Chapter 8

Effect of nickel doping in TiO$_2$ and its photocatalytic antibacterial activity

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“Nobody ever figures out what life is all about, and it doesn't matter. Explore the world. Nearly everything is really interesting if you go into it deeply enough.”
— Richard P. Feynman
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8.1 Introduction

Antibacterial agents are used extensively in hospitals, health care settings, water and air purifications. Modified TiO₂ based photocatalytic disinfection systems are a promising and alternative technology as compared to conventional methods. Conventional methods are time intensive, short term effective, and cannot be standardized. Recently, many studies have been done to improve photocatalytic properties of TiO₂ by doping with transition metal elements. Transition metal ions can provide additional energy levels within the band gap of a semiconductor. Electron transfer from one of these levels to the conduction band requires lower photon energy than in the situation of an unmodified TiO₂ [1]. Although the use of TiO₂ in photocatalytic disinfection and degradation of dyes have been studied extensively, but there is no report on photocatalytic disinfection using Ni–doped TiO₂ (Ni–TiO₂) under visible light irradiation. In the present chapter, photocatalytic antibacterial activity of Ni–TiO₂ nanoparticles has been discussed. We reveal that Ni–TiO₂ nanoparticles exhibit efficient photocatalytic antibacterial activity against gram–positive Staphylococcus aureus, (S. aureus) Bacillus subtilis (B. subtilis) and gram–negative Escherichia coli (E. coli), Salmonella abony (S. abony) under fluorescent visible light irradiation.

8.2 Characterizations

The X–ray diffraction patterns (XRD) of the samples were recorded on a Bruker AXS D8–Advance X–ray diffractometer with Cu–Kα radiation of wavelength 1.5406 Å in 2θ range from 20° to 80°. X–ray photoelectron spectroscopy (XPS) was used for determining the surface compositions of the photocatalysts, using a Physical Electronics 5600 Multi–technique System with monochromatic Al Kα radiation. UV–Visible absorbance spectra of all
samples were obtained using a UV–visible spectrophotometer (UV3600, Shimadzu, Japan) in the range of 200–800 nm. TEM images of the samples were recorded on a Tecnai F30 field emission transmission electron microscope operating at 300 kV. Elemental composition was determined from EDS analysis. The photoluminescence (PL) spectra of the sample was recorded by using JASCO F.P.–750 Model, (Japan) spectrofluorometer.

8.3 Photoassisted inactivation of bacteria with Ni–TiO$_2$ nanoparticles

For the photocatalytic inactivation test, four common pathogenic bacteria, gram positive *Staphylococcus aureus*, *Bacillus subtilis* and gram negative *Escherichia coli*, *Salmonella abony* were used.

For all experiments, a suspension of each bacterial species with a concentration of ~ $10^8$ cfu mL$^{-1}$ (identified by UV–Vis spectrophotometer) was used. For photocatalytic inactivation experiment, a multilamp borosilicate glass reactor having eight fluorescent tubes (Philips 8 W WW T5, $\lambda$>400nm) was used. All samples were exposed to a light intensity of ~0.5 mW cm$^{-2}$. For all experiments, Ni–TiO$_2$ nanoparticles (1.0 g L$^{-1}$) were suspended in 5 mL saline (0.9% of NaCl at pH 7.0) containing bacterial suspension and exposed to visible light. 100µL of bacterial suspension was pipetted at regular time intervals and spread on freshly prepared Mueller–Hinton agar plates and incubated at 37°C for 24 h. Then the grown colonies were counted. During all these experiments, control test were performed under the same irradiation conditions without nanoparticles and a dark experiment with nanoparticles without light exposer was carried out simultaneously. Each set of experiments was performed in triplicate.
8.4 Results and discussion

8.4.1 X-ray diffraction studies

Figure 8.1 shows the XRD patterns of Ni–TiO$_2$ nanoparticles. The diffraction peak at $2\theta = 25.07, 37.59, 47.98, 53.72, 54.86, 62.58, 68.54, 70.05, 75.05, 82.65$ corresponds to the $(101), (004), (200), (105), (211), (204), (116), (220), (215), (224)$, respectively for plane of tetragonal anatase TiO$_2$ (JCPDS 21–1272). There is no peak which corresponds to rutile phase or oxide of the dopant metal ions. The crystallite size was calculated using Debye–Scherre formula. The average crystallite sizes are 9.67 nm, 8.59 nm, 7.96 nm for 1.0 mol%, 2.0 mol%, 3.0 mol% doping of Ni$^{2+}$ in TiO$_2$, respectively. It can be seen that, with increasing dopant concentration the crystallite size decreases. The decrease in crystallite size can be correlated to increase in structural defects that prevent particle growth [1]. The ionic radius of Ni$^{2+}$ is different from that of Ti$^{4+}$. Doping of Ni$^{2+}$ generates oxygen vacancies in the lattice of TiO$_2$ to maintain charge neutrality. In this work, the nickel dopant is incorporated by replacing few Ti$^{4+}$ ions in TiO$_2$ crystal lattice. These results clearly confirm the successful substitution of few Ti$^{4+}$ ions by Ni$^{2+}$ ions.
8.4.2 X–ray photoelectron spectroscopy

The valance state, substitution and contents of Ni$^{2+}$ ions in TiO$_2$ matrix were examined by XPS analysis as shown in Figure 8.2. The XPS survey scan spectrum of the Ni3–TiO$_2$ sample shows the existence of Ni, O and Ti ions in an almost stoichiometric composition as shown in Figure 8.2(a). Figure 8.2(b) shows the core level Ti 2p spectrum of Ni–TiO$_2$ sample. For TiO$_2$, Ti 2p$_{3/2}$ and Ti 2p$_{1/2}$ peaks are observed at 457.21 and 462.94 eV, respectively. The splitting between the Ti 2p$_{1/2}$ and Ti 2p$_{3/2}$ is 5.73 eV, demonstrating a normal state of Ti$^{4+}$ in the sample. Figure 8.2(c) shows the peaks at 529.44 eV which is
characteristic of O 1s electron binding energy arising from titanium lattice while peak at 531.47 eV is attributable to the surface absorbed hydroxyl species. The XPS spectrum shows complex structure 853.69 eV, 861.08 eV, for Ni2p\textsubscript{3/2}, and 872.34 eV, 877.96 eV for Ni2p\textsubscript{1/2}, respectively and theses are attributed due to the multiplet splitting. The peak at 861.08 eV is due to the O 2p → Ni 3d charge transfer transitions [3]. The binding energy of a Ni atom is deduced to be 853.69 eV on TiO\textsubscript{2} host lattice and which is 7.39 eV smaller than that of atomic Ni in the gas phase [4]. The Ni 2p\textsubscript{3/2} peak position at 853.69 eV is quite different from that of metallic Ni (852.7 eV), NiO (853.8 eV) and Ni\textsubscript{2}O\textsubscript{3} (856.7 eV). The binding energy difference between Ni 2p\textsubscript{3/2} and Ni 2p\textsubscript{1/2} core level is 18.65 eV, which is different from the value of metallic Ni (17.27 eV) and NiO (17.49 eV) [5–7]. The change in the Ni2p peak and a shift of Ti2p peaks corresponds to the rearrangement of Ti\textsuperscript{4+} ions and Ni\textsuperscript{2+} ions. These results give evidence that the few Ti\textsuperscript{4+} ions are successfully substituted by Ni\textsuperscript{2+} ions without forming any detectable impurity phase, such as Ni, Ni\textsubscript{2}O\textsubscript{3} and NiO. Moreover, XPS results are correlated with the EDS results, confirming the existence of Ni\textsuperscript{2+} ions upto 3.0 mol\% dopant concentrations into TiO\textsubscript{2} host lattice.
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(a) Survey Spectrum

(b) Ti(2p) Spectrum
Figure 8.2 XPS for the Ni3–TiO2 (a) survey; (b) Ti 2p; (c) O 1s configuration; (d) Ni 2p configuration.
8.4.3 UV–Visible diffuse reflectance spectroscopy

Figure 8.3 shows the UV–Visible absorption spectra in the range of 300–700 nm for nickel doped TiO$_2$ nanoparticles. The absorption spectra of the doped samples show a stronger visible light absorption indicating that the band gap was decreased upon doping. This visible light absorption ascribed the new energy level for Ni$^{2+}$ below the conduction band and above the valance band edge of TiO$_2$ [8]. Such new energy levels and oxygen vacancies generated by metal doping, induce the bathochromic shift in the band gap transition and the visible light absorption through a charge transfer between a dopant and conduction or valance band or a d–d transition in the crystal field according the energy level [9–11]. The calculated band gap energy for Ni1–TiO$_2$, Ni2–TiO$_2$, and Ni3–TiO$_2$, is 2.95, 2.58, and 2.36 eV, respectively. After doping with nickel metal ions in TiO$_2$, the absorption is shifted in the visible light region due to introduction of new energy level in TiO$_2$ semiconductor. This causes the color change in the doped samples from white to pale yellow.

![UV–Visible diffuse reflectance spectra of Ni–TiO$_2$ samples.](image)

Figure 8.3 UV–Visible diffuse reflectance spectra of Ni–TiO$_2$ samples.
8.4.4 Transmission electron microscopy and EDS analysis

TEM image of the Ni3–TiO2 nanoparticles and corresponding SAED pattern are shown in Figure 8.4(a) and 8.4(b), respectively. It is evident from TEM micrograph that the synthesized nickel–doped TiO2 have uneven and non–spherical particles. The particle size was obtained from TEM micrograph within the range 8–10 nm. This is in close agreement with the average crystallite size obtained from XRD analysis. The homogeneous distribution of nickel in TiO2 matrix was confirmed form TEM micrograph. SAED pattern confirms the crystalline nature of Ni3–TiO2 nanoparticles.

EDS analysis was performed to ensure the existence of nickel in TiO2 matrix. Figure 8.4(c) show the EDS spectrum of Ni3–TiO2 nanoparticles which indicates nickel metal ions have been successfully integrated into TiO2, in an atomic ratio very close to that mentioned in the experimental section for synthesis of Ni–TiO2 nanoparticles.
Figure 8.4 (a) TEM image of Ni₃–TiO₂; (b) SAED pattern of Ni₃–TiO₂

Figure 8.4 (c) EDS spectrum of Ni₃–TiO₂ nanoparticles
8.4.5 Photoluminescence spectroscopy

The PL spectra show broad emission peaks in the region of 440–600 nm. These emission signals are due to the surface defects and the charge transfer transition from an oxygen vacancy trapped electron [12]. Interestingly, the PL spectra of Ni–TiO$_2$ samples show a gradual decrease in the peak intensity with increasing nickel concentration in TiO$_2$. The surface defects reduce the recombination rate of photogenerated charge carriers, the excited electron are trapped by the oxygen vacancies and holes are trapped by dopant metal ions. Additionally, the excited electrons can migrate from the valance band to the new energy levels introduced nearer to the conduction band by nickel doping, and also reduce, the PL intensity [13]. Our PL results reveal that lower the PL intensity, higher is the photocatalytic inactivation activity.

![PL spectra of Ni–TiO$_2$ samples](image)

**Figure 8.5** PL spectra of Ni–TiO$_2$ samples
8.5 Effect of nickel–doping on photocatalytic inactivation of bacteria

In previous chapter, we found the highest photocatalytic antibacterial activity of copper–doped TiO$_2$ nanoparticles under visible light irradiation against *S. aureus* and *E. coli* bacteria. Metal modified TiO$_2$ photocatalyst attracted more attention because of its extended visible light absorption. For this reason, in this work, the inactivation of different bacterium with nickel–doped TiO$_2$ was investigated in presence of visible light.

We characterized the photocatalytic inactivation of the different gram strain bacteria with Ni–TiO$_2$ (Ni1–TiO$_2$, Ni2–TiO$_2$, and Ni1–TiO$_2$) nanoparticles. The photocatalytic inactivation of the bacterial strains was increased with increased nickel dopant concentration in TiO$_2$ host lattice. The survival number of all bacteria species is not affected in dark (with nanoparticles) and light condition (without nanoparticles). This reveals that antibacterial activity testing method itself did not inactivate bacteria during experiment. This characteristic pattern is observed in all tested bacterial strains.

For each of the species under this investigation the reduction in colony forming units following irradiation in the presence of Ni–TiO$_2$ are displayed in Figures 8.6 – 8.9. Bacterial inactivation was observed for all four bacterial samples in presence of Ni–TiO$_2$ with light only. For all four bacteria, Ni3–TiO$_2$ nanoparticles show efficient photocatalytic inactivation than Ni2–TiO$_2$ and Ni1–TiO$_2$ nanoparticles. This might be due the band gap narrowing as a result of new generated energy levels within the TiO$_2$ band gap which induces more visible light absorption.

Figure 8.6 shows the inactivation of gram positive *S. aureus* by the photocatalytic antibacterial activity of Ni–TiO$_2$ nanoparticles after visible light exposer. In dark test, *S. aureus* cells were not inactivated by contact with Ni–TiO$_2$ within the experimental time scale. No *S. aureus* inactivation was
observed under visible light alone within the experimental time scale. However, *S. aureus* inactivation was observed when visible light was irradiated in presence of nanoparticles. After 2 h light irradiation, a significant level of *S. aureus* inactivation was observed. After 3 h few colonies were still found, while for complete inactivation of *S. aureus* 4 h light irradiation is required. There are two major stages in the inactivation of *S. aureus*: slow inactivation within first 2h followed by a faster inactivation after 2 h of light irradiation.

Similar experiment performed for gram positive *B. subtilis* inactivation under visible light irradiations using Ni–TiO$_2$ samples (Figure 8.7). In dark and control experiment, the viability of *B. subtilis* did not seem to be affected within experimental time scale. More than 70% bacteria were inactivated within 120 min of irradiation by Ni3–TiO$_2$ sample.

![Figure 8.6 Inactivation of S. aureus as function of time](image)
Additionally, photocatalytic inactivation of two gram negative species was also evaluated in this study. Figure 8.8 shows the survival of *E. coli* as a function of time. The 100% reduction time for *E. coli* at initial cell concentration of $2.7 \times 10^4$ cfu mL$^{-1}$ was 300 min (Figure 8.8). Photocatalytic inactivation of *S. abony* under visible light irradiation is shown in Figure 8.9. In case of *S. abony*, the complete inactivation was observed within 360 min of light irradiation. The time required for complete inactivation of *S. abony* is higher than the time required for *E. coli* inactivation.
**Figure 8.8** Inactivation of *E. coli* as function of time

**Figure 8.9** Inactivation of *S. abony* as function of time
There are some controversies in the literature regarding the photocatalytic inactivation of the microorganisms due to different experimental settings [14]. It is well known that the photokilling of bacteria is caused by the attack of reactive oxygen species [15, 16]. It is proposed that the photocatalytic killing mechanism first damages the bacterial cells membrane. The internal bacterial components then leak from the cells and ultimately, the photocatalytic reaction oxidizes the cell components [17–19]. For a semiconductor photocatalyst, when energy is provided larger than the band gap, the electron / hole pairs are generated and react with O$_2$ and H$_2$O to form superoxide anion radicals (O$_2^.-$) and hydroxyl radicals (-OH). These oxidative species (h$^+$, -OH, and O$_2^.-$) are all highly reactive, which are considered to be the dominant oxidative species contributing to the mineralization of microorganism cells [9].

The results obtained in the current study showed that the rate of inactivation of gram positive species using Ni–TiO$_2$ nanoparticles in the presence of visible light is more than that of gram negative species. In all four species, for gram negative S. abony species much higher time was required. The gram positive species are more susceptible than the gram negative species and this is in agreement with previously published reports [20–22].

The difference is commonly ascribed to the difference in cell wall structure. The thick cell wall in gram positive bacteria composed of a many layers of peptidoglycan and teichoic acids whereas the cell wall of gram negative bacteria is a relatively thin with an outer membrane containing lipopolysaccharides and lipoproteins bilayers. Also this may relate to different affinities for photocatalyst and cell wall of bacteria [21]. Gram negative bacteria are relatively more resistant because of the nature of their cell wall, which restricts absorption of many molecules to movements through the cell membrane [22, 23]. Consequently, a higher number of hydroxyl radical attacks for gram negative bacteria are needed to get the complete bacterial inactivation.
8.6 Conclusions

This study demonstrates visible light assisted photocatalytic inactivation using Ni–TiO$_2$ nanoparticle and represents the most effective way to remove pathogenic bacteria. Nanocrystalline anatase TiO$_2$ nanoparticles doped with nickel metal ions were prepared by sol–gel method. The nickel metal ions were successfully incorporated in TiO$_2$ matrix. The UV–Visible diffuse reflectance spectroscopy analysis confirms that the nickel doping in TiO$_2$ extends optical absorption in the visible region. It was found that the Ni–TiO$_2$ nanoparticles exhibited photocatalytic antibacterial property towards gram positive, *S. aureus*, *B. Subtilis* and to a lesser extent of gram negative *E. coli* and *S. abony*. The nickel metal modified TiO$_2$ nanoparticles which have been tested under weak visible light are a promising way for future environmental and biomedical photocatalytic disinfection applications.
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References


