PHOTOCATALYTIC DEGRADATION OF MOXIFLOXACIN USING TITANIA NANOTUBES

by

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This thesis is dedicated to my parents and beloved sister for endless love, prayer and support and my teacher Dr. Ishtiaq A. Qazi for his guidance

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List of Abbreviations

BET	Brunauer Edward Teller
CIP	Ciprofloxacin
DTNPs	Doped Titania Nanoparticles
DTNTs	Doped Titania Nanotubes
EDS	Energy Dispersive Spectroscopy
ESI	Electro Spray Ionization
EU	Europe
FDA	Food and Drug Administration
Fe	Iron
FQs	Fluoroquinolones
GRP	General Purpose Reagent
HPLC	High Pressure Liquid Chromatography
LEVO	Levofloxacin
MOX	Moxifloxacin
SE	Secondary Electron
SEM	Scanning Electron Microscopy
TiO_2	Titanium Dioxide
TNPs	Titania Nanoparticles
TNTs	Titania Nanotubes
TOC	Total Organic Carbon
US	United States
UV	Ultraviolet
XRD	X-Ray Diffraction

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Abstract

Fluoroquinolones are a family of antibiotics, with moxifloxacin among one of the fourth generation. Fluoroquinolones are efficient but are incompletely processed during human treatment. They store in environment because of incomplete elimination from sewage and wastewater treatment plants. Present study focuses on photocatalytic degradation of moxifloxacin using TiO_2 nanostructures. For this, pure and 1% iron doped titania nanotubes The nanostructures were characterized using Scanning Electron Microscopy (SEM), Xray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS). Experiments were performed by adding different dosages (10, 20, 30, 40and 50mg) of nanostructures and placed under ultraviolet and visible light for 120 minutes. Samples were collected after 30 minutes and readings were recorded using UV/V spectrophotometer. Finally degradation of both nanoparticles and nanotubes were compared. The degree of mineralization was confirmed by High Performance Liquid Chromatography (HPLC) and Kirby- Bauer antibiotic test. Results showed maximum degradation under pure and doped Titania Nanotubes using ultraviolet light.

INTRODUCTION

1.1 Background

Existence of pharmaceuticals in environment has been receiving attention recently due to the potential negative impact these cause to humans and animals. Nevertheless, there is anecdotal evidence that the release of pharmaceutical products in human and veterinary medicines into the environment has steadily increased. Copious samples have been branded in the environment globally and are detected in surface water, ground water, sewage effluents, sea water and soil. Because of their accretion, living beings are at a greater risk of getting unsympathetic effects pertaining to health issues (Nikolaou *et al.,* 2007). Among these pharmaceutical products, Fluoroquinolones are frequently detected in water at elevated meditation s as these were extensively used from the last 20 years (Golet*et al.,* 2002).

Quinolones belongs to a family of synthetic antibacterial drugs. The trendy ones in medical use belong to the fluoroquinolones, which have a fluorine atom attached to the innermost ring system (Gul *et al.,* 2013). Furthermore, among the five major classes of antibiotics, fluoroquinolones are one of them (Diaz-Cruz and Barcelo, 2006 and Khetan and Collins, 2007) and their existence has been avowed in drinking water (Ye *et al.,* 2007), ground water (Barnes *et al.,* 2008), surface water (Kolpin*et al.,* 2002) and wastewater (Gros*et al.,* 2007) worldwide. From many years, multiplicity of contagious diseases has been treated using fluoroquinolones because of their high antibacterial commotion (Gul *et al.,* 2013).Thus their ample procedure had led to higher bacterial confrontation to fluoroquinolone in metropolitan mess slush effluents, hospitals and livestock wastewater and river creating more susceptibility of microbes towards human and animals (Hu *et al.,* 2008, Polk *et al.,* 2004;

Reinthaler*et al.,* 2003; Taylor *et al.,* 2008). Researchers have alienated quinolones into four cohorts and among these;Moxifloxacin belongs to fourth generation fluoroquinolone (Mather *et al.,* 2002). Third and fourth generations fluoroquinolones demonstrate lofty action against gram positive bacteria whereas first and second generations are vigorous against gram negative bacteria (Blondeau*et al.,* 2000).

Studies show that the second generation Ciprofloxacin was the widely used antibiotic in Europe for diverse skin and urinary infections before 2003. Their amplified consumption and exposure had increased bacterial resistance which lead to hunt for new compounds. One of those new FQ is moxifloxacin (MOX) which is well-known to be the last remedy at the moment when all other antibiotics have disastrous (Rubinstein, 2001). Due to an unmitigated antibacterial spectrum, the consumption of this fourth generation moxifloxacin (Avelox) is mounting which make it more advantageous in a wider array of applications such as acute sinusitis and community-acquired pneumonia (Ferech*et al.,* 2006). Fluoroquinolones are not biodegradable by microorganisms present in river and stream water (Al-Ahmad *et al.,* 1999). Their incidence in aquatic environment may lead to expansion of antibiotic resistant to aquatic bacteria and will cause serious risk to human health through drinking water or food chain (Adamson*et al.,* 2011).

1.2 Scope of the Study

The captivation of fluoroquinolones in aquatic environment has been confirmed in European countries like France, Italy, Sweden, and Greece (Andreozzi*et al.,* 2003) and Switzerland (Golet*et al.,* 2002a). Pakistan, China, India and Bangladesh, among the Asian countries, are

the largest pharmaceutical producers and also the consumers of fluoroquinolones (Rehman*et al.,* 2013). Wastes that were not appropriately handled after production and wrapping also cause the contamination in water bodies leading to serious problem these days (Garcia *et al.,* 2010).

1.3 Hazards

Approximately 300-600 mg dosage of fluoroquinolone (FQ) is suggested for therapeutic patients daily (Seifrtová*et al.,* 2008). FQs are set free through patient's excreta as an unaffected parent compound into the hospital, sewage and municipal waste effluents (Lindberg *et al.,* 2004). Antibiotic deliberations calculated in hospital wastes are equal to other contaminants that are susceptible to pathogenic bacteria because main cause of contamination in water originates from hospital (Kümmererand Henninger, 2003).Their high concentration in hospital, sewage and municipal treatment plants may become the resistant bacterial reservoir. Improper sewage treatment and poorly removal by treatment plants causes the release of effluent into the surface and ground water; which finally reach to drinking water and cause the drug pollution (Hartmann*et al.,* 1999). Due to this drug pollution, diverse effects can be seen concerning the direct toxicity to microfauna, microflora, and formulation of resistance against drug in water bacteria and also a great health risk to human when they devour contaminated water, fauna and flora (Rigos*et al.,* 2004).Thus treatment of sewage and municipal waste water must be done for their complete removal.

1.4 Treatment Methods

A variety of treatment methodologies have been followed for the segregation of fluoroquinolones from waste water. One of such method is conventional wastewater treatment including activated sludge process but this is not effective in complete elimination of such compounds (Westerhoff*et al.,* 2005). To triumph over such flaws other methods were launched to eliminate the contaminants and renovate them into less toxic form such as ozonation, membrane separation, activated carbon adsorption and fenton oxidation (Ikehata*et al.,* 2008).The most sophisticated technique following now is photocatalysis under ultra violet light using Titania nanostructures for the remedy of fluoroquinolones (Michael *et al.,* 2010).

1.5 Proposed Solution

After all, what have been discussed above, Titania was preferred for the current work because of its degrading aptitude. Broad range of studies may be seen in research field for the photocatalytic degradation of fluoroquinolones using Titania like ciprofloxacin, norfloxacin, enrofloxacin and many others belong to all four generations (Paul *et al.,* 2007). However, degradation of moxifloxacin has been done using stochastic cellular automaton (Van der Weeen*et al.,* 2012). Photocalytic degradation using Titania nanotubes and particles and their evaluation under ultra violet and visible light has not been reported yet.

There has been a wide range of research on the TiO_2-mediated heterogeneous photocatalysis because right now no other element is superior to TiO_2 as a photocatalyst. It is because of its high photocatalytic activity and chemical stability in aqueous solution under UV and visible light irradiation (Gerischer and Heller, 1991). Among the three main crystalline forms of Titania (i.e. anatase, rutile and brookite), only anatase and rutile are used for photocatalysis

as both are semiconductors having band gap of about 3.2 and 3.0 eV (385 and 410 nm) (Stathatos*et al.,* 2001). Doping Titania nanotubes (NTs) and nanoparticles (NPs) has also been approved and recurrently practiced method to condense the Titania band gap thus can enhance the photodegradation rate (Arabatzis*et al.,* 2003).

1.6 Present Study

- Synthesis and characterization of pure and doped TNTs and TNPs
- Photocatalytic degradation of Moxifloxacin (optimization of parameters)
- Photocatalytic degradation of Moxifloxacin (Zone of inhibition)

Both pure and doped TNTs and TNPs were geared up in laboratory using hydrothermal method and their characterization were accomplished using different techniques such as SEM, EDS and XRD. Comparative analysis was done for pure and doped TNTs and TNPs for checking their efficiency. Degree of mineralization was confirmed using Liquid Chromatography Mass Spectrophotometry (LCMS). Degradation of drug was confirmed using Kirby-Bauer antibiotic test (KBT).

Chapter 2

LITERATURE REVIEW

2.1 Antibiotics in Environment

History of antibiotic is not so far as first time it was brought in the 20th century with the breakthrough of the Penicillin in 1928 by Alexander Flemings. This discovery penetrated the formulation of thousands of new more antibiotics in the market and this became the turning point in the field of medicine and human history (Davies and Davies, 2010).This is because they can:

- Prevent bacterial growth
- Cure many contagious diseases
- Used for treating bacterial infection both in animals and human
- Helpful in growth of livestock (Sarmah*et al.*, 2006)

Based on their diverse benefits, medical fields introduced variety of antibiotics in market and their usage amplified with the passage of time leading their amputation in waste water. It is now became an international issue because these have been detected in sewage, hospital, municipal effluents. Improper sewage and municipal treatment technologies does not

completely remove antibiotics which then get mixed with surface and ground water that are used for drinking purposes (Watkinson *et al.*, 2009).

2.1.1 Sources and Pathways

The key source of antibiotic detection in environment is due to anthropogenic activities because it is an extensively used product nowadays by therapeutic patients. Other sources engross the inappropriate techniques of sewage and municipal treatment which botched in their complete diminution from water (Li *et al.,* 2012). As these were used for animals too, livestock and agriculture is also a source contributing to the drug or antibiotic pollution in aquatic environment (Sarmah*et al.,* 2006).

a) Producers

One of the antibiotic sources in water is because of the manufacturers or the pharmaceutical companies owing to their good manufacturing practices (GMP). The high demand leads to the release of toxic chemicals as production of antibiotic results in some toxic end product, which is then dumped in land fill or released in water bodies (Kümmerer, 2008).

b) Hospitals

It is evident that hospitals are the prime source of antibiotic release in environment. It may diverge from country to country and consumption rate by the patients. Inadequately handled hospital waste becomes a grim apprehension at global level (Molstad*et al.*, 2002).

c) Households

Household drainage systems are another source in discharging expired pills and their leftovers into the water. Approximately, one third of medicines sold in Germany and 25% in Austria is disposed of in household drain (Greiner and Ronnefahrt, 2003). A survey carried

out in UK showed that about 400 household members dispose of expired medicines either through sink, toilet or waste (Bound and Voulvoulis, 2005). It is reported that human faeces contain high concentration of antibiotic ranging from 3 to 300mg/kg. This is because the major portion of drug that is consumed by human body remains unchanged after several reactions. Household drains are the reasons of about 70% antibiotic release in environment (Wise, 2002).

d) Landfills

Household or hospital waste are dumped into the land area and covered with soil. At one side, it may be a good practice to remove such waste from houses and hospitals but at the same time leachates from landfills can contaminate ground water aquifers (Verstraeten*et al.,* 2003).

e) Veterinary medicines

For animal production variety of veterinary drugs have been used and removed with manure. To enrich the fields farmers make use of these manure and sewage slurry causing their entry into soil. In heavy rains they reach to surface water as runoff from soil (Kreuzig*et al.,* 2002).

f) Aquaculture

Antibiotics are also consumed in fish farms for their growth and as a feed stabilizer causing direct source of their release into the aquatic environment (Hallin*get al.,* 1998).

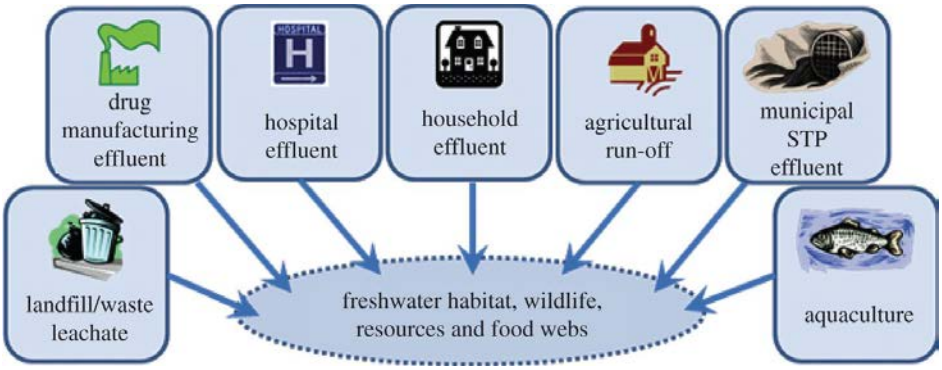

Figure 2.1: Pathways of Pharmaceutical Releases to Freshwaters

(Shore *et al.*, 2014)

2.2 Quinolone

The quinolones belong to a family of synthetic antisepticmedicines. Based on different antibacterial range, it is divided into four generations. However, the first generation was introduced in 1962 by George Lesher with the discovery of nalidixic acid used for the cure of infections correlated to urinary tract in humans. Their antibacterial effect caninhibit bacterial DNA to unwind and duplicate. For clinical use, major class of quinolones belongs to fluoroquinolones which have a fluorine atom bind to the central loop system (Hooper, 2001). Due to severe noxiousness problems by the makers, a number of the 2nd, 3rd and 4th generation medications have been removed from medical practices. Most regularly prescribed drugs today consist of Avelox (moxifloxacin), Cipro (ciprofloxacin), Levaquin (levofloxacin) and to some extent their general equivalents (Refat*et al.*, 2013).

2.2.1 Fluoroquinolones

The fluoroquinolones have been used comprehensively for respiratory and urinary tract infections treatment. These are active against a wide range of gram-positive and gram-negative organisms. Gram-positive contains Staphylococci, Streptococcus pneumoniae and viridans etc. Gram negative comprisesPseudomonas aeruginosa, Neisseria meningitides, Haemophilus influenza and many other species. Following figure is representing the basic makeup of quinolone in which the blue drawn remainder of R is piperazine. When a Fluorine atom is attached to the central ring at the 6th position or on the C-7 position, it is called a fluoroquinolone. The red color fluorine is clearly shown in the figure (Ball, 2000).

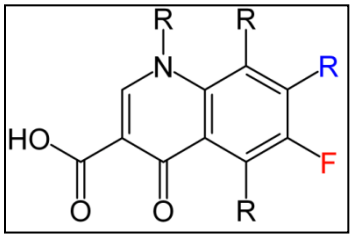

Figure 2.2.1: Fluoroquinolone Structure

Fluoroquinolones are advised by the doctors on numerous bacterial infections like pneumonia, urinary tract infections, bacterial bronchitis, intra-abdominal infections, sinusitis, joint and bone infections, typhoid fever, soft tissue and skin infections, urethral and gynecological infections, and pelvic inflammatory disease and several other infectious conditions. Extensive and inappropriate use may escort to the problem for the future in causing swift maturity of confrontation, predominantly among staphylococci and P. aeruginosa.

2.2.2 Mechanism of Action

Fluoroquinolones are inimitable among antimicrobial agents in medical use because they are good at inhibition of type II DNA toposiomerases (gyrases) that are indispensable for production of bacterial mRNAs (transcription) and DNA duplication. They reveal little inhibition of human, host enzymes and have had an excellent safety record. The mechanism starts when the drug inhibits the DNA duplication by interacting with either of two target enzyme i-e DNA gyrase and topoisomerase IV that are responsible for their duplication (Hiasa*et al.,* 1996).

2.2.3 Classification of Fluoroquinolones

Following are the classes of fluoroquinolones present in market now days.

1. First generation: nalidixic acid, oxolinic acid and cinoxacin.

2. Second generation: ciprofloxacin, enrofloxacin, marbofloxacin, danofloxacin, difloxacin, norfloxacin and enoxacin.

3. Third generation: orbifloxacin, levofloxacin, sparfloxacin and grepafloxacin.

4. Fourth generation: trovafloxacin, gatifloxacin, moxifloxacin, gemifloxacin and sitafloxacin (Owens and Ambrose, 2002a).

Currently, far and wide used and available fluoroquinolones in market are ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, norfloxacin, and ofloxacin (Halkin, 1998).

2.3 Moxifloxacin

Moxifloxacin belongs to the fourth-generation fluoroquinolone developed by Bayer AG (initially called BAY 12-8039) and have been accepted for clinical use since 1999. Globally marketed under the brand names Avelox, Avalox, and Avelon. Third and fourth generation differ from second generation as they show high activity against Gram-positive pathogens

such as staphylococci, streptococci, pneumococci and enterococci. Also it is available in an ophthalmic solution (eye drops) under the brand names Vigamox, and Moxeza for the treatment of conjunctivitis (pink eye). It is also known as “respiratory quinolone” because of its strong activity against the common respiratory pathogen Streptococcus pneumoniae. Moxifloxacin hydrochloride ophthalmic solution 0.5% has recently been approved by the US Food and Drug Administration. A number of infectious diseases are treated using Moxifloxacin such as respiratory tract infections, cellulitis, anthrax, intra-abdominal infections, meningitis, and tuberculosis. About 400mg per day oral dose of moxifloxacin has been observed in clinical use for acute bacterial infections and various other skin infections (Keating and Scott, 2004).

2.3.1 Side effects

Approximately, 45% of an oral dose of moxifloxacin is excreted throughout human body as an unchanged drug (20% in urine and 25% in faeces). However, a total of about 96% of an oral dose is excreted as either unchanged drug or known metabolites. Resultantlu, it may affect Central Nervous System as soon as after taking the first dose of AVELOX.Beside this, consumption of drug may cause serious allergic reactions, skin rash, serious heart rhythm changes, intestine infection, changes in sensation and possible nerve damage. These are also related with an amplified risk of tendinitis and tendon rupture in all ages. Older patients usually over 60 years of age are more vulnerable to this risk and patients with heart, lung or kidney transplants (Keating and Scott, 2004).

2.3.2 Moxifloxacin (Fluoroquinolones) in Environment

The major source of Moxifloxacin release to sewage treatment plants (STPs) is from residential areas, healthcare services, and animal farms as a result of the excretion of unmetabolized residues via feces and urine (Brown *et al.*, 2006; Heberer, 2002). It is because the collapse of drugs in natural systems is difficult (Carballa*et al.*, 2004; Hapeshi*et al.*, 2010). Moreover, the biotransformed metabolites can retain their indispensable composition of mother complexes (Robson, 1992). Therefore, presence of drug in the aquatic environment will contribute to the overall environmental risk. Also deficient treatment of industrial effluent containing pharmaceutical products also plays part in release of antibiotics to STPs (Kümmerer, 2009a). Table 1 is representing the unchanged degree excretion of drug in urine after single dose of moxifloxacin.

Table 1–Degree of excretion of unchanged pharmaceuticals and their metabolites from human body (urine and feces) after single dose treatment

Pharmaceutical	Excreted (%)		Reference
	Unchanged	Metabolites	
Moxifloxacin	41–55	45–59	Stass and Kubitza (1999)
Pefloxacin	<10*	60–85	Robson (1992)
Difloxacin	10*	22	Granneman et al. (1986)
Gatifloxacin	80–100	–	Grasela (2000)
Amoxicillin	80–90	10–20	Hirsch et al. (1999)
Ampicillin	30–60	20–30	Hirsch et al. (1999)
Penicillin G	50–70	30–50	Hirsch et al. (1999)

* Only urine excretion

2.3.2.1 Bacterial Resistance

As these antibiotics released into the aquatic environment structurally unchanged, they cause defiant to vigorous bacteria due to their long term exposure and further obscure

contamination control efforts (Gao *et al.,* 2012; Wellington *et al.,* 2013). The unremitting exclusion into the aquatic environment makes infection control efforts problematic as it quickens the emergence of antibiotic resistant bacteria, leading to the formulation of new antibiotics a costly process. There has been seen an increased evidence of dominance of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in sewage treatment plants (STPs) effluent (Rizzo *et al.,* 2013; Wellington *et al.,* 2013).

2.3.2.2 Aquatic life

However, various aquatic organisms such as fish are also pretentious by the release of antibiotic drugs into the surface water. It was observed that after 6 weeks of exposure to a diluted wastewater effluent, zebra-fish (Danio rerio) made a noteworthy decrease in embryo production (Galus*et al.* 2013). Similarly, another study conducted on a fish named "fathead minnow" showed a significant decline in their fertility after 21 days of toxicity test (Margiotta-Casaluci*et al.,* 2013).

2.3.2.3 Effects on Soil

Furthermore, when soil is exposed to pharmaceutical residues; the toxic properties of these products can cause undesirable effects on soil dwelling organisms. Human beings take food from such plants (food chain) causing adverse effects in them too. In 2005, Thiele-Bruhn and Beck reported that there is 10% inhibition of microbial activity due to the presence of pharmaceutical products in soil. In a 21st day test period, it also caused major effects on soil microorganism enzymatic. Many appetizing plants such as carrot, cabbage, green onion, lettuce, and corn were also affected by soil exposed to such pharmaceuticals, pointing

towards the risk on the direct use of manure and sludge to agricultural soil (Boxall *et al.,* 2006; Kumar *et al.,* 2005).

2.3.2.4 Effects on Human

As underdeveloped and developing countries are at a greater risk of waterborne infections causing high morbidity and mortality rate and approximately 80% of the infectious diseases are waterborne in these countries. The presence of fluoroquinolones in water contributes to serious diseases in human beings like gastroenteritis, diarrhea, typhoid, and dysentery (Kudoh andZen, 1977). Approximately, 2.2 million people pass away due to basic hygiene related diseases. Improper sewage sludge treatment releases such pharmaceuticals into the water bodies which then get mixed with drinking water. Universal practice to overcome these infections is the use of antibiotics but extensive use of antibiotics leads to drug resistance in these microbes, which permits the steps to avert public health risk (Tambekar and Banginwar, 2005).

2.3.2.5 Degradation

Based on the toxic effects of antibiotics like fluoroquinolones, their amputation from the water must be done. Livestock and household actions are the main sources of pharmaceutical presence in water. Thus the processes like hydrolysis can be helpful as an important method for degradation of such drugs. However, this method failed because fluoroquinolones show no activity towards increased temperature and hydrolysis. Furthermore, they are also not susceptible towards biodegradation because of their high sorption rate for organic materials found in soil and manure usually has slow rates of degradation because they aren’t available to be degraded by microorganisms (Viola *et al.,* 2004).

2.4 Treatment Methodologies

The antibiotics cannot be biodegraded in sewage treatment plants (STPs) and their failure provided the opportunity for many researches to focus or discover other methods for their degradation (Burhenne*et al.,* 1997a). Activated sludge process does not remove Moxifloxacin and thus more advanced options were introduced. Various techniques have been presented for their complete removal like ozonation, membrane separation, and activated carbon adsorption.

2.4.1 Membrane Separation

Many types of membrane separation techniques have been introduced in past using ultra filtration, reverse osmosis, microfiltration, nanofiltration and membrane bioreactors. However these techniques failed to remove organic contaminants. It is because their pore size ranges from 100-1000 times bigger than the micropollutant, allowing them to pass through (Bellona and Drewes, 2007; Snyder *et al.*, 2007).

2.4.2 Activated Carbon Adsorption

Activated carbon is one of the conventional techniques used for the removal of pharmaceutical residues from water (Hrubec*et al.,* 1983; Annesini*et al.,* 1987). It is functional in a powdered form in packed bed filters for both adsorption and filtration. However, it cannot remove organic contents from water completely. Main flaw of using this technique is that the filter pores are blocked with the dissolved organic compounds and carbon separation from water still an issue; (Zhang and Zhou, 2005; Snyder *et al.*, 2007).

2.4.3 Ozonation

Ozonation process utilizes the strong oxidizing power of ozone for the exclusion of organic compounds from drinking and wastewater (Kishimoto*et al.*, 2005). Ozone only reacts with a limited number of compounds e.g. compounds with C=C bond or aromatic compounds having electron donor groups (phenol, alkyl, or methoxy) are greatly susceptible to ozone attack, whereas organics with amide and carboxylic groups are resistant (Nakada*et al.*, 2007). Less literature is found on this process as it is costly for the user (Larsen *et al.*, 2004; Cokgor*et al.*, 2004). The key downside of this process is that it doesn't entirely neutralize the organic compounds and transformed into toxic products which require additional filtration (Dantes*et al.*, 2008).

2.5 Photocatalytic Oxidation

2.5.1 Photolysis

Photolysis derived from Greek dictionary made of two words; 'photos' means light and 'lysis' means: to decompose or breakdown. By captivating light parent compound break down in this process. Indirect photolysis occurs when photo sensitizers, such as dissolved organic matter, absorb light and produce oxygenated radicals that degrade other compounds (Legrini*et al.*,1993).

2.5.2 Photocatalysis

Photocatalysis involves the breakdown of organic content in the presence of light by utilizing a photocatalyst. Catalyst is activated by the light which enhances the chemical reaction without getting involved in it. Heterogeneous photocatalysis involves a previously formed boundary between the fluid containing reactants and products and the solid photocatalyst i.e. a semiconductor or metal. The photoreaction happens at the surface of a catalyst which

supports the excitation of the electron, enhancing chemical activity (Mills and Le Hunte, 1997).

2.5.3 TiO_2Photocatalysis

During 1970 and 1980s, broad knowledge was gained during the formulation of semiconductor photo electrochemistry by Heller which greatly emphasized on the development of photocatalysis. TiO_2 has turned out to be excellent for photocatalytically breaking down organic molecules (Heller, 1981).From environmental point of view TiO_2 is an ideal photocatalyst because it is relatively inexpensive, nontoxic, and highly stable chemically and have highly oxidizing photo generated holes. In addition, photo generated electrons are reducing enough to produce superoxide from dioxygen (Gaya and Abdullah, 2008).

2.5.4 Mechanism of Action

Titania has a basic electronic band structure with a highest occupied energy band called valence band (vb), and the lowest empty band, called conduction band (cb). However, both are estranged by a band gap i.e. distance between valence and electron band. Furthermore, when light falls on a semiconductor or it absorbs a photon of energy higher or equal to the band gap energy, an electron from the valence band jump to the conduction band. It creates a hole (h^+) in the valence band and an electron (e^-) in the conduction band. The positive holes breakdown the water molecules producing hydroxyl radical (OH^-) and hydrogen gas (H_2) whereas negative electrons react with oxygen molecule to form super oxide anion. These hydroxyl radicals are highly oxidizing and responsible for the degradation. Wavelength for TiO_2 in this process is 3.2eV (band gap energy) (Litter, 1999).

Following reaction showing photocatalysis:

$UV + TiO_2 \rightarrow TiO_2 (h^+ + e^-)$

$h^+ + H_2O \rightarrow H^+ + {}^{\bullet}OH$

$2\ h^+ + 2\ H_2O \rightarrow 2\ H^+ + H_2O_2$

$H_2O_2 \rightarrow HO^{\bullet} + {}^{\bullet}OH$

$e^- + O_2 \rightarrow {}^{\bullet}O_2^-$

${}^{\bullet}O_2 - + HO^{\bullet}{}_2 + H^+ \rightarrow H_2O_2 + O_2$

$HOOH \rightarrow HO^{\bullet} + {}^{\bullet}OH$

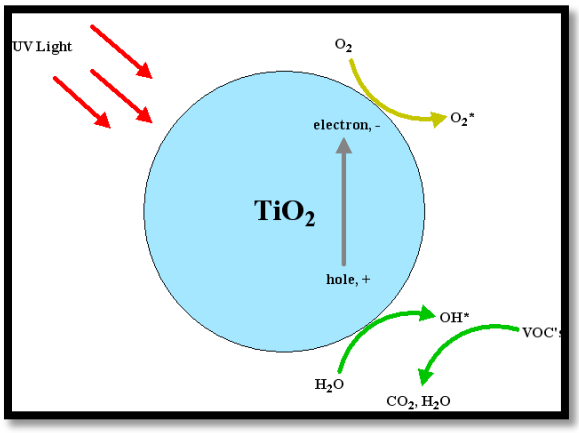

Figure 2.5: TiO_2Photocatalysis

2.5.5 Titania Polymorphs

Titania exists in three polymorphs i.e. anatase, brookite and rutile. It has been reported that only anatse and rutile phases participate in photocatalytic degradation. When differentiated from each other, anatase phase has always shown a higher photocatalytic activity than that of rutile in the presence of oxidizing agent O_2 (Augugliaro*et al.*, 1988). TiO_2 anatase surface is generally much hydroxylated and under thermal treatment it can easily lose water at the expense of superficial hydroxyl groups under thermal treatment (Kobayatawa*et al.*, 1990).

2.5.6 Obstacle

For photocatalysis, wavelength of less than 380nm is required. Photons lie only 40% to 45% in ultraviolet part of the solar spectrum in visible region. Pure TiO_2 has a larger band gap and thus it required photons of domain ($\lambda \leq 380$ nm for anatase) for their activation. Around 5% of the incident radiation has enough energy for this process if natural sunlight is used for the photoexcitation. To overcome this problem, doping of titanium dioxide with transition metal ionsis gaining attention for the band gap shift (Litter and Navio, 1996).

2.5.7 TiO_2 Doping

Doping of titania has been reported in several papers using numerous transition metal ions like Co, Cr, Cu, Fe, Mo and V. TiO_2 consumes only a small portion of the solar spectrum due to its band gap energy. Doping with transition metals improved its capability for the maximum absorption of light in the visible region (Serpone*et al.,* 1994). Choi found that doping quantum-sized TiO_2 with Fe^{3+}, Mo^{5+}, $Ru^{3+,}$ $Re^{5+,}$ V^{4+} and Rh^{3+} boosts the photocatalytic activity both for the oxidation and the reduction reactions (Choi *et al.*,1994).

2.6 Nanotechnology in Waste Water Treatment

Water is the most vital constituent for all biota and a valuable resource for human. It is a basic human right to get access to clean and reasonable water. Globally, approximately 780 million people still lack access to safe drinking water sources (WHO, 2012). Developing countries or underdeveloped countries have no proper waste water setup so there is a need to implement water treatment technologies. Moreover, existing water and wastewater treatment

technologies and infrastructure are reaching their limit for providing satisfactory water quality to meet human and environmental needs.

Nanotechnology advancements have offered many opportunities to develop next-generation water supply systems. Our current water treatment, distribution, and discharge practices are no longer sustainable. Nanotechnology, through its highly capability and multifunctional processes can offer high performance; affordable water and wastewater treatment solutions that less relies on large infrastructures. Nanomaterials are smaller than 100 nm in at least one dimension. Due to their novel size, fast dissolution, high reactivity and high adsorption rate, they can remove organic contents from the waste water. This is because nanomaterials have relatively larger surface area, good surface chemistry, many active sites, pore volume and its small intra particles diffusion distance (Qu *et al.,* 2013).

2.7 Nanostructures

2.7.1 Nanoparticles

The size of nanoparticle is between 1 and 100 nanometers. TiO_2 is much more effective as a photocatalyst in the form of Titania Nanoparticles. Nanoparticles have diameters lesser than the wavelength of the incident light and are optically transparent enough to reduce the scattering of light. Thus nanosize semiconductor particles have the additional benefit of providing transparent solutions for detailed systematic studies of photoredox processes by photocatalysis techniques (Beydoun*et al.,* 1999). It was described that with the declining of particle size, the fascination edge blue band shifts and the redox capacities of the photogenerated electrons and holes in semiconductor particles increased (Mill and Hunte, 1997).

2.7.2 Nanotubes

With the detection of carbon nanotubes by Iijima in 1991, a variety of incredible properties encouraged the journey for the synthesis of nanotubular structures of other substances like V_2O_5, SiO_2, TiO_2, Fe_2O_3, ZrO_2 and MoO_3. Among these, titanium dioxide (titania) has appealed great attention since the discovery of its photosensitivity by Fujishima and Honda in 1972. However, titania nanotubes has shown bestresultsas compared to any other form of titania for applications in water and air purification, photocatalysis, sensing, water photoelectrolysis for hydrogen generation, photovoltaics, photoelectrochemical solar cells, electronics, optics, tissue engineering and molecular filtration. Nanotubes show high degradation rate as compare to nanoparticles because the planner surface of nanoparticles causes the optical loss. To excite the photocatalyst, photons of light are required and nanoparticles planner surface is incapable to utilize maximum photons for its excitation. However the tubular structures of nanotubes have a high light trapping capability which results in more excitation pf photocatalyst. Doping of nanotubes with metal ions can enhance the photocatalytic reaction (Cao, 2004).

2.8 Nanotubes Synthesis

Various methods have been reported for the synthesis of Titania Nanotubes. Some of them are listed below. Hydrothermal method is better option as it is relatively simplest methods synthesis i.e. temperature and pressure are moderate and easy to maintain (Guo*et al.,* 2008).

- Hydrothermal Method
- Sol–gel method
- Anodization
- Chemical treatment of fine titania particles (Morgan *et al.*, 2008)

2.9 Fluoroquinolone Degradation

A lot of research has been done for the photocatalytic degradation of fluoroquinolones using Titania as catalyst. Sturini and his coworkers has degraded fluoroquinolones (ciprofloxacin, danofloxacin, levofloxacin, enrofloxacin, marbofloxacin and the most recent moxifloxacin) under natural sunlight in untreated river water using titania catalyst in photocatalysis (Sturini*et al.,* 2012). Moxifloxacin have reported to be removed using advanced oxidation process from the wastewater matrices (Haylamicheal, 2013).Xander Van Doorslaer and coworkers worked on heterogenousphotocatalysis at different concentration of moxifloxacin (Van Doorslaer*et al.,* 2012). Using UV-A and UV-C Xander Van Doorslaer and coworkers performed photocatalysis in the aqueous ciprofloxacin and moxifloxacin and checked their adsorption rate (Van Doorslaer*et al.,* 2011).

Most of the work is limited to the use of pure nanoparticles and nanotubes. This creates a baseline of research to degrade the drug using doped titania nanoparticles, doped titania nanotubes and their comparison.

Chapter 3

MATERIALS AND METHODS

3.1 MATERIALS

For the preparation of Titania nanoparticles (TNPs), General Purpose Reagent (GPR, BDH Chemicals Ltd. Poole England) was used. Then Titania Nanotubes (TNTs) were synthesized using prepared TNPs material. Iron oxide was used to formulate doped Titania Nanoparticles (DTNPs) and from this, Doped Titania Nanotubes (DTNTs) were prepared. Analytical grade NaOH was used for the synthesis of pure and doped Titania Nanotubes and analytical grade HCl was used for the complete dissolution of drug into the water. Target organic compound Moxifloxacin in the form of "Avelox" (Bayer Schering Pharma AG) was purchased from the market for the degradation purpose. For Kirby Bauer Test (KBT) of antibacterial drug, pure culture of *Bacillus Subtilis*(gram-positive) was obtained from Microbiology Environmental Teaching Laboratory Institute of Environmental Science and Engineering.

3.2 STOCK SOLUTION (MOXIFLOXACIN)

Primarily, Moxifloxacin stock solution was prepared by crushing 400 mg tablet of Avelox and then dissolved in 100 ml of 0.1N HCl. Solution was then allowed to stir for 30 minutes for complete dissolution, poured to 400 ml flask for volume makeup, followed by sonication for 20 minutes and filtered accurately which gave 1mg/ml stock solution displaying fresh yellow color.

3.3 TITANIA NANOSTRUCTURES SYNTHESIS

Synthesis of Titania nanostructures is described underneath:

3.3.1 Titania Nanoparticles

For preparation ofTitania Nanoparticles, 20 mg of Titania powder was added in 100 ml distilled water and allowed to stir for 24 hours to mix well. Resulting slurry was placed in oven for 12 hours at 105 ^{o}C for drying. Dried slurry was crushed and placed in NEY-525 SRIES II muffle furnace for 6 hours at 550^{o}C for calcination. Resultant powder was allowed to cool down which gave clear crystalline form of Titania nanoparticles (Danish, 2012).

3.3.2 Titania Nanotubes

Hydrothermal method was used for the synthesis of Titania Nanotubes (TNTs). 2.5 g of TNPs were added in 100ml of 10 M NaOH and allowed to stir for 2 hours at 250 rpm. Then placed in high ultra-sonication for 1 hour and transferred to Teflon lined autoclave at 135^{o}C for 24 hours. Resulting solution was washed with 0.1M HCl and then with distilled water to adjust the pH in the range from 6 to 7. Sample was then placed in an oven at 105^{o}C for 24 hours to remove remaining moisture and placed in muffle furnace at 500^{o}C for 1 hour (Yoshida *et al.,* 2005).

3.3.3 Iron doped TNPs and TNTs

For 1% Fe-doped Titania Nanotubes (DTNTs), Doped Titania Nanoparticles (DTNP) were prepared by adding 0.2g Fe and 18g Titania powder in 100 ml distilled water and followed the same procedure as for pure TNPs. Doped Titania nanotubes were prepared using precursor material (DTNPs) and the procedure for pure TNTs was followed (Fu *et al.,* 2013).

3.4 CHARACTERIZATION OF TITANIA NANOSTRUCTURES

Characterization of prepared nanostructures was carried out using different instruments to observe the physical properties. However, physical features of pure and doped nanostructures

involve the phase identification (rutile, brookite or anatase), their tubular or spherical structures and elemental composition of compounds present in them.

3.4.1 Scanning Electron Microscope (SEM)

It is the type of electron microscope that employs focused beam of electron to scan a sample for image production. It has the resolution of less than 1 nm. When the electrons strikes with atoms in the sample, it generates various signals that may be sensed and thus sample's structure, size, shape, topography and atomic patterns may be detected. Following are the main signals produced by SEM:

- SE - Secondary Electron
- BSE - Backscattered electrons (reflected from sample)
- Specimen current and transmitted electrons
- X-rays and with the light and heat as well

SE supports in generating the image of sample or in other words provides the structure and morphology of the sample because itoriginates from the upper layer of the sample. From the deeper layer, BSE comes and contribute to bright or faint signals of the specimen to give compositional contrast to several phases. However, X-rays inform about the elemental and chemical composition of the sample. Following figure is representing the principle mechanism of SEM (Goldstein *et al.*, 1981).

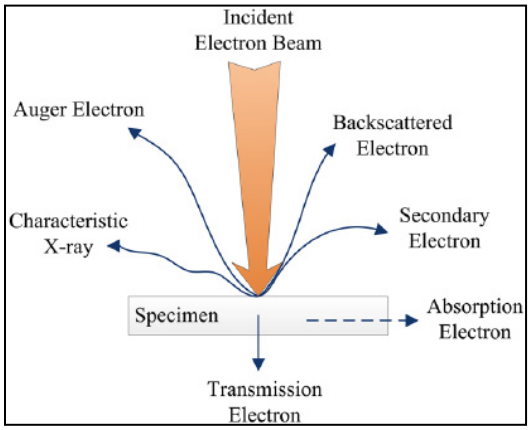

Figure 3.4.1: Electron Beam and Specimen Contact

3.4.1.1 Present Study

JEOL JSM-6460 scanning electron microscope was used at a filament current of 80mA and with the acceleration voltage of 20kV.

3.4.2 X-Ray Diffraction XRD

X-ray diffraction is an instrument that naked information about crystallographic structure and phase of the sample compound. It can make out more than one phase in a single sample. This is based on the intensity of the scattered rays from the specimen. The typical X- ray Diffractor had already saved pattern of peaks of known phases, making easy to determine the phase of target material (Renault, 2010).

Depending upon the density, approximately 5mg to 1g amount of sample is required for examination under XRD. Basic features of XRD involve:

- Measuring the distance between atom rows or sheets.
- Determining the position of a single crystal or particle.
- Find the crystalline arrangement of an unidentified material.
- Measure the size, shape and internal stress of small crystalline regions.

- Using half width, crystalline size can be found using Scherrer formula. Detectable size ranges from 2 to 100 nm.

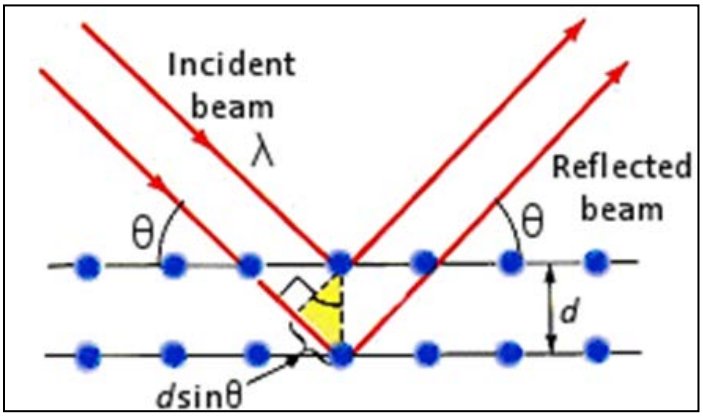

Figure 3.4.2: XRD Principle

The x-rays fall on the specimen atoms and diffracted into many directions. Measuring the intensities and angles of diffracted rays, a 3D image generated determining the crystallinity of the substance and electron density. This electron density helps in shaping the atoms position in the crystal, bonds, and other arrangements. Furthermore an intense peak of the phase is used in order to determine the calculation of grain size particle. In case of anatase phase, it is considered as 101. For this purpose, Scherrer formula is used (Behnajady*et al.*, 2008):

$$D_p = \frac{0.94\lambda}{\beta_{1/2}\cos\theta}$$

Where,

Dp is the average grain size

λ = 0.154056nm X-ray wavelength employed (CuKα1)

$\beta 1/2$ = Full Width of a diffraction line at one Half of Maximum intensity (FWHM) in radian

θ = Diffraction angle of crystal phase (Guinier, 1994).

3.4.2.1 Present Study

Present study was accomplished using JEOL JDX-II, X- ray diffractometer using CuKα radiation with the current and voltage of 30mA and 40 kV. Examination was performed at 20^o to 80^o.

3.4.3 Energy Dispersive Spectroscopy (EDS)

Energy dispersive spectroscopy (EDS) is an analytical technique used for the elemental examination or chemical characterization of the target compound. It is attached with the SEM. The principle of EDS involves the electron beam which falls on the sample atom. Due to the excitation in the inner shell of atom, every element produces x-rays. This moves the outer shell electrons to move towards inner shell to cover the gap. This difference in outer and inner shell is released in the form of x-rays. These x-rays form the specific peaks for each element which is detected by EDS as each element has a different atomic structure with different peaks on x-ray atomic spectrum. Thus, from the controlled beam of electron it can also tell us about elemental composition of a selected area. Energy Dispersive Spectroscopy graphs shows the X-ray energy on the horizontal axis whereas number of counts on the vertical axis. The percentage composition of specific element can be determined by the number of counts on the graph (Goldstein *et al.,* 2003).

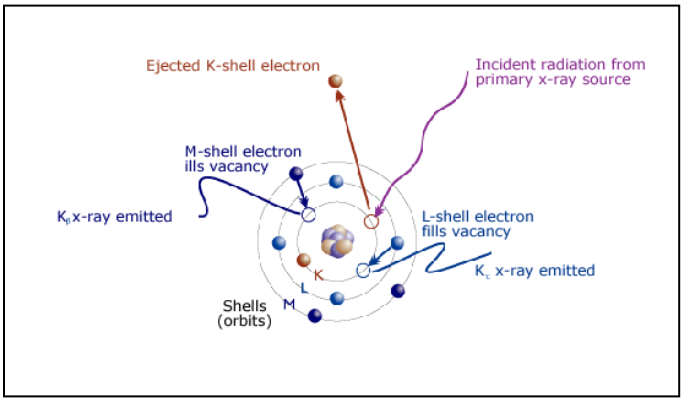

Figure 3.4.3: EDS Principle

3.4.3.1 Present Study

Energy Dispersive Spectroscopy system was attached with (SEM) JEOL JSM-6460 and used as an instrument to determine the elemental composition and level of purity of synthesized Titania nanostructures.

3.5 EXPERIMENTATION

3.5.1 Preparation of Stock Solution

Stock solution was primed and using this, different dilutions were made with a concentration ranging from 2 to 20ppm. Each dilution was run in spectrophotometer to check their absorbance. The solution which gave reading near to 1 absorbance was selected for the experiment.

3.5.2 Experimental Setup

Around, 350 ml of selected concentration (14 ppm) was prepared and poured to cylinder (500 ml) placed at a magnetic stirrer for constant stirring. Different dosages of TNPs, TNTs, DTNPs and DTNTs were added. UV and visible lamps were dipped in each cylinder (not touching the bottom) and then cylinders were covered with aluminum foil to kept reaction in

dark. After 120 minutes of illumination, samples were taken in test tubes, filtered and then centrifuged for the separation of TNTs, TNPs, DTNPs and DTNTs. Finally absorbance of each sample was measured using UV/Vis Spectrophotometer (HACH DR 2400) at λmax of 296nm. The absorbance recorded before the experiment was taken as a reference to show the percentage decrease in the concentration of the drug. It was further analyzed by HPLC (High Performance Liquid Chromatography).

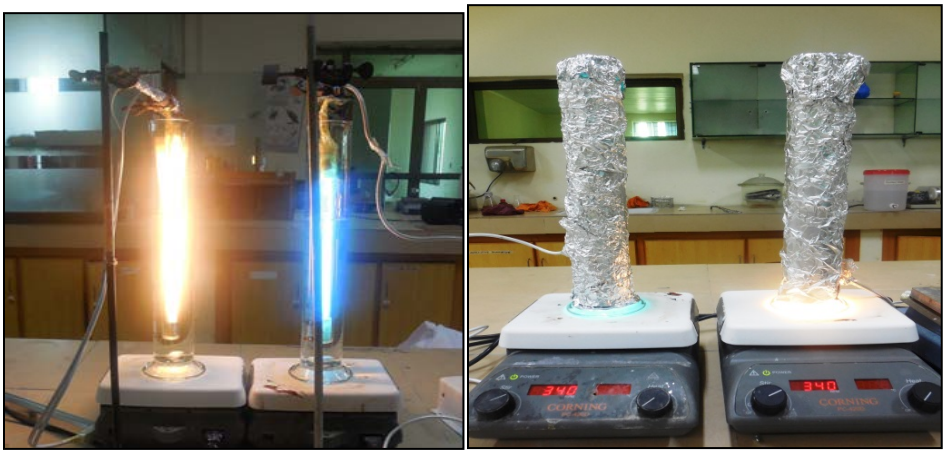

Figure 3.5.2: Experimental Setup (UV and Visible lamp)

3.5.3 Degradation Efficiency

By calculating difference of absorption before and after the experiment through spectrophotometer, the degradation efficiency of pure and doped Titania Nanoparticles and TitaniaNanotubeswas achieved. Following formula was used to calculate degradation percentage:

$$D = 100\% - (\frac{A_f}{A_o} X\ 100)$$

Whereas,

D is the percentage degradation of drug.

A_o is the absorbance before the experiment.

$A_f$is the absorbance after experiment.

3.6 INSTRUMENTATION FOR ANALYSIS OF MOXIFLOXACIN

All the instruments used for the analysis of moxifloxacin solutions will be discussed in this section.

3.6.1 Spectrophotometer

For the quantitative analysis of organic compounds, UV/Vis spectrophotometer is commonly used instrument. It is based on the absorption phenomenon in which UV and visible light absorbs the portion of wavelength of the sample compound.

Figure 3.6.1: UV/V Spectrophotometer

3.6.1.1 Principle of UV/V Spectrophotometer

Electrons in the molecules absorb the energy in the form of ultraviolet or visible light and get excited to high energy orbitals. It utilizes light in the visible range i.e. UV and near-infrared ranges. The reflectance of the sample molecule in the visible range directly affects the apparent color of the chemicals involved. If the electrons are more easily excited, longer they

can absorb the wavelength of light while transition of electrons from ground to higher state, their absorption is recorded by comparing the light intensity that is transmitted before and after passing through the sample(transmittance). It is noted that transmittance is inverse toabsorbance of the sample (Perkampus, 1992).

3.6.1.2Beer-Lambert law

According to this law the concentration of the absorber is directly proportion to the light it absorbs and the path length. With increasing the concentration the absorbance will also increase.

$$A = \varepsilon .c. L$$

Where,

A is the absorbance noted

C is the concentration of absorber

L is the path length

ε is the absorption coefficient

εis specific to each compound present in the solution.

3.6.1.3Present Study

For the present work the UV/V is Spectrophotometer (HACH DR 2400) was used to measure the percentage degradation of Moxifloxacin at λmax of 296nm.

3.6.2 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is a technique that is widely used for the quantitative and qualitative analysis of organic components in a sample. It enables the separation, identification and quantification of the mixture in the sample. Basically HPLC is

based on adsorption, partition and ion exchange method by using mobile and stationary phase, and it identifies each component of the sample solution.

3.6.2.1 Principle

HPLC involves mobile phase and a stationary phase. Sample mixture passes through the pump by a column filled with a solid adsorbent material which contains the pressurized liquid solvent. While passing through the adsorbent material, each component in the sample interacts with it differently which cause difference in the flow rate and sample components from the mixture got separated.

3.6.2.2 Procedure

Following are the working procedures of High performance Liquid Chromatography and the basic components of HPLC have shown in figure 3.6.2.

(i) Mobile Phase

- The solvent is held in a moving reservoir called as a mobile phase.
- A high pressure pump is used to generate and meter (milliliters per minute) a specific flow rate.
- Sample is injected into the continuously flowing mobile phase streams through injector or a sample manager. This carries sample into the HPLC column.

(ii) Stationary Phase

- Stationary phase involves the packing material which is held in place by column hardware.
- Separation is carried out by chromatographic column material.

- From the HPLC column, a detector is needed to get the separated compound bands as they are releasing from this column (most components are colorless, cannot be seen with eyes).
- During this process the mobile phase exits the detector and can be sent to waste collector, as desired
- The detector reads the concentration changes from the sample solution and converts into signal voltage.
- With the passage of time, change in the voltage is recorded on the computer through a signal cable and traced on a paper in the form of a chromatogram. Detected elements may be shown in rising and falling of the peaks.

It is to be noted that high-pressure tubing and fittings are used to interconnect the pump, injector, column, and detector components to form the channel for the mobile phase, sample, and separated compound bands (Lough and Wainer, 1995).

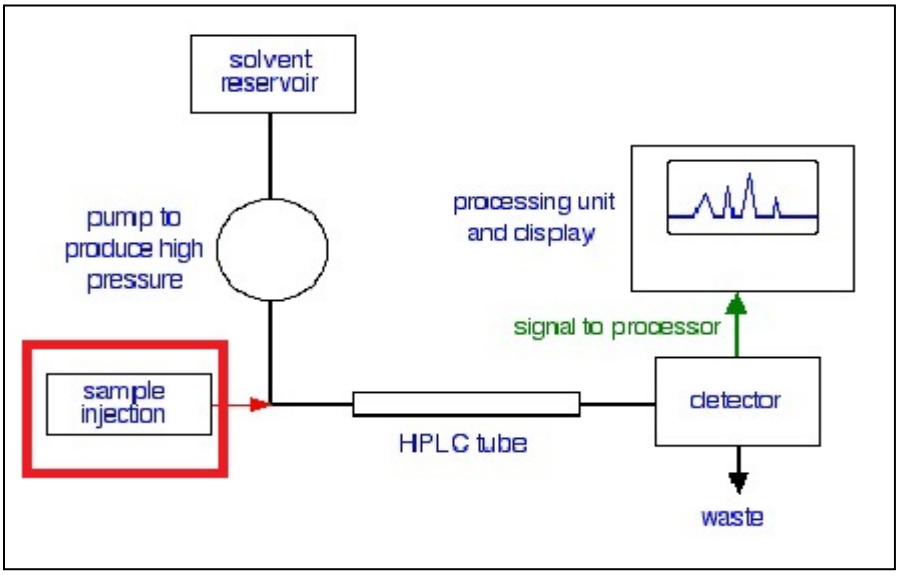

Figure 3.6.2: HPLC Components

3.6.2.3 Present Study

HPLC Agilent Technologies 1200 series was used for the research purpose.

3.6.3 Kirby-Bauer Antibiotic Test or Zone of Inhibition

Kirby-Bauer antibiotic testis a widely used method to determine the sensitivity of microorganisms towards various antibiotic drugs. It is very helpful technique in the current research as it confirms the degradation of antibacterial or antibiotic drugs from the water. The degraded drug area has either no zone or very small zone of inhibition, which confirms that drug has been degraded. This means larger the zone area smaller the amount of drug has been degraded.

3.6.3.1 Steps

Following are the steps involved in Kirby-Bauer Antibiotic test:

- Firstly, an agar plate is prepared and then the bacterial suspension is spread over the agar plate with the help of a swab which should be highly sterile to avoid environmental contamination.
- At the center of the plate, put the pure drug soakedpaper disc and place it in incubator for 24 hours. Replicate the same procedure for the treated solutions as well.
- After 24 hours of bacterial growth, the plate with pure untreated drug will show clear zone around the paper disc indicating the presence of drug and high antimicrobial activity.

- Other plate with degraded or treated drug paper disc will show no sign or very small zone around it. This means drug is no more active as it degraded completely (Bauer *et al.,* 1959).

Chapter 4

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF NANOSTRUCTURES

This section consist of the outcomes of characterization of synthesized nanostructures (TNPs, TNTs, DTNPs and DTNTs) and their detailed discussion regarding SEM image, crystallinity, elemental composition and their different sizes in nanorange.

4.1.1 Scanning Electron Microscope (SEM)

SEM images of prepared nanostructures were carried out using JEOL JSM 6460 Scanning Electron Microscope at different magnifications.

4.1.1.1 Pure and Doped Nanotubes

Pure and Doped Titania Nanotubes were acquired from JEOL JSM 6460 Scanning Electron Microscope at 30,000 to 75,000 magnifications. Images clearly indicating tubular and long structured as shown in Figure 4.1 (a, b, c, d).The average size of TNTs was 22nm.

4.1.1.2 Pure and Doped Nanoparticles

Similarly, pure and doped Titania Nanoparticles were also examined from JEOL JSM 6460 Scanning Electron Microscope at 10,000 and 30,000 magnifications showing in Figure 4.1 (e, f). The average size for nanoparticles was 78 nm.

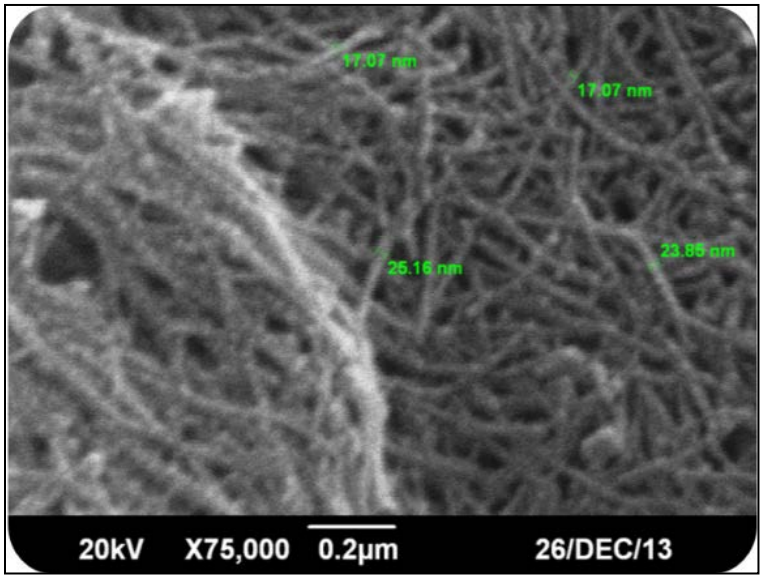

Figure 4.1(a): SEM image of Pure TNTs at X75,000

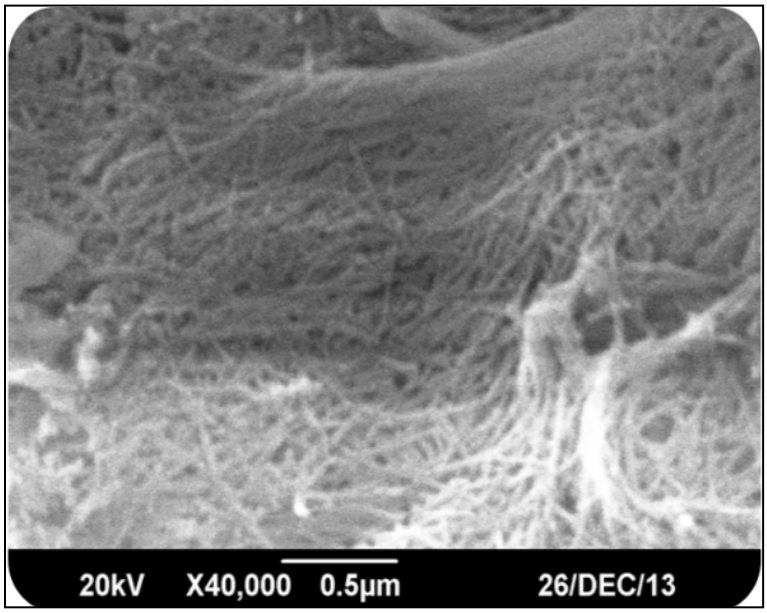

Figure 4.1(b): SEM image of Pure TNTs at X40,000

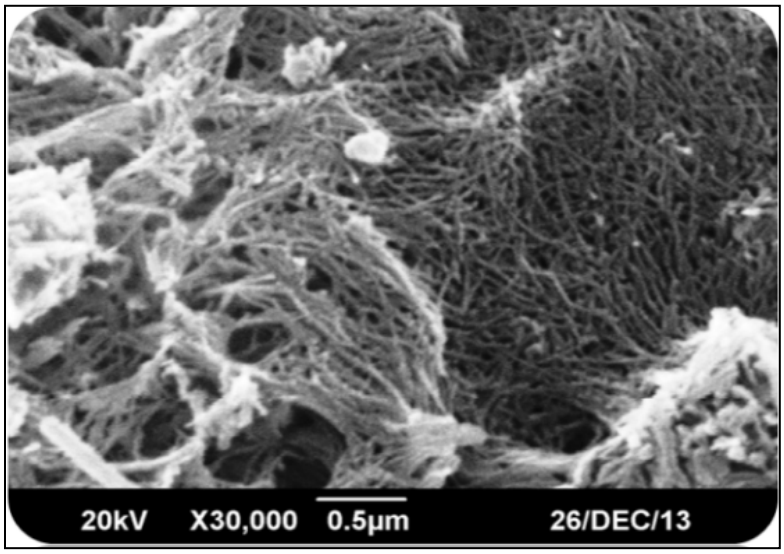

Figure 4.1(c): SEM image of Fe doped TNTs at X30,000

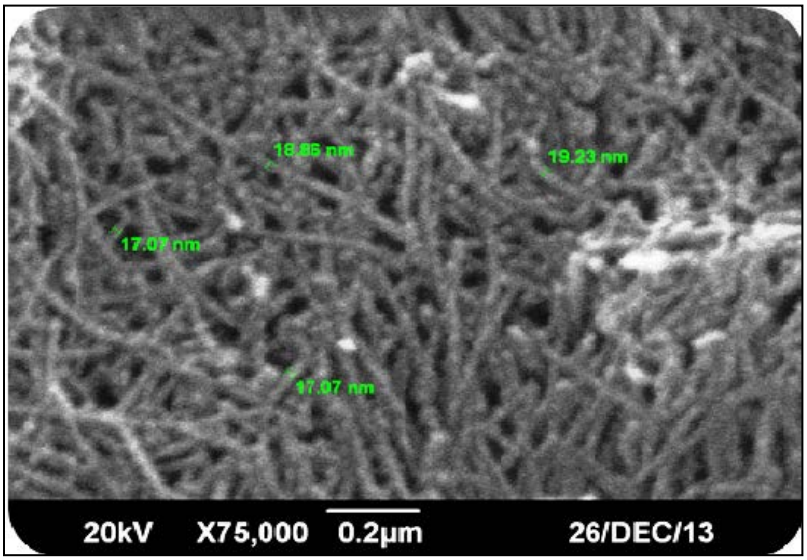

Figure 4.1(d): SEM image of Fe doped TNTs at X75,000

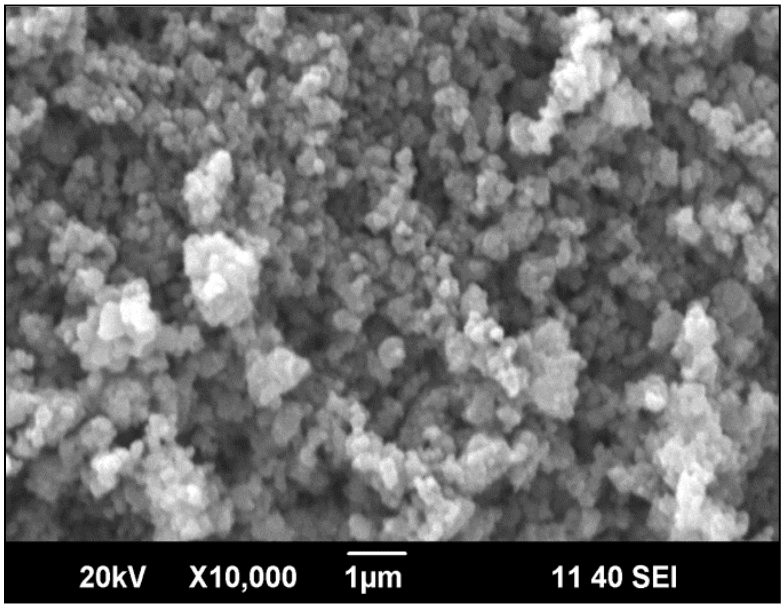

Figure 4.1(e): SEM image of Pure TNPs at X10,000

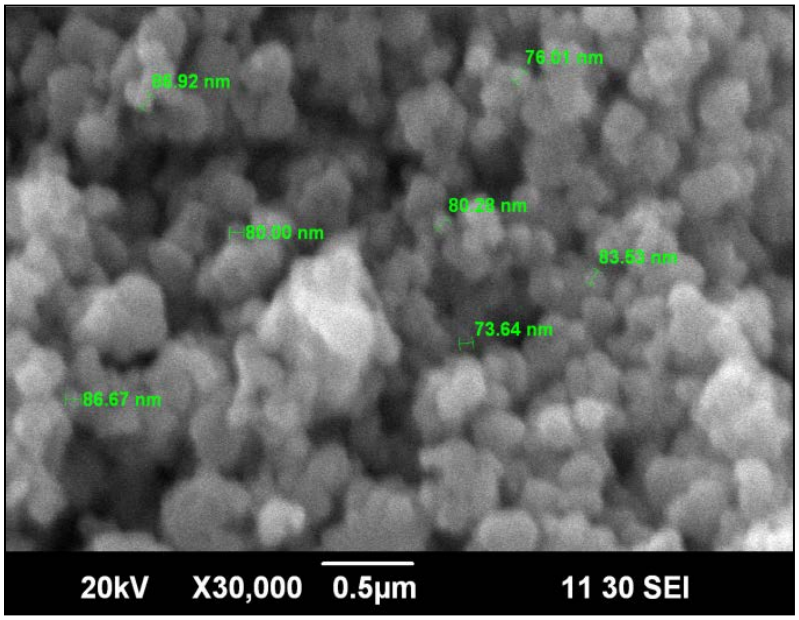

Figure 4.1(f): SEM image of Fe Doped TNPs at X30,000

4.1.2 Energy Dispersive Spectroscopy (EDS)

Energy dispersive spectroscopic (EDS) spectra of TNPs and TNTs were obtained by using JEOL JSM 6490A logical station to analyze the essential composition. Figure 4.2 (a and b) presenting the main elements of Pure TNTs (81% Titania, 18% Oxygen) and Doped TNTs (62% Titania, 34% Oxygen and 2% Iron). Similarly, pure and doped Titania Nanoparticles EDS has been shown in Figure 4.2c displaying 49% Oxygen, 48% Titania in case of pure TNPs and Figure 4.2d showing 72% Titania, 2% Iron and 24% Oxygen in DTNPs respectively.

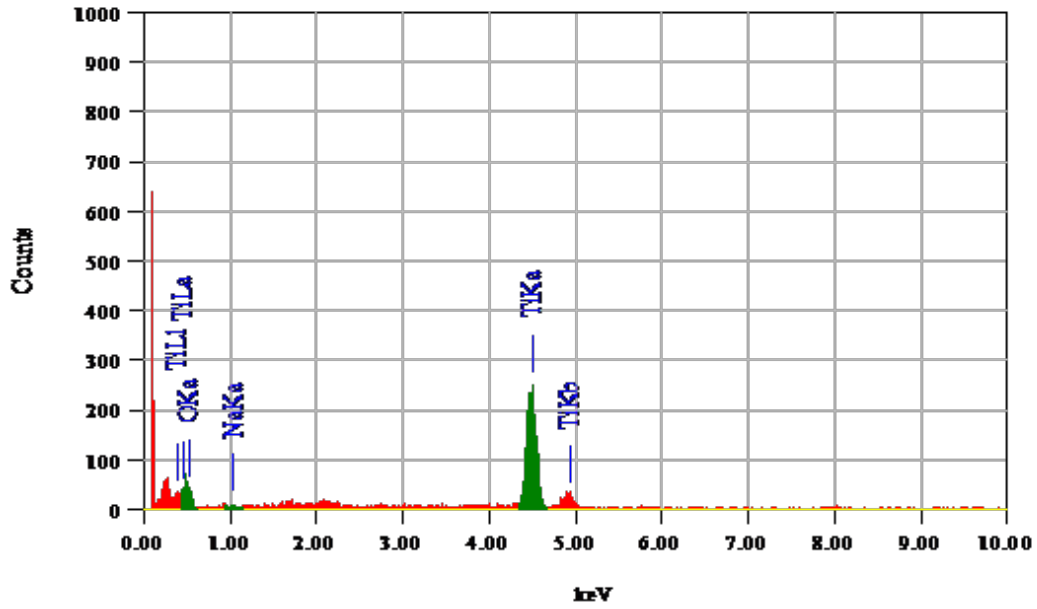

Figure 4.2(a): SEM image of Pure TNTs

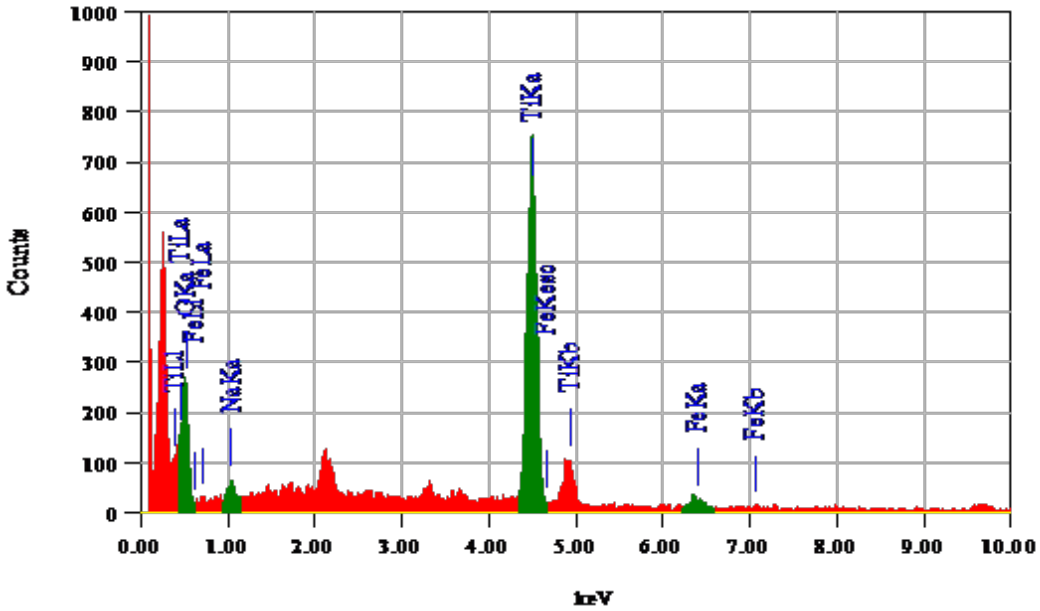

Figure 4.2(b): SEM image of Fe Doped TNTs

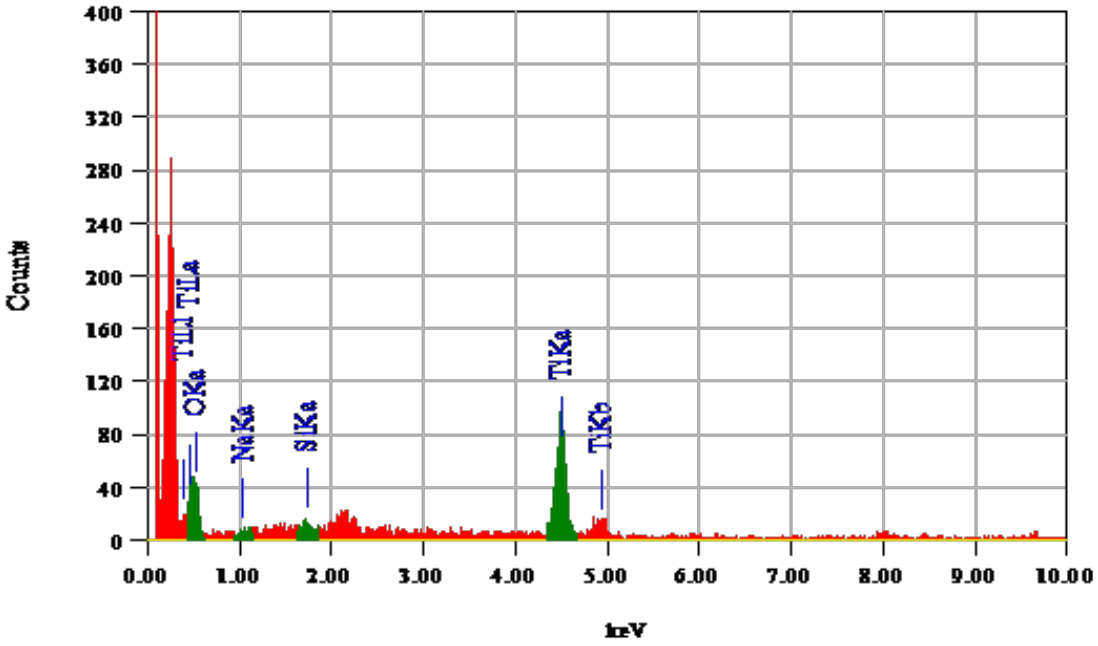

Figure 4.2(c): SEM image of Pure TNPs

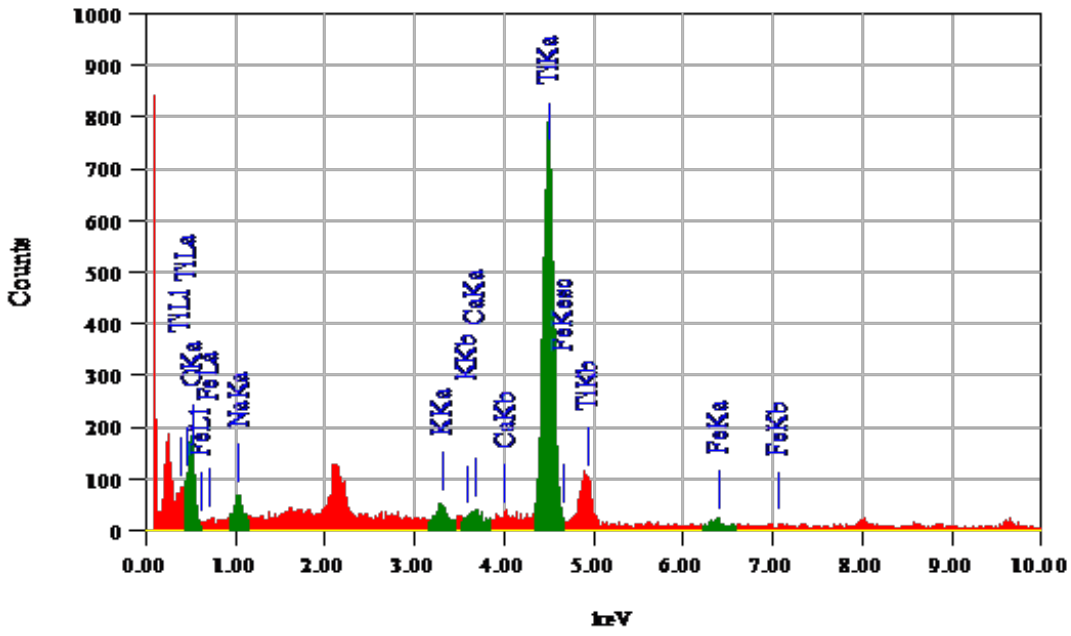

Figure 4.2(d): SEM image of DTNPs

4.1.3 X-Ray Diffraction Analysis

For the determination of crystalline size and phase of nanostructures, JEOL JDX-II X-ray diffractometer (XRD) was used with Cu-Kα radiations at an angle of 2θ from 10^{o} to 80^{o} for both pure and doped Titania Nanostructures concerning accelerating voltage 15kV and the applied current 20 mA respectively. Calcinations is a very important factor in developing the anatase phase because it enhance the crystallinity and photocatalytic activity of catalyst (He*et al.,* 2011).Using Scherer equation ($L=k\lambda/(\beta \cos \theta)$, crystalline size was determined (Zhang*et al.,* 2005). Figure 4.3 (a, b, c and d) confirmed the anatase phase of nanostructures.

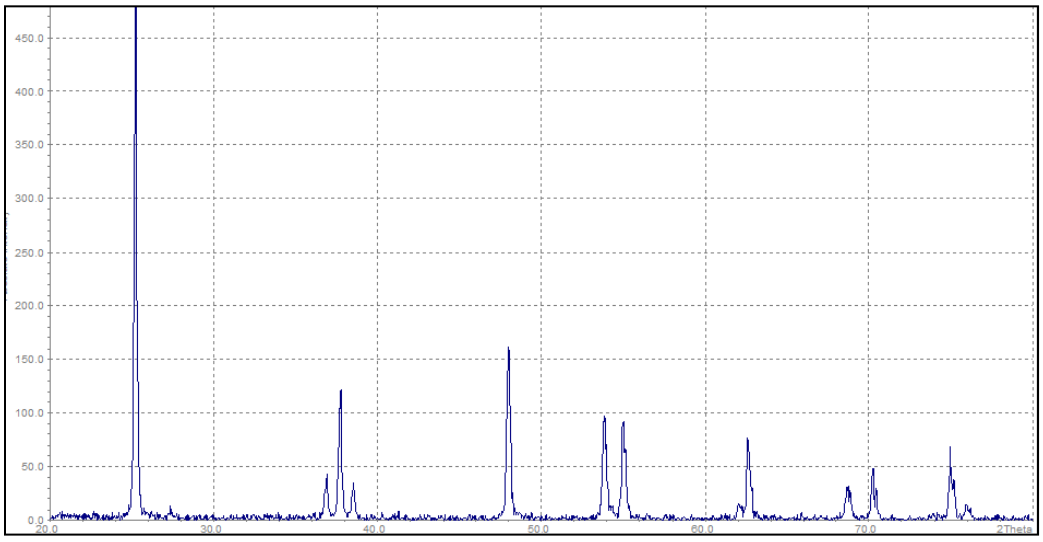

Figure 4.3(a): XRD Graph for Pure Titania Nanotubes

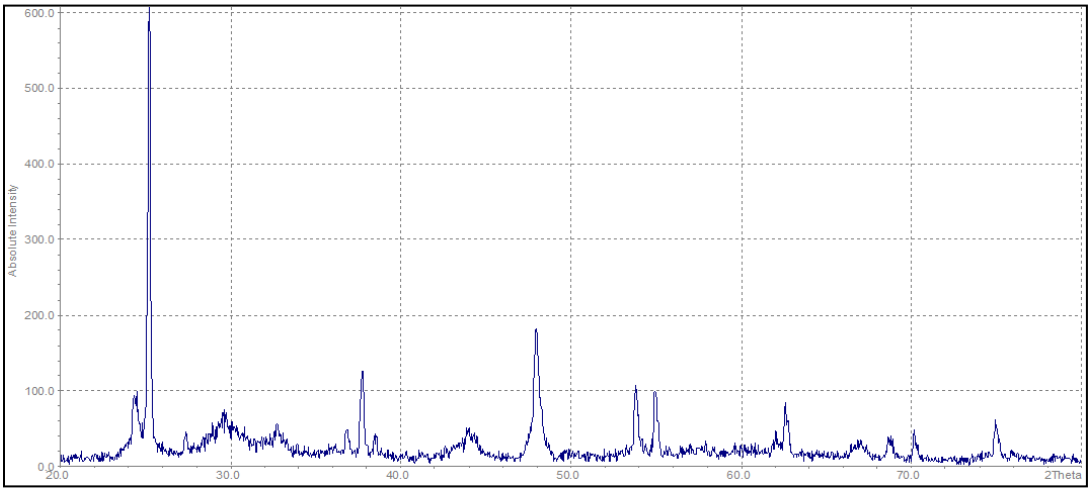

Figure 4.3(b): XRD Graph for Doped Titania Nanotubes

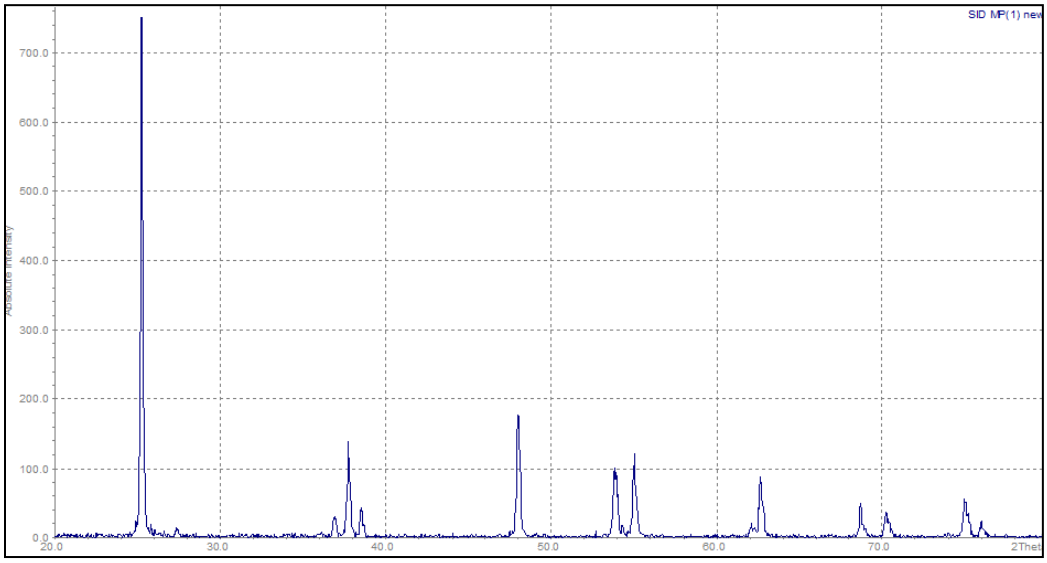

Figure 4.3(c): XRD Graph for Pure Titania Nanoparticles

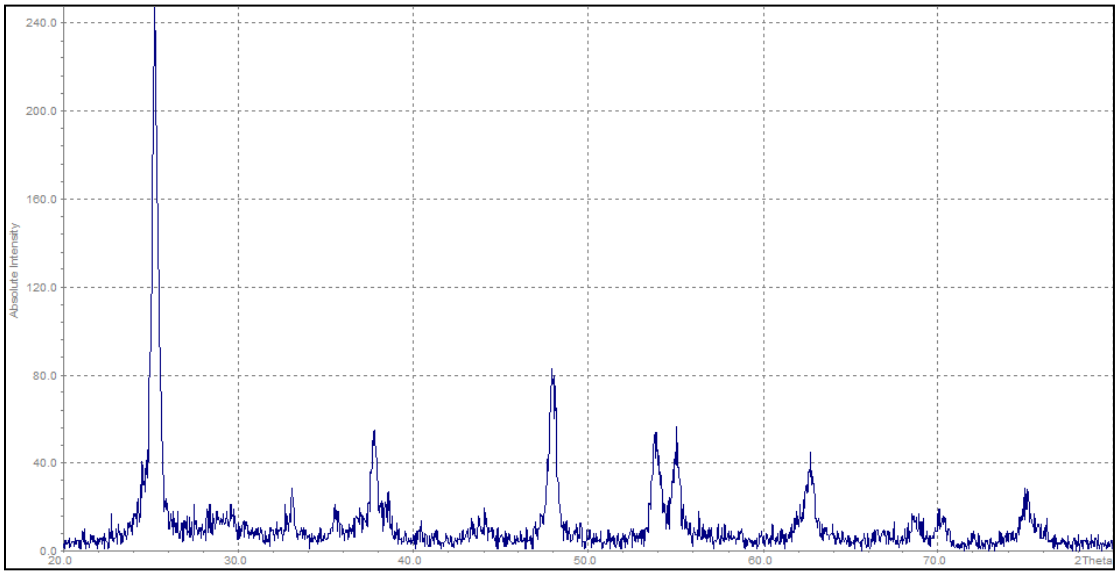

Figure 4.3(d): XRD Graph for Doped Titania Nanoparticles

4.2 ANALYSIS & RESULTS

4.2.1 λmax of Moxifloxacin

Every compound has its particular λmax. To execute experiments at the outset it is imperative to find out the λmax of Moxifloxacin at this wavelength, it gives maximum absorbance in light. By means of this wavelength, absorbance readings may be compared before and after degradation and percentage degradation may be calculated. To measure the absorbance, wavelength was deposited between 250nm to 450nm.

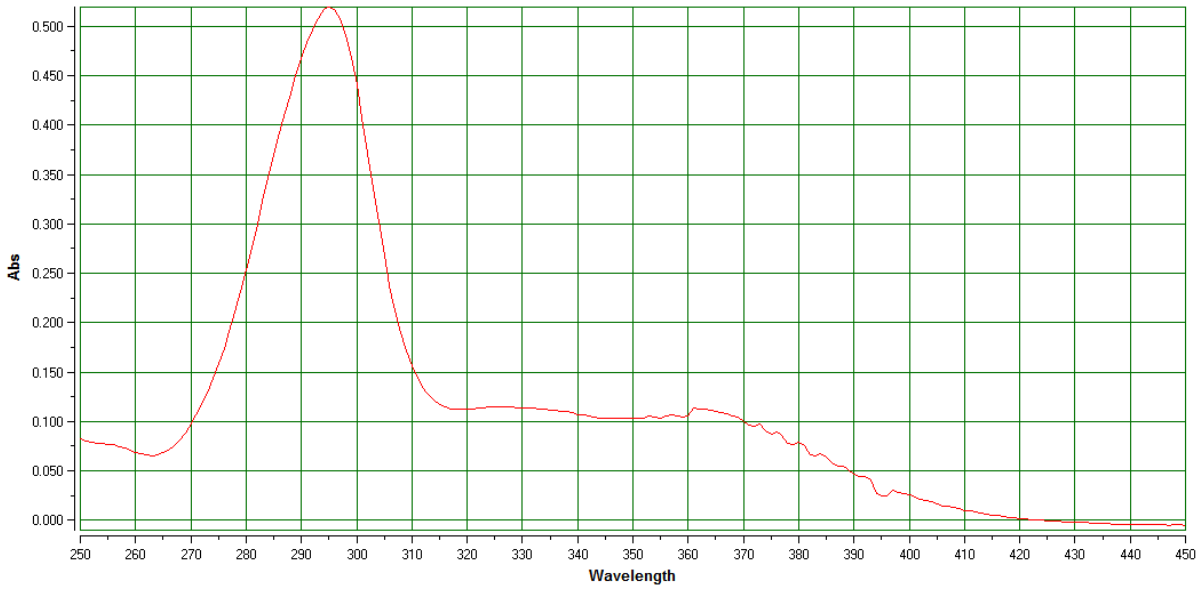

Figure 4.2.1: Maximum Wavelength of Moxifloxacin

Figure 4.2.1 is showing maximum peak of Moxifloxacin at 296nm and this is also supported by literature (Motwani*et al.*, 2007; Lemoine*et al.*, 2000).

4.2.2 Beer's Law Curve

In order to prepare the calibration curve, working standards were prepared from the moxifloxacin stock solution which described the dependence of absorbance on the concentration of the solution.

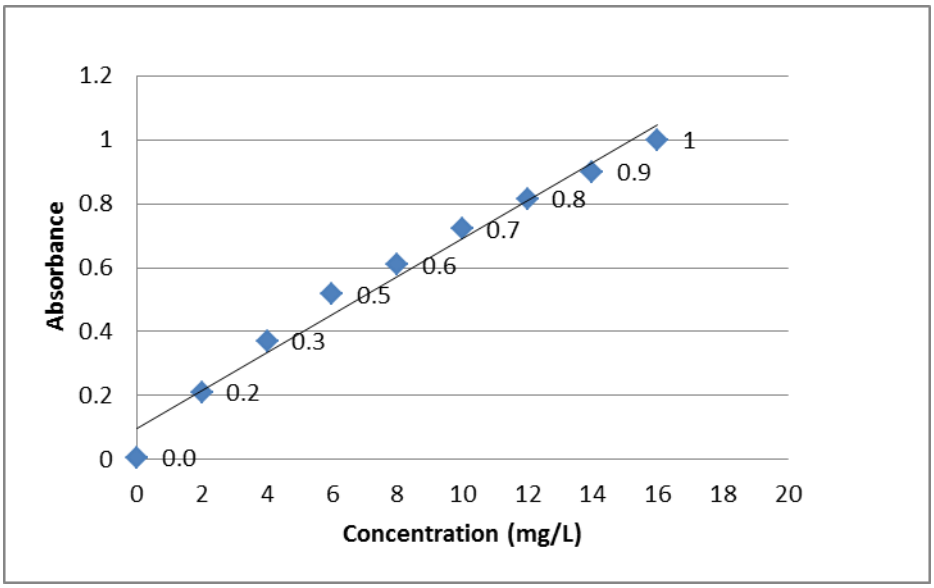

Figure 4.2.2: Calibration Curve

Figure 4.2.2 is presenting calibration curve at various concentration. For experimental purpose 14 mg/L concentration was preferred as it is near to absorbance 1.

4.2.3 Degradation Vs Irradiation Time

Figure 4.2.3 shows the maximum degradation of moxifloxacin attained at 120 minutes using 50 mg Doped Titanianoparticles. However, further degradation was not seen after this time.

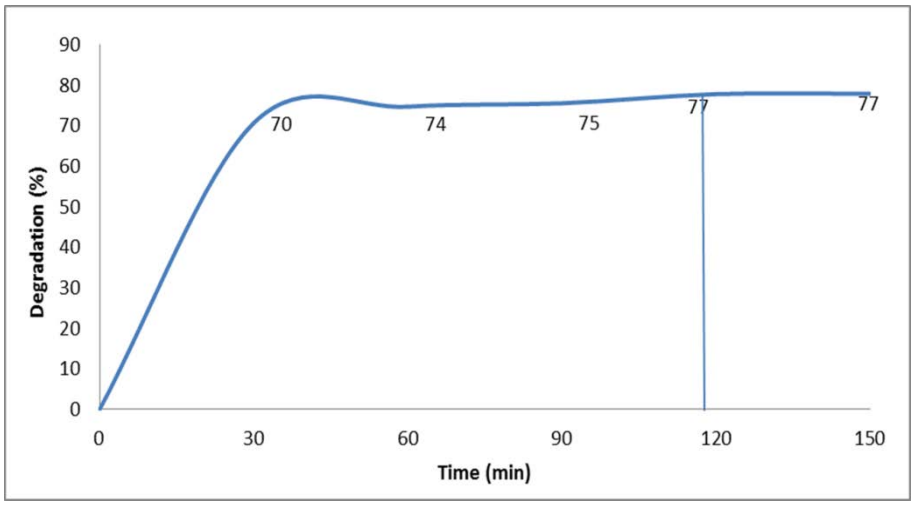

Figure 4.2.3: Irradiation Time Optimization

4.2.4 Pure and Doped NPs and NTs Comparison

Photocatalytic degradation results were accomplished by measuring absorbance for each degraded sample on UV spectrophotometer. Samples that were treated with Titania nanotubes showed the highest degradation rate as compare to nanoparticles. Larger surface area of nanotubes enhanced the degradation rate as compared to nanoparticles that have smaller surface area.

4.2.4.1 UV Light

Figure 4.2.4.1 is showing the comparison between nanoparticles and nanotubes for both pure and doped under UV light at 120 minutes of time interval. TNPs showing degradation of about 62%, DTNPs degradation percentage was 77%, TNTs having 94 % of degradation and maximum degradation was attained at DTNTs showing 98% of degradation respectively.

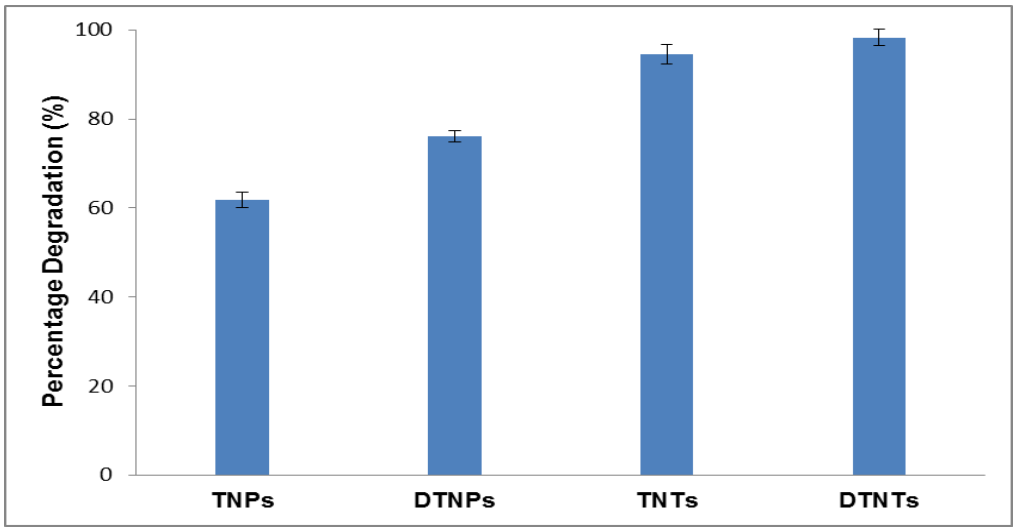

Figure 4.2.4.1: Comparison between Nanostructures under UV Light

4.2.4.2 Visible Light

Figure 4.2.4.2 representing the degradation percentage under visible light at the time interval of 120 minutes. TNPs showing least degradation percentage 15%, DTNPs having 20% of degradation, TNTs had degraded 59% and DTNTs showing 74% of degradation.

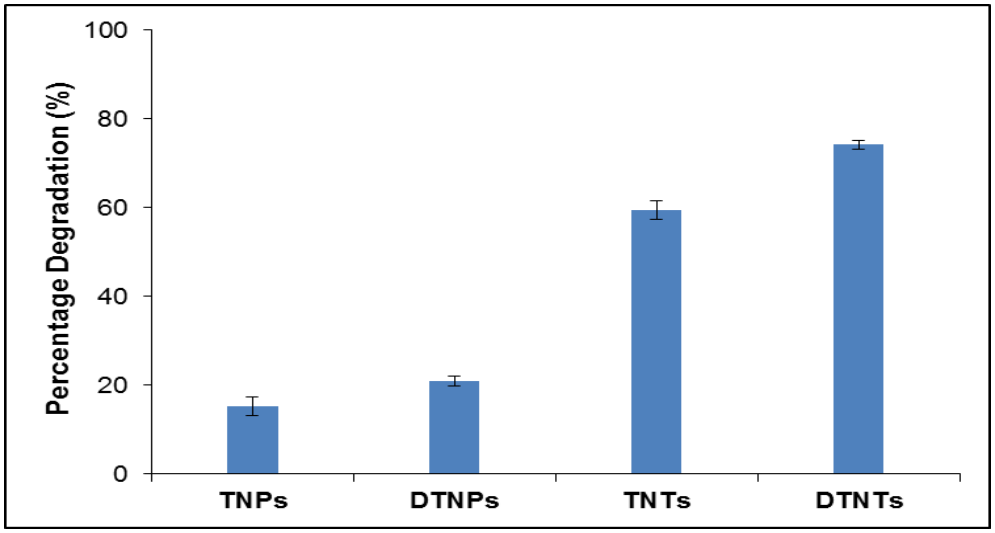

Figure4.2.4.2: Comparison between Nanostructures under Visible Light

4.2.5 High Performance Liquid Chromatography (HPLC)

Treated and untreated samples were tested in High Performance Liquid Chromatography to specify the change and verify the degradation of Moxifloxacin. Figure 4.2.5 (a) is representing the untreated sample of drug in which peak is clearly visible at 7th minute. Figure 4.2.5 (b) is displaying graph of treated sample under UV light, in which no peak was appeared which confirms thatmoxifloxacin has been degraded completely.

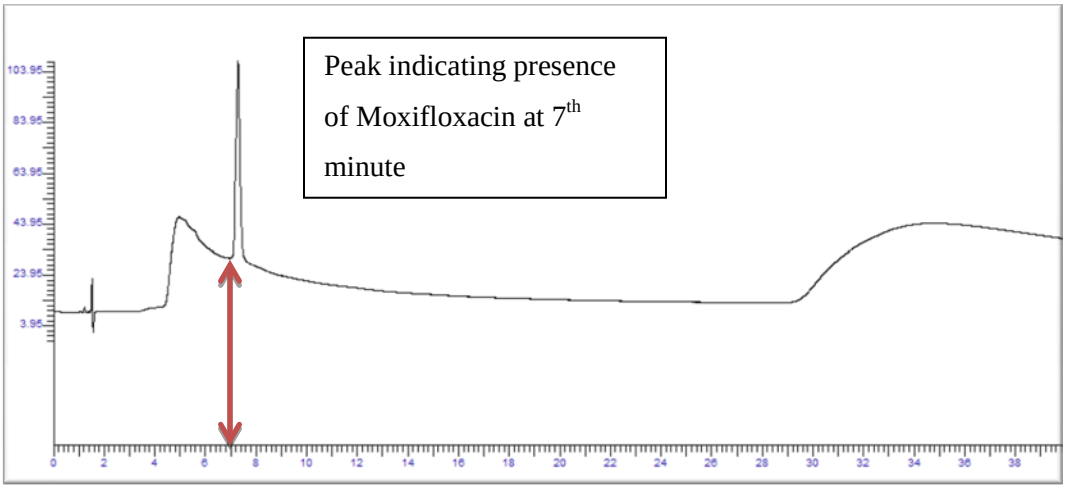

Figure 4.2.5 (a): Untreated Sample

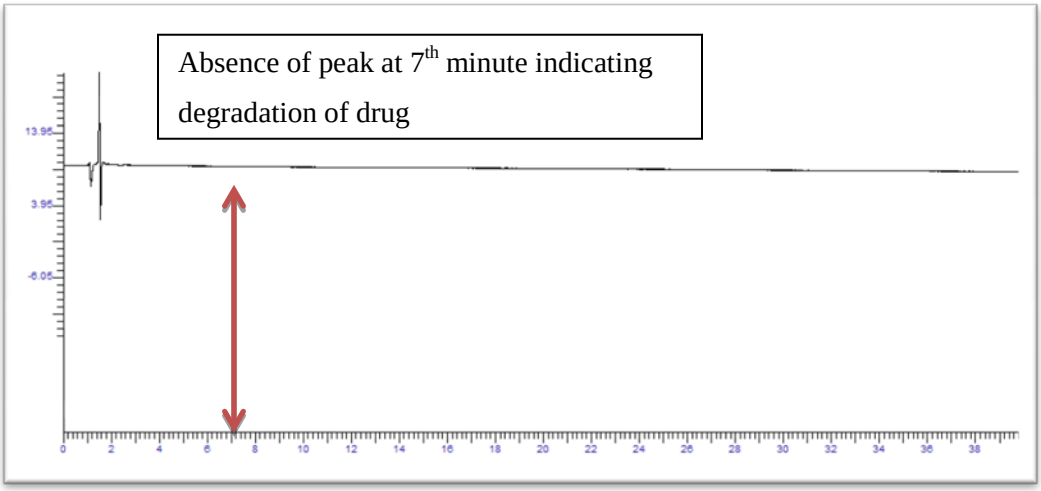

Figure 4.2.5 (b): Treated Sample under UV light and DTNTs

4.2.6 Kirby-Bauer Antibiotic Test

Kirby Bauer Test or Zone of Inhibition was performed in order to appraise the antibacterial property of the degraded Moxifloxacin. Previous families of fluoroquinolones (second and third generation) showed high activity against gram negative bacteria. However, Moxifloxacin is entirely dormant towards gram negative bacteria and very active towards gram positive bacteria. Experiments were performed using *BacillusSubtilis* and samples were placed in incubator for 24 hours. In Figure 4.2.6 (a and b) untreated area clearly presenting a zone, signifying the presence of drug and treated area are without any zone representing the absence of drug.

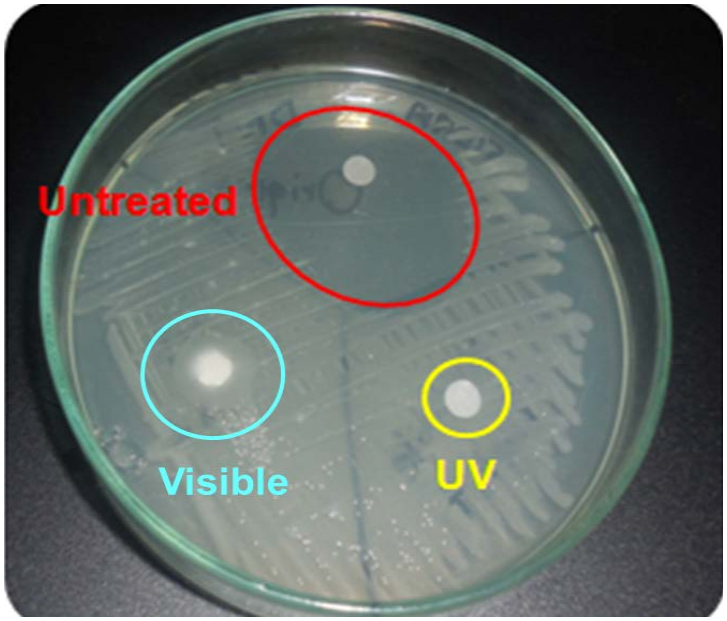

Figure 4.2.6 (a): KBT treated with TNTs

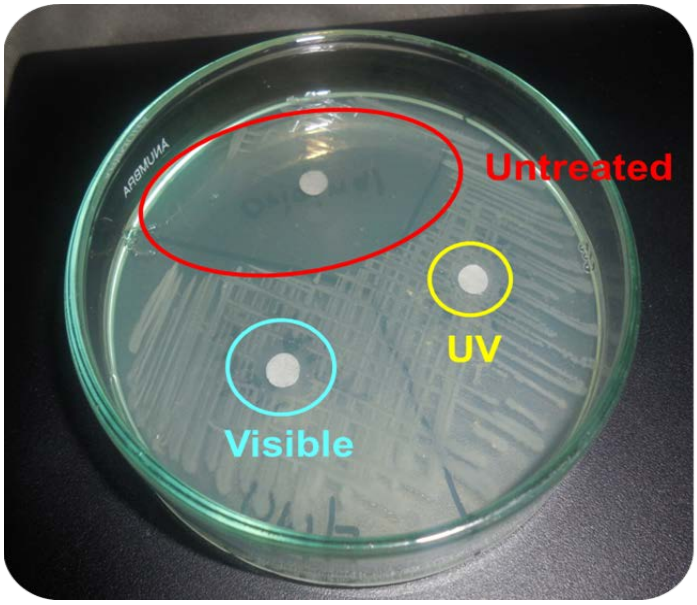

Figure 4.2.6 (b): KBT treated with DTNTs

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

After taking into account the photo-degradation outcomes by pure and doped Titania Nanoparticles and Nanotubes, it is concluded that:

- Doped nanotubes showed good degradation of Moxifloxacin under both UV (98%) and visible light (74%)
- Degradation rate increased with increasing Titania nanostructures dosage (10 to 50 mg)
- UV and visible light irradiation time periods also influenced degradation rate
- Nanotubes are effective than Nanoparticles for degrading antibacterial drug

5.2 Recommendations

- At IESE degradation of second, third and fourth generation of fluoroquinolones had been studied. It would be appealing to pertain the same degradation technique to other groups of antibiotics such as Sulfonamides, Tetracyclines and many others related to water pollution.
- The probability of red shift due to the charge transfer complexation existed in case of Ciprofloxacin, Levofloxacin and Moxifloxacin. Using this property, it would be helpful to degrade other pharmaceutical products.
- Lab scale reactor may be developed for degradation of real pharmaceutical wastewater

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