum of TiO$_2$ and TiO$_2$: piperine nanoparticles solution has an absorption edge in the range of (340-355) nm, suggesting that the TiO$_2$ colloidal solution obtained is anatase phase. The band gap energies ($E_g$) of the prepared TiO$_2$ nanoparticles (3.46 eV) are higher than the value of (3.2 eV) for bulk TiO$_2$, as shown in Fig. 5. This can be explained by the fact that the band gap of semiconductors is particle size dependent. The band distance widens as particle size decreases, and the absorption edge shifts to a higher energy (blue shift) as particle size decreases. The absorption onset of the present samples can be attributed to the direct transfer of electrons in TiO$_2$ nanocrystals, based on the blue change of the absorption location from bulk TiO$_2$. The absorption spectrum of UV-Visible (wavelength versus...
absorbance) exhibits two peaks at wavelength of 260 nm and 325 nm, that its unique of TiO$_2$ NPs well in an agreement with the reported results [35, 36]. UV-vis spectroscopy has also been used for the confirmation of TiO$_2$ NPs formation in aqueous solution.

**Fourier transformation-Infrared spectroscopy (FTIR) results of TiO$_2$ nanoparticles**

The results of Fourier transformation-Infrared spectroscopy provide detail. About the structure of the phases and how oxygen binds to metal ions. The FTIR spectra of TiO$_2$ nanoparticles are shown in the figure below (see Fig. 6). The peaks in the FTIR spectrum of TiO$_2$ nanoparticles corresponding to 3454 cm$^{-1}$ are due to the stretching of the H-bond of the O-H (Alcohol) group, as shown in Fig. 6. C=C corresponds to the peaks seen at 1627 cm$^{-1}$ and 1419 cm$^{-1}$ (medium weak multiple bands). The stretching and vibration modes of O–Ti–O are shown in the TiO$_2$ sample by the peaks corresponding to 665 cm$^{-1}$ and 648 cm$^{-1}$. As Ti atoms collide with oxygen, the mean kinetic energy of the Ti atoms decreases, resulting in the formation of (O$^{2+}$) ions (through energetic change reaction of Ti with O$_2$ molecules). As a result, chemical bonding such as (O–Ti–O) stretching modes are formed.

This absorption peak in between the wavenumber range 650-700 cm$^{-1}$ is the characteristic peak of
TiO$_2$ NPs anatase phase as cited in the literature [26, 37]. The peaks appearing in between the range of 1000-1300 cm$^{-1}$ can be associated to mainly Ti–O–Ti vibrations which clearly showed O–Ti–O bond. According to literature, the FTIR spectrum of TiO$_2$ nanomaterials prepared with and without CHE present similar FTIR spectra with peak around 450 cm$^{-1}$ assigned to stretching vibration of Ti–O–Ti bonds. The broad band at 3428 cm$^{-1}$ can be ascribed to the –OH stretching vibration of the adsorbed water molecules on TiO$_2$ surface and the peak around 1630 cm$^{-1}$ is attributed to the bending vibrations of –OH.

**PL spectra**

To study the separation efficiency of photogenerated electrons and holes, the room temperature PL spectra of the as-synthesized composite structure TiO$_2$ were carried out, respectively. Figure 7 exhibits the PL spectra of all samples. The decrease of PL intensity indicates the efficient electron-hole separation and long-lived carriers.

**Antibacterial Activity of Nanoparticles**

The antibacterial activity of the nanoparticles was investigated using *S. aureus* and *E. coli*. The zones of inhibition after exposing the organisms to different concentrations of nanoparticles were measured and presented in Figs. 8(a) and 8(b). The antibacterial activity of TiO$_2$ NPs and TiO$_2$: pilipine was tested on the following Bacteria *S. aureus* and *E. coli*. A figure reveals the zone of inhibition diameter of TiO$_2$ NPs and TiO$_2$: pilipine NPs on bacteria strains, in which that TiO$_2$: pilipine NPs exhibited higher effect on *E. coli* and *S. aureus*. The inhibition zone increased significantly with increasing the concentration of TiO$_2$: pilipine NPs. *S. aureus* was more sensitive to TiO$_2$: pilipine NPs.

![Fig. 7 PL spectra for TiO$_2$ nanoparticles.](image1.png)

**Fig. 7** PL spectra for TiO$_2$ nanoparticles.

![Fig. 8 The antibacterial activity of the nanoparticles was investigated using *S. aureus* and *E. coli* (a) TiO$_2$ NPs and (b) TiO$_2$: Pilipine NPs.](image2.png)

**Fig. 8** The antibacterial activity of the nanoparticles was investigated using *S. aureus* and *E. coli* (a) TiO$_2$ NPs and (b) TiO$_2$: Pilipine NPs.
piperine NPs than *E. coli*. *E. coli* is a Gram-negative bacterium and it is less susceptible to antimicrobials than Gram-positive bacteria like *S. aureus* because it is more resistant to lipophilic and amphiphilic inhibitors than those Gram positive, including dyes, detergents, free fatty acids, antibiotics and chemotherapeutics agents may be attributed to the presence of the outer membrane. The pore channels slowed the penetration of small hydrophilic solutes and the low fluidity of the lipopolysaccharide layer decreased the rate of Trans membrane diffusion of lipophilic solutes.

**Conclusions**

The synthesis of TiO$_2$ NPs characterized by SEM, TEM, FE-SEM, EDX, various results confirmed the spherical shape and clustered form of TiO$_2$ NPs. SEM images TiO$_2$ sample prepared chemically showed agglomeration of TiO$_2$ particles comparing to piperene modified TiO$_2$ particles. The diameter of the TiO$_2$ particles was noticed to be smaller for piperene modified TiO$_2$ nanoparticles (8-10 nm) as compared to chemically prepared TiO$_2$. The TEM results were found to be in good agreement with the XRD measurements. The intensities diffraction peak decreases and becomes broader for TiO$_2$: piperine nanoparticles indicating smaller particle size which is important for quality and decreasing TiO$_2$ toxicity.

**Conflict of Interests**

The authors declare that no competing interest exists.

**References**


[21] Ramakrishnan, V.M., Natarajan, M., Santhanam, A.,


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