DEGRADATION OF A FLUOROQUINOLONE BASED PHARMACEUTICAL PRODUCT BY PHOTOCATALYSIS USING TITANIA (TiO_2) NANOTUBES

By

Khadija Nawaz

(2011-NUST-MSPhD-EnvS-12)

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science

In

Environmental Science

Institute of Environmental Sciences and Engineering (IESE)
School of Civil and Environmental Engineering (SCEE)
National University of Sciences and Technology (NUST)
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This thesis is dedicated to my parents, my sister and two of my friends Fahad Ali Asif and Sadaf Tehmina

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List of Abbreviations

APCI	Atmospheric Pressure Chemical Ionization
API	Atmospheric Pressure Ionization
APPI	Atmospheric Pressure Photo Ionization
BET	Brunauer Edward Teller
BSE	Backscattered Electrons
CIP	Ciprofloxacin
EDS	Energy Dispersive Spectroscopy
ESI	Electro Spray Ionization
EU	Europe
FDA	Food and Drug Administration
Fe	Iron
FEDESA	European Federation of Animal Health
FQs	Fluoroquinolones
GRP	General Purpose Reagent
HPLC	High Pressure Liquid Chromatography
LCMS	Liquid Chromatography Mass Spectrometry
LEVO	Levofloxacin
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

The presence of trace amount of antibiotics in soil, surface water and ground water is of real concern as it results in the resistance of many pathogenic bacteria. There is a need, therefore, to purify the waters containing such compounds before their discharge to the environment. In the present study Photo-catalysis, using Titania, was found to be an effective technique for the degradation of Levofloxacin, a Fluoroquinolone based antibiotic. Pure and 1% iron doped Titania Nanotubes were synthesized by Hydrothermal method and characterized by Scanning Electron Microscopy (SEM), Xray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS), Surface Mapping, Brunauer-Edward-Teller (BET) surface area analysis and band gap energy calculation. Degradation of the compound was studied using pure and 1% iron doped was under ultraviolet and visible light. Comparative analysis was done between Titania Nanoparticles and Nanotubes. Factors that affect the degradation i.e. pH, reaction time and amount of catalyst was monitored. The degree of mineralization of the drug has been confirmed by using instruments like Liquid Chromatography Mass Spectrometry (LCMS) and Total Organic Carbon (TOC) analyzer. Titania Nanotubes were found to be very effective as compared to Titania Nanoparticles. Maximum degradation using Titania Nanotubes was observed under acidic range. Degradation efficiency further increase with 1% iron doping of Titania Nanotubes. Significant degradation has also been observed under visible light. The degradation of drug, both under ultraviolet and visible light was verified through the Kirby-Bauer antibiotic test.

Chapter 1

INTRODUCTION

1.1 Background

The elimination of trace amounts of pharmaceutical products from water is receiving a lot of attention in recent decades as the presence of these products, even at minute level, is harmful for aquatic ecosystem as well as for humans. The occurrence of these products in water was reported more than two decades ago and Fluoroquinolones (FQs) along with many other pharmaceutical products were frequently detected (Sievers *et al.,* 1977).

Fluoroquinolones, evolved from quinolone, are group of antibiotics used for the treatment of multiple bacterial infections in both humans and animals. Their broad spectrum action, against many bacterial strains, makes them most frequently used antibiotic. Among these, Levofloxacin (LEVO) is a recently developed compound belonging to the third generation of quinolones. The first and second generations of compounds are active against the gram negative bacteria while the third and fourth generation quinolones are more advanced and active against gram positive bacteria as well (Ferech *et al*., 2006).

The most prescribed Fuoroquinolones in the Europe till 2003 was Ciprofloxacin. The trend is now shifting towards the third generation Moxifloxacin and LEVO due to their even broader spectrum action (Ferech *et al*., 2006). Like other drugs fluoroquinolones cannot be metabolized entirely within the animal and human body, due to which a considerable amount of drug is released in the wastewater in its pharmaceutically active form (Kümmerer, 2009).

1.2 Scope of the Study

As discussed above the occurrence of Fluoroquinolones in wastewater has been confirmed in countries such as The United States, China, Switzerland, Australia and some other (Watkinson *et al.,* 2007 and Ikehata, 2008). Asian countries, especially Bangladesh, China, Pakistan and India are the rising pharmaceutical manufacturers and user of Fluoroquinolones. Untreated waste from production and packaging units is therefore contributing to this problem.

1.3 Hazards

The discharge of FQs in environment is a hazard for human health as they induce drug resistance in the native bacterial strains (Chee-Sanford *et al.,* 2009). This resistance is highly undesirable as Fluoroquinolones are preferred to be as "last resort" when other remedies are not effective (Cardoza *et al*., 2005). On the other hand their discharge is matter of a concern because such discharge ultimately gets mixed with the water body used for a drinking purpose and high concentration of the compounds could be harmful to the consumer (Hooper and Wolfson *et al*., 1985). Fluoroquinolones are also known for their genotoxicity and have been found to be toxic to plants (Brain *et al.,* 2004) as well as aquatic organisms (Robinson *et al.,* 2005). It is, therefore, important to consider their elimination while treating waste water.

1.4 Treatment Methods

Conventional wastewater treatment techniques are not very effective for remaining pharmaceutical compounds and a high concentration of drugs remains in the sludge, disposal of which is a big problem (Yang *et al.,* 2011). There is a need therefore to

develop a method of treatment which could convert the antibiotic into less active products. Oxidation methods such as Fenton and photo-fenton oxidation (Gonzalez *et al.,* 2007), heterogeneous photo catalysis (Palominos *et al.,* 2009), H_2O_2 enhanced photolysis (Andreozzi *et al.,* 2003) and ozonation (Yargeau and Leclair, 2007) are thus fairly popular. One of the techniques receiving a lot of attention these days is the photocatalysis using Titania with UV light which has been found to be very efficient in degrading organic compounds.

1.5 Proposed Solution

Taking account of what has been discussed above, use of Titania Nanotubes (TNTs) to degrade FQs was selected for the present work. There have been extensive studies on the degradation of other Fluoroquinolones like Ciprofloxacin and Ofloxacin (An *et al.,* 2010; De Bel *et al.,* 2009; Hapeshi *et al.,* 2010), where as photocatalytic oxidation of LEVO, using Titania Nanotubes has not been reported yet.

Titania is considered as one of the popular and efficient catalyst but its ability to absorb only ultraviolet portion of light limits its use. Detailed studies have been done on modifying Titania so that it would be able to absorb light in visible range (Pan *et al*., 2010). Dopping of NPs with different metals has been commonly used and proved to be a sucessful method in reducing the bandgap of Titania, however doping of TNTs is comparitively a new area and less work has been done in this regrad (Fu *et al*., 2013).

The use of anatase Titania, in nanoparticles form usually requires larger dosage as well as its recovery is difficult after the photo catalysis process by simple filteration (Costa and Prado, 2009). For this reason the aim of study is focused on the efficient

recovery of Titania Nanotubes after the reaction as well as transformation of the antibiotic agent into less active, non-toxic or completely mineralized form.

1.6 Present Study

The objectives of this research work can be summarized as:

- Synthesis and characterization of pure and doped TNTS
- Photocatalytic degradation of LEVO– optimization of parameters
- Photocatalytic degradation of LEVO – Kirby-Bauer test

Chapter 2

LITERATURE REVIEW

2.1 Antibiotics in the Environment

Discovery of antibiotics has always been considered as the wonder of the 20th century. There is no doubt about it but in the present scenario the problem being faced is the environmental contamination and antibiotic resistance in communities and hospitals due to their overuse (Davies and Davies, 2010). Since the discovery of penicillin in 1928 by Alexander Fleming, thousands of other antibiotics have emerge in the market with the following major uses:

- For human and animals to treat diseases
- For fees efficiency improvement
- As growth promoters (Addison, 1984)

Due to their diverse use the risk associated with the release of these antibiotics now become a global concern as significant quantity of up to few µg/L have been detected in aquatic environment (Halling-Sorensen *et al.,* 1998). Once used, they are excreted partially metabolized and enter the sewage system. These drugs cannot be easily removed by the conventional waste water treatment plants and would ultimately mix with the receiving water bodies (Kümmerer, 2003).

2.1.1 Sources & Pathways

Since the antibiotics are being very widely used today by humans and animals, hence the sources for antibiotics are abundant. They have especially an important role to

play in such industries as livestock and agriculture e.g. antibiotics are being abundantly used for improving efficiency of feed and for improving growth in livestock (Levy and Levy, 1992).

2.1.1.1 Manufacturers

The study done on ground water of a landfill used for pharmaceutical waste by Holm *et al.,* (1995) showed a varied forms of antibiotics with a concentration as high as 5mg/l. Though, the most prominent source of antibiotics are pharmaceutical manufacturers, however, there role in contamination is minimum because of being limited to only a certain area and being only a point source.

2.1.1.2 Human Medicine (Hospitals and Households)

Different countries have different ratio of consumption for humans in each compound. The rates for intake of prescribed antibiotics and those for the unprescribed one are considerably different for different countries. (Mölstad *et al.,* 2002). The remainders of expired medicines are mostly disposed off through household drains. A study showed that almost one third of the total pharmaceuticals being sold in Germany and almost 25 % of that in Austria were disposed off through the drain or along with household waste. One of the most common routes includes the disposal of unused pharmaceuticals through toilet or sink or with household waste (Bound and Voulvoulis, 2005). The drugs which are absorbed by the body of organisms undergo several reactions like hydroxylation, glucoronidation etc but even then, a major portion of the drug would not be processed by the body and would be excreted as such and will go to sewage. The human feaces may contain antibiotic in as high a concentration as 300 mg/kg.

Unlike the common assumption, hospitals aren't the most common source of contaminating the sewage with pharmaceuticals (Kümmerer, 2009 and Schuster *et al.,* 2008). It was estimated that, 70% use of antibiotic can be attributed to household. This percentage is 75% in US (Wise, 2002).

2.1.1.3 Animal Production &Veterinary Medicine

It is routine practice to use antibiotics for prevention of diseases in livestock. The feed and the water of animals also contain antibiotics in subtherapeutic concentrations for enhancing growth (Cromwell, 2001).

Out of the total antibiotics used in EU in 1996, 50% was used in growth promoters in veterinary as was reported by European Federation of Animal Health (FEDESA).

The application of livestock manure on land also results in the introduction of antibiotics on a huge scale in the environment. Waste slurries and manure can have considerable quantities of antibiotics and they can have persistant presence in soil after application (Donoho, 1984; Gavalchin and Katz, 1994).

2.1.1.4 Aquaculture

The considerable use of antibiotics in aquaculture accounts for such a wide range of routes for antibiotics to be introduced in water. The use of antibiotics by fisheries in foods and against diseases results in introduction of antibiotics in surface water (Halling-Sorensen *et al*., 1998). Antibiotics are majorly used for therapeutic reasons in aquaculture. They can be introduced through feed or water. Moreover, the unused drugs mostly go to sediments and would hence degrade (Lai *et al.,* 1995) or would go to the

water in surroundings (Smith and Samuelsen, 1996). The cases of persisting antibiotics and changing microbial resistance as observed earlier in medicated feeds is attributed to aquaculture (Husevag *et al.,* 1991 and Nygaard *et al.,* 1992).

2.1.1.5 Plant agriculture

The use of antibiotics against diseases in high value plants, ornamental plants and vegetables can be dated back to 1950s. An antibiotic can be considered useful against disease if it remains active inside or on the plant and can tolerate UV irradiation, oxidation, high temperatures and rainfall. These properties are the main reason for causing environmental problems. However, the situation varies in different countries due to a difference in regulations (Kümmerer, 2009).

So the sources of dissemination of antibiotics in environment can be both human and agriculture through flushing of expired prescriptions, excretion, medical waste, leakage of septic system, discharge coming out of wastewater treatment facilities and waste storage structures of agriculture sector. The other sources and pathways include application of agricultural waste and human waste on land and surface runoff (Sarmah *et al.,* 2006).

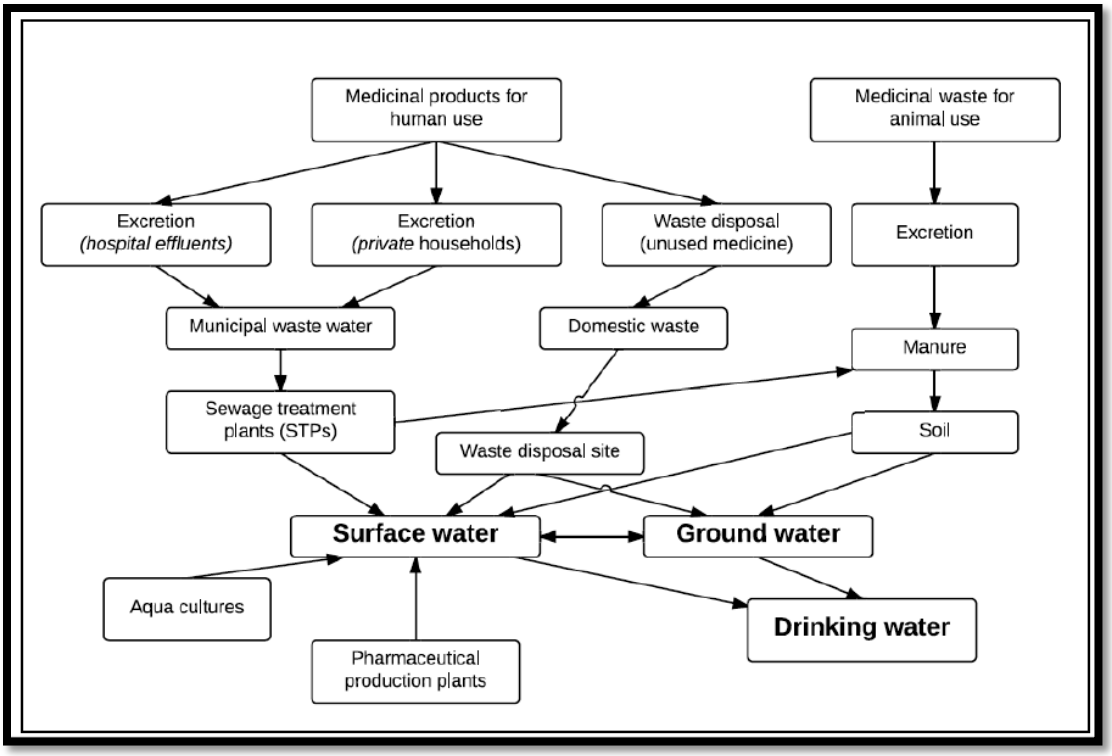


Figure 2.1: Possible pathways for antibiotic exposure to environment (Heberer,2002)

2.2 Quinolones

It is the class of a synthetic antibiotics used to treat a number of bacterial diseases. Its clinical use can be dated back to 1960. There first generation was started when nalidixic acid was introduced for treating infections of urinary tract in humans in 1962. The use of Quinolones was limited to managing infections of urinary tract due to their small spectrum of activity and poor concentrations in tissue and serum in early years. They can be divided into four generations till today, depending upon their activity against a vast range of the pathogens or spectrum of their activity. In recent times, market has seen fluoroquinolones having more broad spectrums of activity and enhanced profiles on pharmacokine (Lee and Kanatani, 1999).

2.2.1 Fluoroquinolones

This class of drugs is made by adding fluoride to the quinolone's original antibiotic compound. They have broader antimicrobial spectrum and a better profile for pharmacokinetic properties. This is today the more common subset of clinically used quinolones.

Following figure shows the basic structure of FQs;

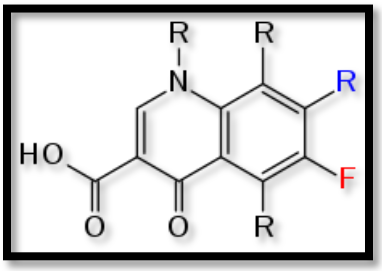


Figure 2.2: Structure of Fluoroquinolones

R represents piperazine and on addition of the fluorine atom in the structure, it would be named Fluoriquinolone.

A broader activity spectrum requires even greater care for avoiding the bacterial resistance. The doctors in Italy have reported event of spontaneous adverse effect making flouroquinolones among the top most agents responsible for adverse psychiatric and neurological effects .These effects are referred by some patient advocacy and support groups as "flouroquinolone toxicity".

In terms of the world revenue, fluoroquinolones are the most rapidly growing antibacterial class which is being used increasingly in hospitals as well as community sectors for treating lots of infections. Due to their improved antimicrobial activity, the

fluoroquinolones are being used beyond the traditional quinolone antibiotic indications for treating the infections of urinary tract. Fluroquinolones are found useful against a broader range of infections like respiratory infections and skin infections etc (King *et al.*, 2000).

2.2.2 Mechanism of Action

Fluoroquinolones are the sole direct inhibitors for bacterial DNA synthesis when it comes to classes of clinically used antimicrobial agents. They inhibit a couple of bacterial enzymes i.e. topoisomerase IV having major role in replication of DNA and DNA gyrase. Quinolones attach themselves to the complex of every enzyme with DNA making a compound containing the drug which blocks the DNA replication enzyme complex from making progress (Drlica and Zhao, 1997). This leads to damaging the bacterial DNA and the bacterial cell dies. Hence, fluoroquinolines are the bactericidal agents.

2.2.3 Variety of Fluoroquinolones

A variety of drugs from the class fluoroquinolone are available today in the market:

- Enrofloxacin
- Flumequine
- Ciprofloxacin
- Lomefloxacin
- Norfloxacin
- Ofloxacin
- Levofloxacin

- Grepafloxacin
- Sparflaxacin
- Trovafloxacin
- Gemifloxacin
- Moxifloxacin

Among the above mentioned fluoroquinolones, ciprofloxacin (CIP) and LEVO occupy 65% of global market of antibiotics (Lodha *et al.,* 2008). The most commonly used fluoroquinolones in USA in 2008 were moxifloxacin, LEVO and ciprofloxacin (Carroll and Carroll, 2008). Ciprofloxacin is from the 2nd generation whereas LEVO is from 3rd generation which is more recent and has wider spectrum. 2nd generation has the antimicrobial spectrum including gram negative organisms (including the *pseudomonas* species), some of the gram positive organisms (*staphylococcus aureus* are included but *streptococcus pneumonia* not included) and a few atypical pathogens. 3rd generation includes, in addition to 2nd generation agents, the more extended gram positive organisms (penicillin sensitive as well as penicillin resistant *S. pneumonia*) and enhanced activity against the atypical pathogens (King *et al.,* 2000). The agents of 3rd generation also have higher potency against the anaerobes also (Bolon, 2009). Owing to this versatility, ciprofloxacin is being replaced by LEVO as the more commonly prescribed fluoroquinolone (Ferech *et al.,* 2006).

2.3 Levofloxacin

LEVO (Levaquin (U.S.), Tavanic (E.U) and others) is an antibiotic with a broad spectrum from the drug class of fluoroquinolone. This spectrum of activity encompasses

most bacterial pathogen strains causing urinary tract, respiratory gastrointestinal and the abdominal infections including the gram negative (*Haemophilus influenza, Escherichia coli, Legionella pneumophilla, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis,* and *Moraxella catarrhalis*), gram positive (*Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis,* and *Streptococcus pneumonia*), and the atypical bacterial pathogens (*Mycoplasma pneumonia* and *Chlamydophila pneumonia*). This vast spectrum of activity, great tissue protection and easy availability in oral as well as intravenous formulations for LEVO and the other fluoroquinolones has increased their value. LEVO alone or combined with other antibacterial drugs can treat many bacterial infections which include urinary tract infections, pneumonia and the abdominal infections (Mandell *et al.,* 2007).

2.3.1 Side effects

LEVO along with other FQs are normally well tolerated, however, in some rare cases they have resulted in reactions that are serious and life threatening and also produced spontaneous ruptures of tendons and peripheral neuropathy (nerve damage) that is irreversible, severe disorders of central nervous system, phototoxicity/photosensitivity reactions, kidney damage, hypoglycemia and rhabdomyolysis (muscle wasting) (Petitjeans *et al.,* 2003). The tendon damage can last even after the months after use can sometimes even cause lifelong disabilities.

Elderly and children are comparatively more prone to such extreme reactions. These adverse reactions can manifest not only during the therapy but also long after the discontinuity of fluoroquinolone therapy. In addition to this, the other resistance to this

drug is another issue resulting due to its overuse e.g. using it in instances where the antibiotic therapy was not necessary or using these drugs when the drugs with narrower spectrum could have produced good results (Petitjeans *et al.,* 2003).

2.3.2 Black Box Warning

People sometimes claim suffering from long term serious adverse effects to health due to the use of fluoroquinilones. On behalf of such individuals, a class action lawsuit has been filed along with the action by the Public Citizen, a consumer advocate group. As a result of such efforts, Food and Drug Administration (FDA) in USA ordered a black box warnings in 2011 on fluoroquinolones which also includes LEVO in order to advise the consumers about high tendon damage risk (Sprandel and Rocivold, 2003) Under this warning, a pharmaceutical company can be required by FDA for placing a boxed warning on the label of prescription drug, or describing it in the literature. This is the strongest warning required by FDA, signifying that the studies have indicated that the drug can cause significant risk of adverse effects which can be serious and can even be life threatening (FDA).

2.3.3 Levofloxacin/ Fluoroquinolones in Environment

The consumed as well as the unused disposed off pharmaceuticals found in our environment can affect our ecosystem as well as our health. In addition to this, some pharmaceuticals in some foods and drinking water are found in low levels, raising concerns about the effects on human health (Oldenkamp *et al.,* 2013).

The LEVO that is excreted through urine mostly remains unchanged in the form of an active drug. Nearly 80% of the excreted LEVO is unchanged drug as only negligible

metabolism takes place resulting in the formation of metabolites which have pharmacological activity (Fish and Chow, 1997). LEVO exhibits chemical stability against high temperature and hydrolysis (Thiele-Bruhn, 2003). Moreover, it also resists biodegradation. LEVO is found in river water in concentrations varying from nanogram per liter to concentrations in microgram of a liter (Andreozzi *et al.,* 2003).

2.3.3.1 Effect on Primary Producers

Though they occupy an important position among the base levels in food webs, the changes in the community of cyanobacteria can have a significant, though indirect, effect on the remaining community of freshwater. This makes them useful for assessing toxicity in environment. According to some recent studies, high toxicity is exhibited by some LEVOs against cyanobacteria. Their toxic effect is seen on the photosynthesis of cyanobacteria, affecting the evolution of O_2. Duckweeds and algae are affected by LEVO even at very low concentrations i.e. 5 to 100 µg/L (Crane *et al.*, 2006).

When it comes to macrophytes, LEVO was particularly found to have elicited phototoxic effects when the range of concentration was 1000 µg L^{-1} (Brain *et al.,* 2004). It may have similar effects on other higher plants because they possess similar apparatus of photosynthesis. Hence LEVO can adversely affect primary photosynthetic producer (Pan *et al.,* 2009).

2.3.3.2 Sorption

Fluoroquinolones are strong absorbers onto soils, sewage sludge and sediments and do not undergo biodegradation with the sediments. Below 1% of the approved fluoroquinolones for poultry disease prevention was removed in 80 days from different

soils because of being highly capable to bind with the soil. Fluoroquinolones used in human medicine are capable of reaching terrestrial environment by way of sewage sludge. It was confirmed by Golet *et al.,* (2002) and Ginger *et al.* (2003) that fluoroquinolones are abundantly found in sewage sludge (concentration range of 1.4 to 2.42 mg kg-1 of the dry matter). They also showed that the FQs persisted in the sludge treated soils even after a month had passed after the application.

2.3.3.3 Environmental Bacteria

A study was performed by Kümmerer *et al.,* (2004) for assessing the effects of various antibiotics on the environmental bacteria. The measurement of effect was done based on the concentration of the antibiotics that resulted in the inhibition of respiration in the bacteria. The results showed that in the test compound, LEVO had the highest effect on bacteria of sewage sludge due to its broader spectrum of activity exhibited against the gram negative and gram positive bacteria. The results showed that concentration that corresponds to 50% respiration inhibition (IC50) for LEVO is lower among all.

Compound	IC50 (mg/l)	
	30 min	20 h
Antibiotics		
Amoxicillin	>100	>100
Benzylpenicillin, sodium	>100	>100
Ceftriaxon, disodium	>100	>100
Cefuroxime, sodium	>100	>100
Chlortetracycline, hydrochloride	>100	10–100
Clarithromycin	>100	10–100
Clindamycin	>100	>100
Erythromycin	>100	10–100
Gentamycin, sulfate	>100	10–100
Imipenem	>100	1–10
Levofloxacin	10	1
Metronidazole	>100	>100
Monensin, sodium	>100	>100
Nystatin	>100	>100
Ofloxacin	>100	1–10
Sulfamethoxazole	>100	>100
Tetracyclin	>100	1–10
Trimethoprime, naphtoate	>100	>100
Vancomycin, hydrochloride	>100	>100

Table 2.1: IC50 for Levofloxacin

Nitrification is an important step in waste water purification, eliminating toxic ammonia. It is also important in agricultural system for the recycling of nutrients. The second step of nitrification, i.e. oxidation of nitrite to nitrate is particularly sensitive and performed by gram negative bacteria. For Gram negative and broad spectrum antibiotics such as fluoroquinolones significant inhibition of this step was observed under uncontrolled conditions which may lead to accumulation of nitrite nitrogen in the plant effluent, a form of nitrogen which is particularly toxic (Kümmerer, 2009).

2.3.3.4 Bacterial Resistance

The evolution of antibacterial resistance in human pathogenic and commensal microorganisms is the result of the interaction between antibiotic exposure and the transmission of resistance within and between individuals.

There are variety of reasons of selecting fluoroquinolones for the present study but most important reason for this is, that according to the ranking of antibiotics for monitoring the emergence of resistance comes under the category of "critically important or high Concern" (FDA).

Ranking of antibiotics for monitoring the emergence of resistance in the U.S.		
Critically important or High Concern	*Highly Important or Medium Concern*	*Important or Low Concern*
Broad spectrum Cephalosporins	Aminoglycosides	Narrow and expanded spectrum Cephalosporins
Fluoroquinolones	Amoxicillin	
Macrolides	Ampicillin	Monobactams
Lincosamides	Glycopeptides	Quinolones
	Streptogramins	
	Tetracyclines	

Table 2.2: Antibiotics ranking for emergence of resistance

2.3.3.5 Degradation

Since the antibiotics are introduced from livestock and household operations through water the hydrolysis can work as an important pathway for degradation, however, fluoroquinolones show insensitivity towards increased temperature and hydrolysis. Moreover, they are even not susceptible towards biodegradation because substances having high sorption for minerals or organic materials found in soil and

manure usually have slow rates of degradation because they aren't available to be degraded by microorganisms.

2.4 Treatment Methodologies

This is an indication that the antibiotics cannot be biodegraded in a sewage treatment plant for complete removal and need some alternate method of removal (Burhenne *et al.*, 1997; Thiele-Bruhn, 2003 and Viola *et al.*, 2004).

The conventional methods for treating wastewater e.g. activated sludge treatment and clarification do not work for LEVO and it hence, attention is being paid today towards more advanced processes of treatment. Advanced oxidation or ozonation, advanced carbon adsorption and membrane separation are among the most well known process of advanced treatment today and are effective in removing a lot of pharmaceutical products which are common in wastewater.

2.4.1 Ozonation

Ozone shows extreme reactivity towards some pharmaceuticals like estrogen and estradiol but is not useful for all types of molecules. Only a little literature is found on the ozonation of the pharmaceuticals in actual wastewater of pharmaceuticals. Furthermore, the information about the required dose of ozone for removing pharmaceuticals is also contradictory. The biggest drawback of ozonation is that it doesn't fully neutralize the target compound but only transforms it and hence can result in producing even more dangerous substances. This leads to the additional requirement of sand filteration for breaking down the reactive products of oxidation. Hence ozonation leads to higher costs (Deegan *et al.*, 2011).

2.4.2 Activated Carbon Adsorption

The common difficulty associated with treatment using powdered activated carbon is found in separating carbon from the water. Different available options include sedimentation, necessitating the usage of precipitants or membrane filtration requiring additional energy. Filtration reduces the blockage of micropores by the compounds having high molecular weight. As a result, powdered activated carbon can only be used for treating the already treated wastewaters or those which are low in organic loading (Deegan *et al.*, 2011).

2.4.3 Membrane Separation

Membrane processes like ultra-filtration and microfiltration are usually not completely effective for removal of organic contaminants because the pore sizes ranges from 100 t0 1000 times bigger than that of micropollutants and hence these can pass through the membrane. Attention is being paid on reverse osmosis and nanofiltration. But there are only a few studies on using these processes for this purpose. Although, both these processes are efficient enough but the main problem is that of the disposal of the sludge which can have more concentrated pollutant (Deegan *et al.*, 2011).

There are some obvious issues associated with the technologies mentioned above and photocatalysis is found to be a feasible alternate option.

2.5 Photo-Catalytic Oxidation

The Greek word photocatalysis is made of two words; the word photo is from ‘photos’ which means light and catalysis is from ‘katalyo’ which means to break apart or decompose. Photocataysis is, therefore, a process whereby we use light to activate a photo catalyst which changes the rate of the chemical reaction while not getting involved

in the reaction itself. In heterogeneous photocatalysis, there is a previously formed interface between the fluid that contains the reactants and products and the solid photocatalyst i.e. a semiconductor or metal.

2.5.1 Titania as a Photocatalyst

An efficient photocatalyst is one with low band gap, it is photoactive, chemically and biologically it is inert, non toxic, inexpensive and photostable (Bhatkhande *et al.*, 2002). TiO_2 is known to be a superior semiconductor for decomposition of organic materials because it possesses good photocatalytic property. Owing to its thermal and chemical stability TiO_2 is today one of the most commonly investigated photocatalyst.

2.5.2 Mechanism

On being illuminated, titania absorbs photons and promotes electrons to conduction band. A hole is left in the valence band resulting in a pair of positive hole (h^+) and negative electron (e^-). Band gap is the energy difference between the conduction band and the valance band. The required wavelength for photo-exitation is:

$$\frac{1240\ (\text{Planck's constant, h})}{3.2\ \text{ev (band gap energy)}} = 388\ \text{nm}$$

The positive hole breaks the water molecule producing hydroxyl radical and hydrogen gas. The reaction of negative electron with oxygen molecule forms the super oxide anion which also has some role oxidation. Degradation performance exhibited by titania is due to hydroxyl radicals which are highly oxidizing (Carp *et al.*, 2004).

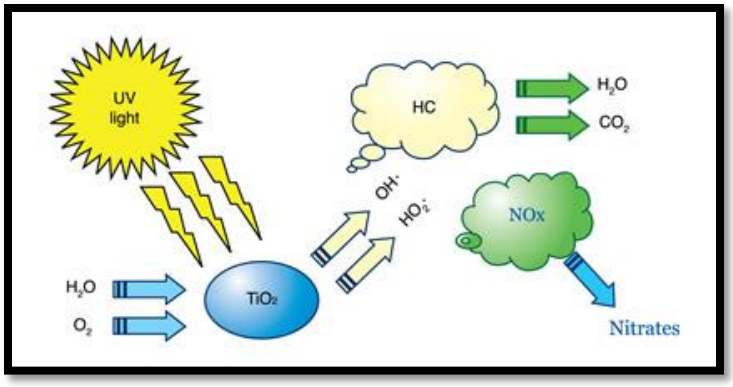


Figure 2.3: Photo-catalysis Process using Titania

2.5.3 Titania Polymorphs

Titania has three different types of polymorphs; brookite, rutile and anatase. Among them, anatase is the preferred polymorph due to highest catalytic activity. According to some studies, it is claimed that better results are achieved from the mixture of rutile and anatase for catalytic degradation of the organic material (Giolli *et al.*, 2007).

2.5.4 Drawback

Pure TiO_2 has a large band gap due to which it cannot be activated until irradiated with photons of UV domain ($\lambda \leq 387$nm for anatase). It limits the solar applications' practical application. Hence, in order to improve solar efficiency for TiO_2, we have to change the nano material for facilitating the absorption of visible light.

2.5.5 Doping

Modifying titania through doping with transition metals like Co, Fe, V and Cr extends its spectral response in visible region along with improvement in photocatalytic activity. Usually, the photocatalytic activity is dependent on the amount and nature of the doping agent. Metals deposition on TiO_2 surface improves photocatalytic activity in visible light

as it acts like an electron trap and hence promotes the interfacial charge transfer and delays electron-hole pairs' recombination.

2.6 Nanotechnology in Wastewater Treatment

A nanomaterial can be typically defined as material which has size smaller than 100 nm of at least one dimension. At this size materials usually possess unique properties which are different from their larger counterparts. The most important size dependant properties include high reactivity, greater surface area and high sorption ability.

Adsorption is the most commonly used properties for the removal of organic compounds from the waste water. The effectiveness of conventional adsorbent is restricted due to its small surface area or potentially active site which affects the reaction kinetics. Whereas nanomaterial is a solution to that problem as they posses high surface area, small intra particle diffusion distance, more active sites and pore volume and its surface chemistry (Qu *et al.*, 2013).

2.7 Nanotubes

Use of nanoparticles is currently receiving a lot of attention not only in treating waste water but in many other fields. The problem regarding planner surface like nanoparticles was the optical loss i.e. photons of light required to excite the photocatalyst. Planner surface is unable to utilize maximum photons for its excitation. Nanotubes, however in addition to high surface area have also a light trapping ability due to this pipe geometry.

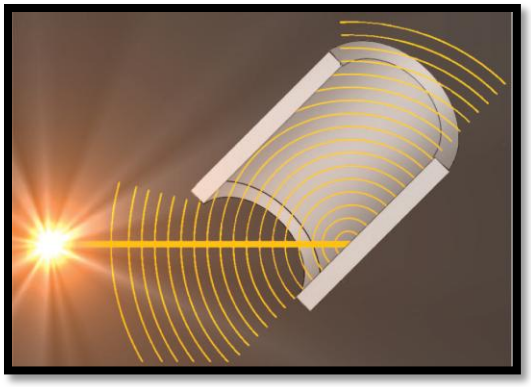


Figure 2.4: Principal of pipe geometry to capture light

This diagram can easily justify the fact that how a tubular structure can trap the maximum photons of light for its excitation which would otherwise be lost in case of planner surface (Shankar *et al.,* 2009).

2.8 Synthesis of Nanotubes

Titania Nanotubes can be synthesized by a variety of methods. Most well known among them are (Morgan *et al.,* 2008; Wang *et al.,* 2007)

- Chemical Vapor Deposition
- Anodic Oxidation
- Hydrothermal Synthesis
- Sol-gel method

Among those, the Hydrothermal method is more preferable as it is relatively simple methods and condition for synthesis i.e. temperature and pressure are moderate and easy to maintain (Guo *et al.,* 2008).

2.9 Degradation of fluoroquinolones

Some work has already been done on degradation of fluoroquinolones which also supports the use of titania as photocatalyst. Nasuhoylu *et al.,* (2012) studied the removal

of LEVO by ozonation and Titania photo-catalysis. They concluded that both methods are efficient but ozonation products are more persistent than those produced during photo-catalysis. Lam and Mabury, (2005) have also studied the photo degradation of atorvastatin, carbamazepine and LEVO in natural waters and their findings were that; the presence of humic matter in natural waters decreases the degradation rate of, specifically, LEVO.

Another study conducted by Sunderland *et al.,* (2001) on the activity of Fluoroquinolones photodegradation products showed that with only LEVO and Ofloxacin, the zone of inhibition size for control solution was significantly less than irradiated solutions which indicates that photo degradation products can possess antimicrobial activity. A study has also been conducted on fluoroquinolones degradation at IESE by Hayder *et al.,* (2012). According to his work Ciprofloxacin degradation is highly efficient in the presence of Titania Nanoparticles with UV exposure of 120 min.

Most of the studies in this regard are restricted to use of nanoparticles only and no work has been done on nanotubes to the best of our knowledge. These studies however provide a baseline for using Titania photocatalyst with high surface area.

Chapter 3

MATERIALS AND METHODS

3.1 MATERIALS

To carry out the research work TiO_2 (GPR, BDH Chemicals Ltd. Poole England) was used to prepare TNPs followed by pure and doped TNTs from this material. Analytical grade NaOH was used for the synthesis of Titania Nanotubes and Iron oxide for doping. Leflox (Getz pharma) was purchased from the local market which was the target organic compound to degrade and analytical grade HCl was used to dissolve the drug in water. Pure culture of *E. coli* and *Pseudomonas Aeruginosa* was taken from Microbiology Laboratory of Institute of Environmental Science and Engineering, National University of Science and Technology, Islamabad, Pakistan for Kirby-Bauer test.

3.2 LEVO STOCK SOLUTION

Stock solution was prepared by grinding 250 mg tablet of Leflox and dissolving in 100 ml of 0.1N HCl solution. The dissolved compound was transferred to a 250 ml flask filled up to the mark with distilled water. The resulting solution was sonicated for 20 mins and filtered giving a clear solution of 1mg/ml of drug which was pale yellow in colour.

3.3 SYNTHESIS OF TITANIA NANOTUBES

First Titania Nanoparticles were synthesized which were used as precursor for TNTs synthesis.

3.3.1 Titania Nanoparticles

TNPs were prepared by mixing the 20 g of GPR Titania in 100 ml of distilled water and stiring for 24 h. The solution was allowed to settle down and the resultant paste was placed in hot air oven for 12 h at 105 ^{o}C for 12 h to remove the moisture in it. The dried solid was then ground to fine powder and placed in NEY-525 SRIES II muffle furnace for calcination at 550^{o}C for 6 hrs. This resulted in the conversion of amorphous Titania to crystalline form. The resulting powder was then allowed to cool down slowly to attain crystal structure in nano size range (Danish, 2012).

3.3.2 Pure Titania Nanotubes

In the present study Titania Nanotubes were synthesized by the hydrothermal method. 1g of prepared nanoparticles were mixed with 100ml of 10M NaOH solution, sonicated for 1h and then transferred into Teflon lined autoclave that was heat, under pressure, for 24hrs at 150^{o}C. The resulting material has washed with 0.1N HCl and then with distilled water until the pH of the solution was in the range of 6.5 to 8. After washing, the sample was placed in a hot air oven for 8 hrs at 100^{o}C. To attain the maximum crystallinity the sample was further calcined for almost 1 hr at 500^{o}C in muffle furnace (Lee *et al*., 2009).

For 1% Fe-doped Titania Nanotubes 32mg of Fe_2O_3 was dispersed in the aqueous solution of NaOH and prepared Titania Nanotpaticles. After 30 mins of sonication the mixture was transferred to the autoclave and same procedure was followed as mentioned for pure nanotubes (Fu *et al*., 2013).

3.4 INSTRUMENTATION FOR CHARACTERIZATION OF TNTs

In order to monitor the physical aspects of the prepared TNTs, different instrumental techniques were used, for, detecting the tubular structure, phase identification of crystalline structure, elemental composition, surface area or pore volume and difference in the band gap of pure and doped TNTs.

3.4.1 Scanning Electron Microscope (SEM)

SEM is a very powerful technique which can have resolution of even less than one nanometer. SEM uses the focused beam of electron in vacuum to scan the sample and produce 3D image. When this primary electron beam strikes the sample it discharges very high energy electron, the pattern of this release of electrons provides information regarding the shape, topography, size, arrangement pattern of atoms and chemical composition. Main signals which it produces are as follows (Reimer, 2000).:

- SE - Secondary Electron (electron from specimen itself)
- BSE - Backscattered electrons (beam which reflected back after hitting nuclei)
- X-rays and with the light and heat as well

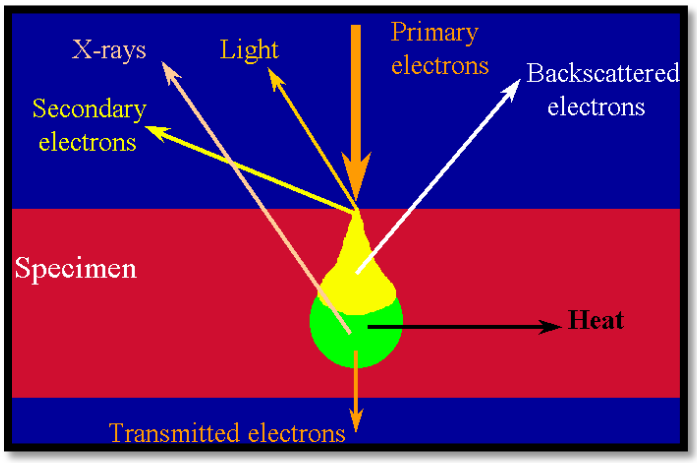


Figure 3.1: Beam Specimen Interaction

These signals give results which we required such as:

- As SE comes from the upper layer of the sample so it helps defining the topography and morphology. In short it aids in producing images of the specimen.
- BSE orgiginate from the inner layers so it gives compositional contrast in the sample having multiple phases, by bright and dull signals
- X-rays tell us about the chemical composition of the sample which will be later discussed in Energy Dispersive Spectroscopy (EDS).

3.4.1.1 Present Study

In this work JEOL JSM-6460 scanning electron microscope was used and the test was done with acceleration voltage of 20kV and filament current of 80 mA.

3.4.2 Energy Dispersive Spectroscopy (EDS) (Goldstein *et al.*, 2003)

EDS is done using an instrument attached to the SEM that gives information about the chemical composition of the sample. The principle on which it works is that when electron beam hits the sample atoms, the inner shell electrons are excited. The electron from the outer shell then fills the gap created. The energy difference between the outer and inner shell is released in the form of X-rays characteristics to each element. These X- rays are detected giving qualitative and quantitative information about the material.

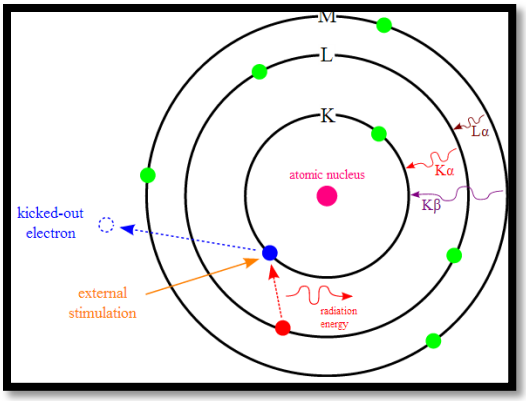


Figure 3.2: Principal of EDS

The most important property of this instrument is that it can also tell about elemental composition of a selected area from the image due to the controlled electron beam. The spectrum or graph produced by the EDS contains X-ray energy (which is characteristic of every element) on horizontal axis where as on vertical it contains number of counts. More the counts more will be the percentage composition of that particular element.

3.4.2.1 Present Study

The instrument used for this research was EDS system attached with JEOL JSM-6460 to find out the level of purity of synthesized Titania Nanotubes.

3.4.3 X-Ray Diffraction XRD

This technique allows determining the crystalline phase of a target sample (Brundle *et al.*, 1992). This can be done by simply comparing the peak position and their intensity with the literature. The standard database already has the pattern of peaks of known phases by which we can easily determine the phase of the target material.

3.4.3.1 Features of XRD

Only a small amount of sample is required for analysis i.e. 5mg or less to 1g (depends upon the density). By a single technique we can get following type of information:

- Orientation of single crystal/ grain
- Crystal structure of unidentified sample
- Crystallite size, determined with the help of Scherrer formula using half width. The range of detectable size is 2 to 100 nm
- Spacing between rows and layers of atoms

3.4.3.2 Principle of XRD (Birkholz, 2004)

The technique is based upon the diffraction of X-rays. As the beam of X-rays hits the crystal, it diffracts. The pattern of all diffracted beams with their angle of diffraction and intensities are recorded when the crystal rotates. Each orientation has its own angle at which it diffracts, with the help of which specific phase can be determined

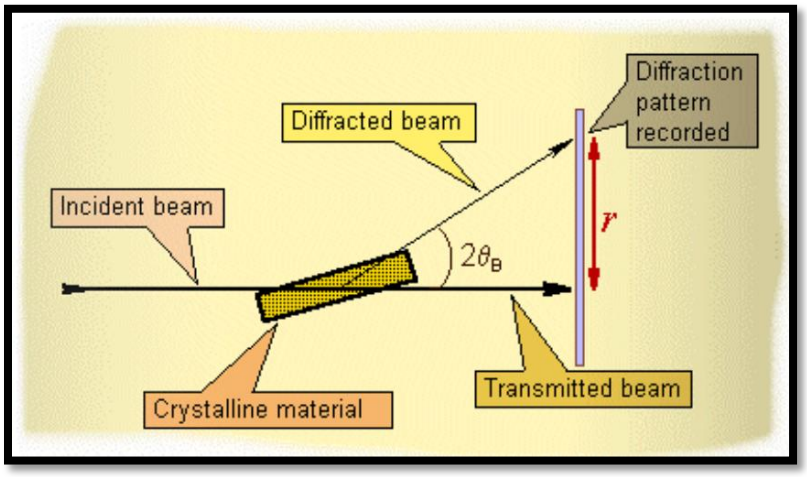


Figure 3.3: Principle of XRD

For the calculation of grain size the more intense peak of the phase is used, which in anatase phase is considered as 101. Scherrer formula is used for this purpose (Behnajady *et al.*, 2008):

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

Where,

D_p 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

3.4.3.3 Present Study

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

3.4.4 Surface Area & Pore Size Analyzer

The surface area and pore size can be analyzed by BET surface area testing. The principle of this test is that the interaction of a solid material is defined by its available surface area on which gas molecules in its surrounding could be adsorbed. BET is based on the physical adsorption of gas molecules on the surface of a solid

sample. By measuring the amount of gas that is adsorbed in a monolayer, the surface area could be derived. The surface area is calculated on the basis of number of gas molecules forming a single layer, by using the dimensions of a molecule (Mikhail *et al*., 1968). For this purpose the gas which is used is Nitrogen at the temperature of 77 k.

The most important step before performing the test is to degas the sample in order to clean the sample of any organic vapors or moisture. The measurement of pore size and distribution is based on the capillary condensation phenomenon. Pore size is the average size of pores which a sample possesses whereas pore distribution is the frequency of existence of pores in the sample. Specific area is the area excluding the pores.

3.4.4.1 Present Study

In this study, BET surface area and pore characteristics of doped and pure TNTs were analyzed by Surface and Pore Size Analyzer NOVA WIN 2200e.

3.5 EXPERIMENTATION

All the aspects regarding experimental setup will be discussed under this section.

3.5.1 Dilutions

Further dilutions were made out of stock solution, with a concentration of 2 to 12ppm. These dilutions were used to calibrate the Spectrophotometer and to select the one which gave the reading close to 1 absorbance.

3.5.2 Experimental Setup

50ml of selected concentration was taken and mixed with optimized amount of TNPs/ TNTs (pure/ doped) in dark. Container was then placed on shaker with a UV lamp fixed over it. RPM were adjusted to 150 and the whole setup was covered with a box with internal lining of aluminum foil, so that the solution could only get exposed to UV light. After 90 mins of illumination the Titania Nanotubes powder was separated by centrifugation. The absorbance reading of the supernatant was taken by UV/Vis Spectrophotometer (HACH DR 2400) at λ_{max} of 294nm. The reading before the experiment was taken as reference to show the percentage decrease in the concentration of the drug. It was further analyzed by TOC analyzer and LCMS as described above. Similar experiment was repeated for degradation under fluorescent light.

Figure 3.4: Experimental Setup

3.5.3 Degradation Efficiency

The degradation efficiency of pure and doped Titania Nanotubes was measured by difference of absorption before and after, by using spectrophotometer. It was calculated by the following formula:

$$D = 100\% - (\frac{Af}{Ao} X\, 100)$$

Whereas D is percentage degradation, A_o is the absorbance before the experiment and A_f is the absorbance after experiment.

3.5.4 pH Effect on Degradation

The reaction was carried out at three different ranges of pH (4, 7, and 12) and degradation efficiency at each pH was recorded. The pH of LEVO solution was adjusted by HCl and NaOH and batch experiments were done for 2 hours of illumination. After the batch reactions the TNTs were separated by centrifugation and absorbance of supernatant was recorded again to find the percentage degradation.

3.5.5 Regeneration of TNTs

The efficiency of TNTs powder, left after separating from the solution was also checked. For this purpose the TNTs powder separated through centrifugation was dried in dark and then mixed again to fresh solution of LEVO in a batch reaction.

3.6 INSTRUMENTATION FOR ANALYSIS OF LEVO

In this section all the instruments used for the analyzing the degradation of LEVO are discussed.

3.6.1 Spectrophotometer

UV/Vis spectrophotometer is an instrument which is commonly used in analytical for the quantitative analyses of organic compounds.

3.6.1.1 Principal of UV/Vis Spectrophotometer

The principal of this technique is based on the absorption of light by molecules that get excited to higher energy state. This absorption for this transition from the ground to higher energy state is measured. This absorbance is taken by the comparison of light intensity that is transmitted before and after passing through the sample (known as transmittance). The transmittance is inverse to absorbance (Burgess, 2007).

3.6.1.2 Beer-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.

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

Here A is the absorbance noted, c is the concentration of absorber, L is the path length and ε is the absorption coefficient which is specific to each compound present in the solution.

3.6.1.3 Present Study

For the present work the spectrophotometer used was Shimadzu spectrophotometer (HACH DR 2400) to measure the percentage degradation of LEVO at λ_{max} of 294 nm.

3.6.2 Total Organic Carbon (TOC) Analyzer

Calculating TOC from TOC analyzer depends upon the principal of combustion chemically or at high temperature. The destruction results in the conversion of all the organic matter into CO_2. This CO_2 released is then calculated and taken as total organic carbon present in the sample (Schumacher, 2002).

3.6.2.1 Present Study

For percentage degradation of LEVO, spectrophotometer was used but limitation of using it was that spectrophotometer does not confirms the degree of mineralization or the compound may converted to some other organic metabolites. To confirm the mineralization Analytik jena TOC multi N/C 3100 analyzer was used by directly injecting the solution after degradation and centrifugation.

3.6.3 Liquid Chromatography Mass Spectrometry (LCMS)

LCMS has become the most popular and preferred instrument for the detection of pharmaceutical compounds as well as their metabolites that result in photo catalytic degradation. It is a separation technique of all the compounds present in the solution and their further detection through Mass Spectrometry. It can separate a variety of compounds from small pharmaceutical metabolite molecules to large peptide chains.

3.6.3.1 Detectors

Conventional detectors used in LCMS can be:

1. Refractive Index
2. Fluorescence
3. Electrochemical
4. UV/Vis Detectors

Most of these detectors produce two dimensional results i.e. signal strength with reference to time. Whereas UV/Vis detector produce three dimensional results i.e. signal strength as well as spectral record at every point with reference to time.

3.6.3.2 Mass spectrometer

It also produces three dimensional results. With signal strength it also generates mass spectral record. The mass spectrum provides a very important information regarding molecular weight, possible structure, impurities and also identifies the components. The two compounds may show same UV spectra but cannot have same mass spectra so using MS confirms the identity of compounds in a very clear way.

3.6.3.3 Ionization

The technique of MS depends upon the ionization of molecules and identification of these ions using their charge to mass ratio (*m/z*). There are varieties of ion sources which are conventionally used for the MS, each of which are specific for different group of compounds. The initiation of a technique called Atmospheric Pressure Ionization (API) results in increasing the range of new compounds which can be analyzed now by LCMS. In this technique the compound is ionized at atmospheric pressure and electrostatic force then separates these from neutral molecules. Some common methods come under this technique are:

1. Electro Spray Ionization (ESI)
2. Atmospheric Pressure Photo Ionization (APPI)
3. Atmospheric Pressure Chemical Ionization (APCI)

3.6.3.3.1 Electro Spray Ionization (ESI)

Basic theme of ESI is that the sample solution from the LC is sprayed (nebulized) in a strong electrostatic field which results in the dissociation of the molecules. At the same time heated dry gas is applied, the purpose of which is to evaporate the solvent. As the droplets shrink the charge density increases which create the stronger repulsive force among ions. This repulsive force overcomes the cohesion force and ions are converted to gaseous phase. These ions are then moved through capillary orifice to the mass analyzer. ESI is the most frequently used ion source as it can easily analyze especially large molecules upto the range of 150,000 µ, but is also efficient for analyzing small molecules.

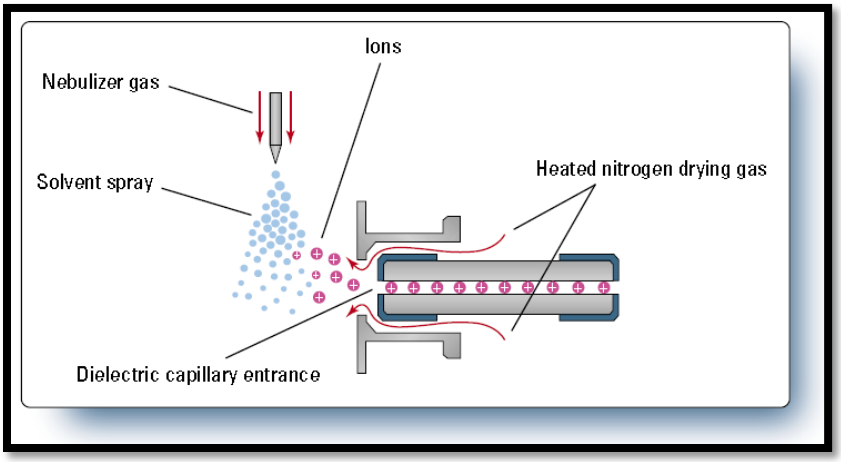


Figure 3.5: Electro Spray Ionization

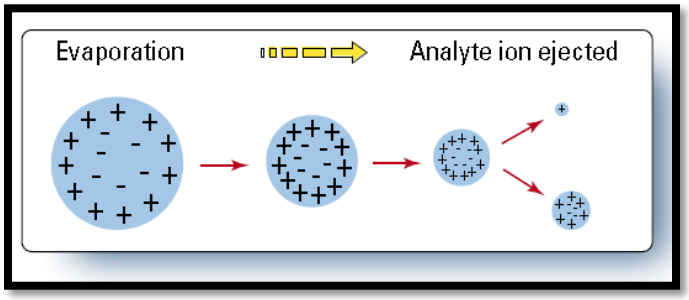


Figure 3.6: Desorption of ions from the solution

3.6.3.4 Mass Analyzer

Most commonly used mass analyzers are as follows

1. Quadrupole
2. Time of flight
3. Ion trap
4. Fourier transform ion cyclotron resonance

3.6.3.4.1 Ion Trap Mass Analyzer

The Ion trap consist of a chamber in which ions are trapped due to electromagnetic field. The selective ions can be ejected by applying another field. The benefit of this mass analyzer is that several stages of mass spectrometry can be done without using any separate analyzer.

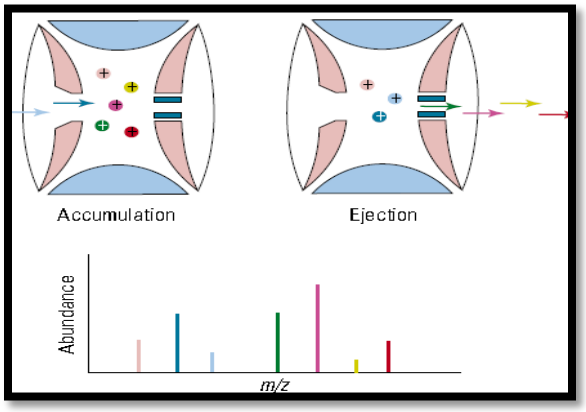


Figure 3.7: Ion Trap Analyzer

3.6.3.5 Present Study

For carrying out this research HPLC Agilent Technologies 1200 series was used with ion source of ESI and mass analyzer was Ion Trap. Following were the specifications for analysis:

- HPLC Agilent Technologies 1200 series

- HPLC Column (Eclipse XDB-C18 4.6x12.5mm 5µl)

Mass spectrometer

- ESI Mode.
- 6310 Ion Trap LC/MS

To carry out analysis Gradient method was used instead of isocratic as it was giving more precise peak for gradient flow. In gradient flow, the percentage of mobile phase (acetonitrile) changes with time where as in isocratic it remains same throughout the flow time. The pattern of mobile phase (B) was as follows:

Time (min)	B%(ACN)
0	10%
15	10%
40	40%
50	80%
60	100%

Table 3.1: Pattern for mobile phase

3.6.4 Zone of Inhibition/ Kirby- Bauer antibiotic testing

This test is commonly used to check the sensitivity of bacteria against antibiotics. It was also helpful in this research to find out whether the drug degraded or not after photo catalysis. The test was performed as follows

1. First of all an agar plate was prepared and bacterial suspension was spread over that plate with the help of swab which should be sterile to prevent the contamination.

2. Drug impregnated wafer was placed at the center of the plate and incubated for 24h. Same steps were repeated for the treated solution (Hayder *et al*., 2012).

RESULTS AND DISCUSSION

2.1 CHARACTERIZATION OF NANOTUBES

In this section results of characterization of the synthesized nanotubes will be discussed in detail, with a focus on SEM images, size, crystalline phase, elemental composition, surface area and band gap will be the main features.

2.1.1 Scanning Electron Microscope (SEM)

Figure 4.1 and 4.2 show the images of pure Titania Nanotubes obtained from JEOL JSM 6460 Scanning Electron Microscope at 20,000 and 40,000 magnifications. These images are clearly showing that the structure is long, tubular and almost uniform.

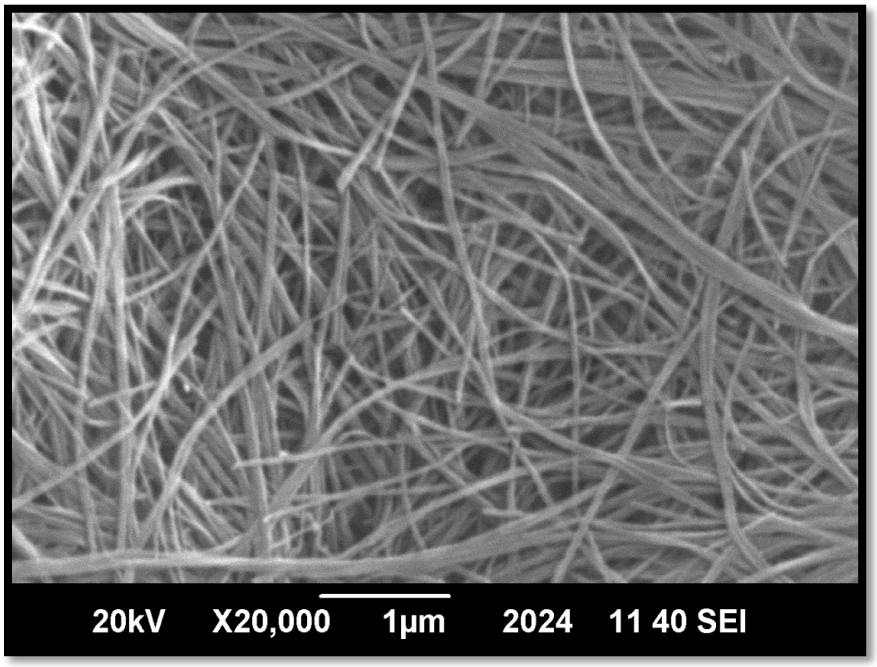


Figure 4.1: SEM image of Pure TNTs at X20,000

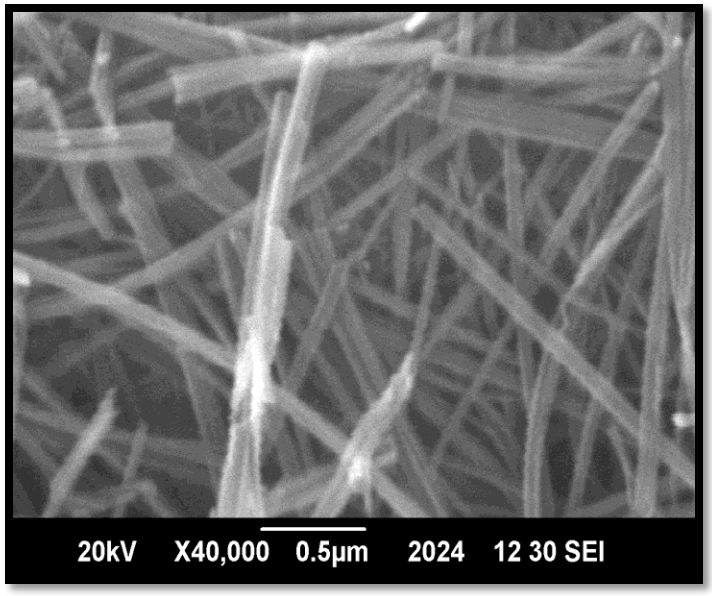


Figure 4.2: SEM image of Pure Titania Nanotubes at X40,000

Figure 4.3 and 4.4 shows the images of Fe-doped Titania Nanotubes at 5,000 and 30,000 magnifications.

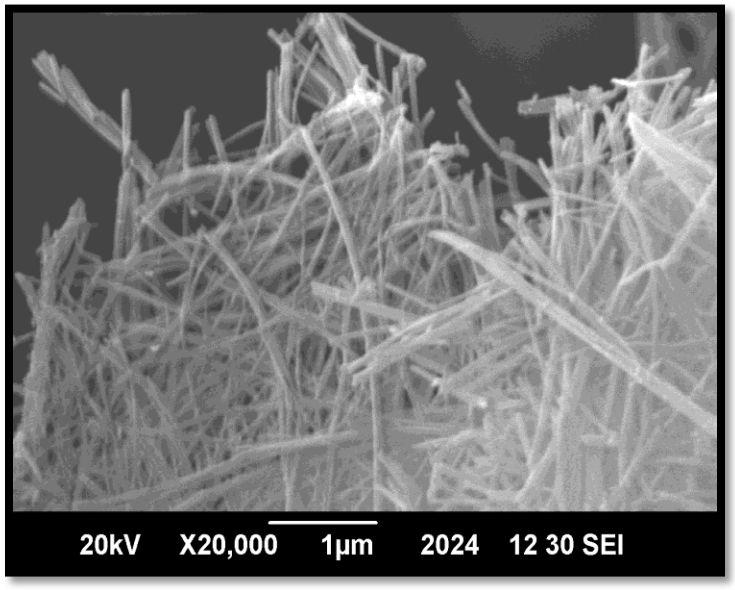


Figure 4.3: SEM image of 1% Fe-doped TNTs at X 20,000

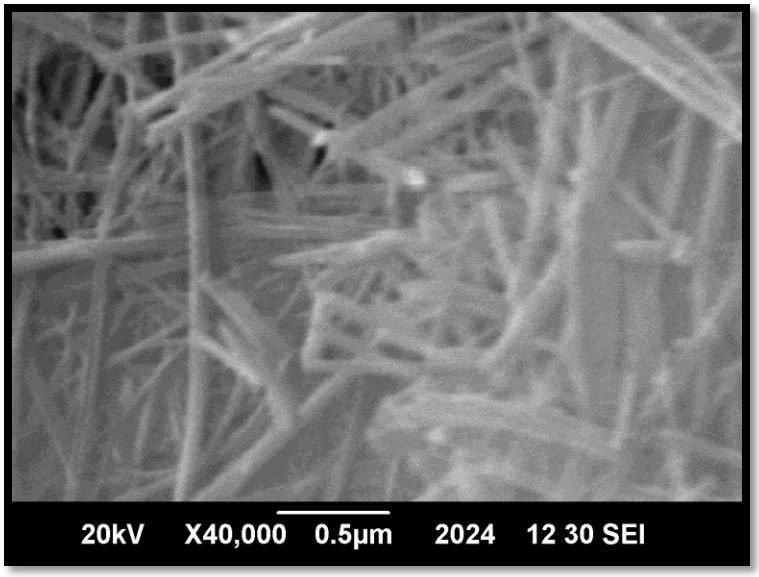


Figure 4.4: SEM image of 1% Fe-doped TNTs at X20,000

2.1.2 Energy Dispersive Spectroscopy

Figure 4.5 and 4.6 are the EDS spectra of pure and doped Titania Nanotubes. EDS results indicate that the chemical composition of pure TNTs is titanium and oxygen only whereas in doped Titania Nanotubes iron is additional. They both do not contain any impurity of sodium which could be present due to the use of NaOH in synthesis process. This confirms that the sample has been washed properly to completely eliminate the presence of any basic specie for batch reactions where the pH is an important factor. Table 4.1 and 4.2 are showing the percentage composition of elements present in the sample. The composition confirms that 1% doping has been achieved successfully.

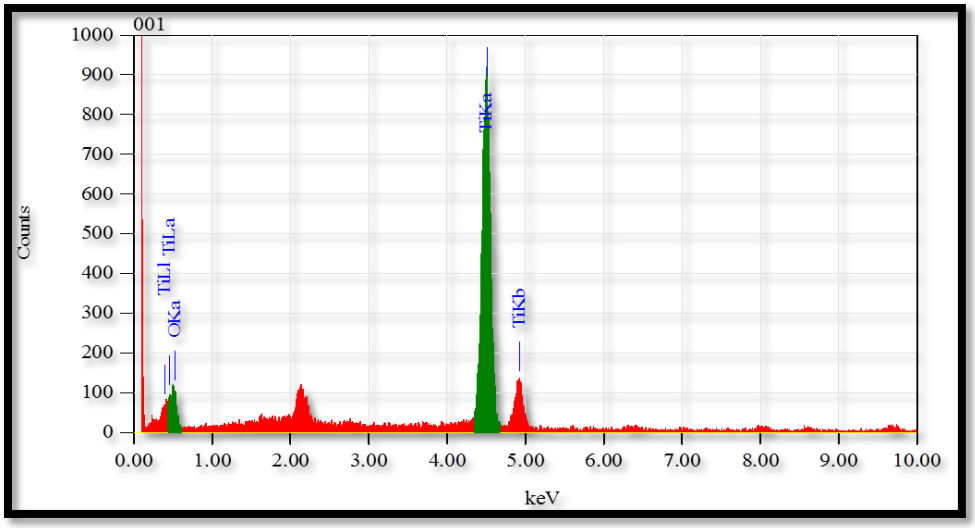


Figure 4.5: **EDS spectra of Pure TNTs**

Elements	Mass Percentage (%)
O	**33.51**
Ti	**66.49**
Total	**100**

Table 4.1: Mass% of Elements in Pure Titania Nanotubes

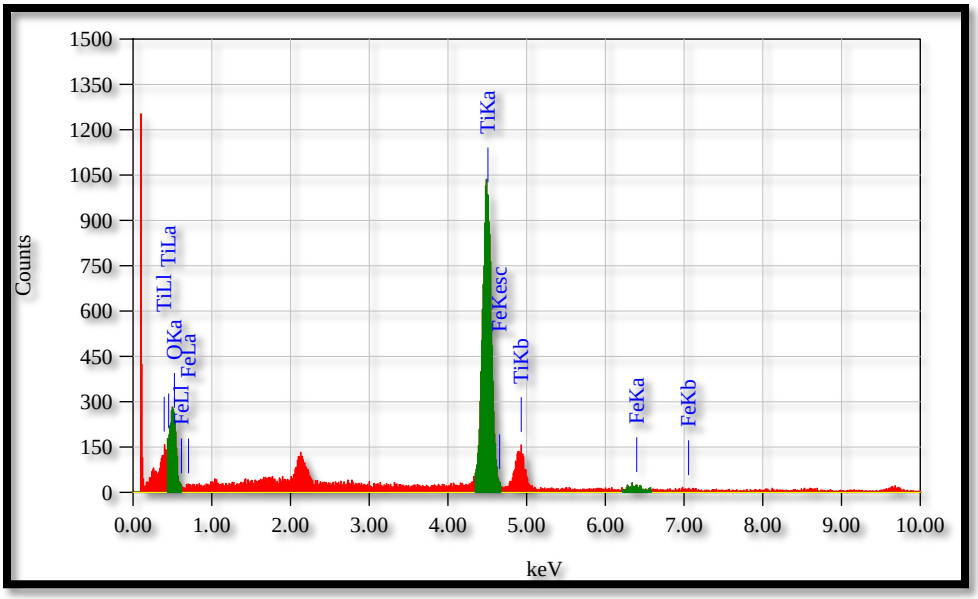


Figure 4.6: EDS spectra of Doped Titania Nanotubes

Elements	Mass Percentage (%)
O	44.64
Ti	51.13
Fe	1.23
Total	100

Table 4.2: Mass% of Elements in Doped Titania Nanotubes

2.1.3 XRD Analysis

X-Ray Diffraction (XRD) was carried out using Cu-Kα radiations at an angle of 2θ from 10^0 to 80^0, for both pure and doped Titania Nanotubes which showed a highly crystalline structure. Peaks at 25^0, 38^0, 48^0, 55^0, 56^0, 63^0, 68^0, 71^0 and 75^0 showed in figure 4.7 are the characteristic peaks corresponding to the (101), (020) planes of the anatase crystalline phase of Titania Nanotubes

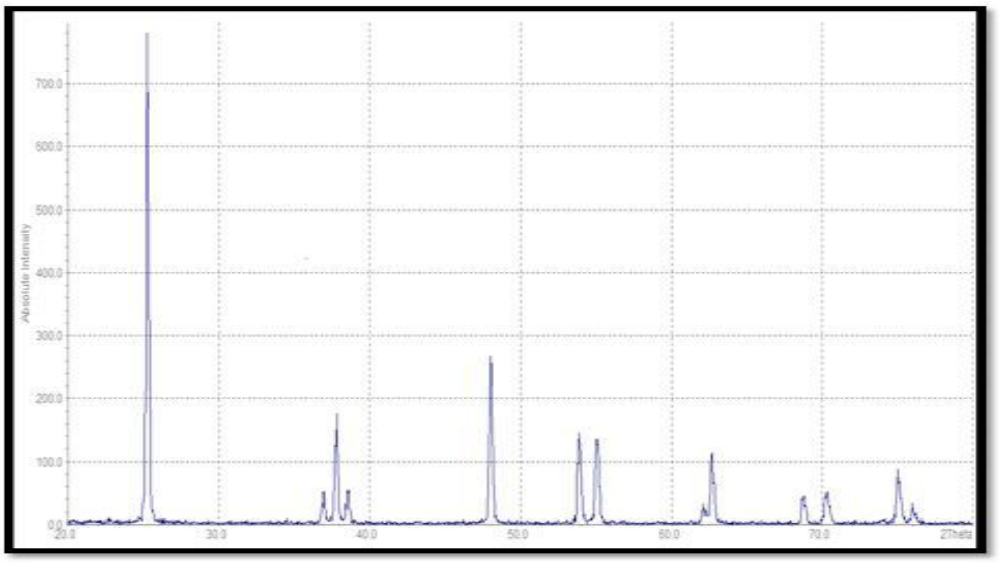


Figure 4.7: XRD Plot for Pure Titania Nanotubes

Crystallinity is achieved due to the calcination of Nanotubes at 500^0C for 2 hours which is the last step in the synthesis process.

In case of 1% Iron doped Titania Nanotubes peaks are found at the same angels as for pure Nanotubes indicative of the fact that iron doped Titania Nanotubes also possess a characteristic anatase phase of high crystallinity.

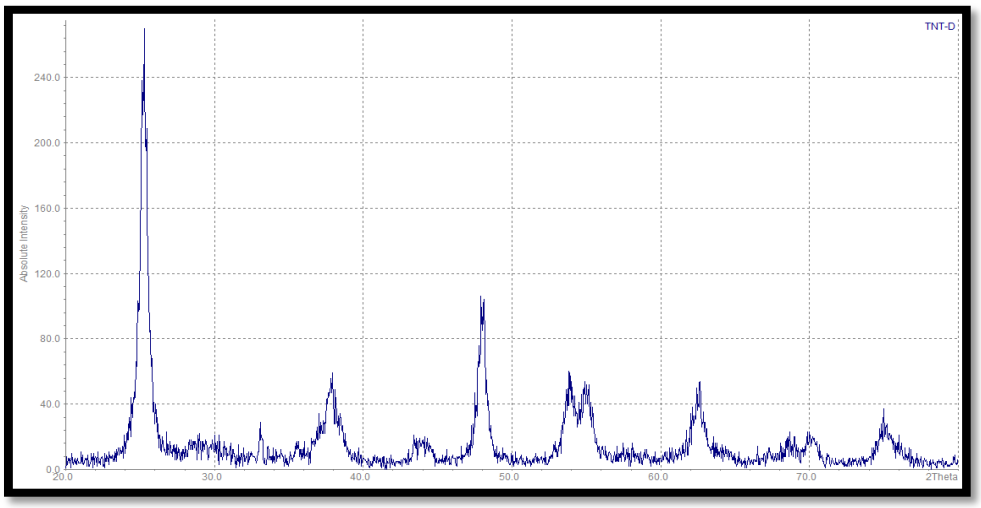


Figure 4.8: XRD Plot for 1% Doped Titania Nanotubes

It has been reported that calcinations is a very important factor in developing the anatase phase hence enhancing the crystallinity and photo-catalytic activity of catalyst (Fanchun *et al.,* 2011).

2.1.4 Surface Mapping of 1% Doped TNTs

In order to confirm the homogenized doping, surface mapping through the same instrument (JSM 6460 Scanning Electron Microscope) was performed. With the help of this analysis, it can be seen that whether the dispersion of doped metal is even or not. Figure 4.9 is showing the pattern of distribution.

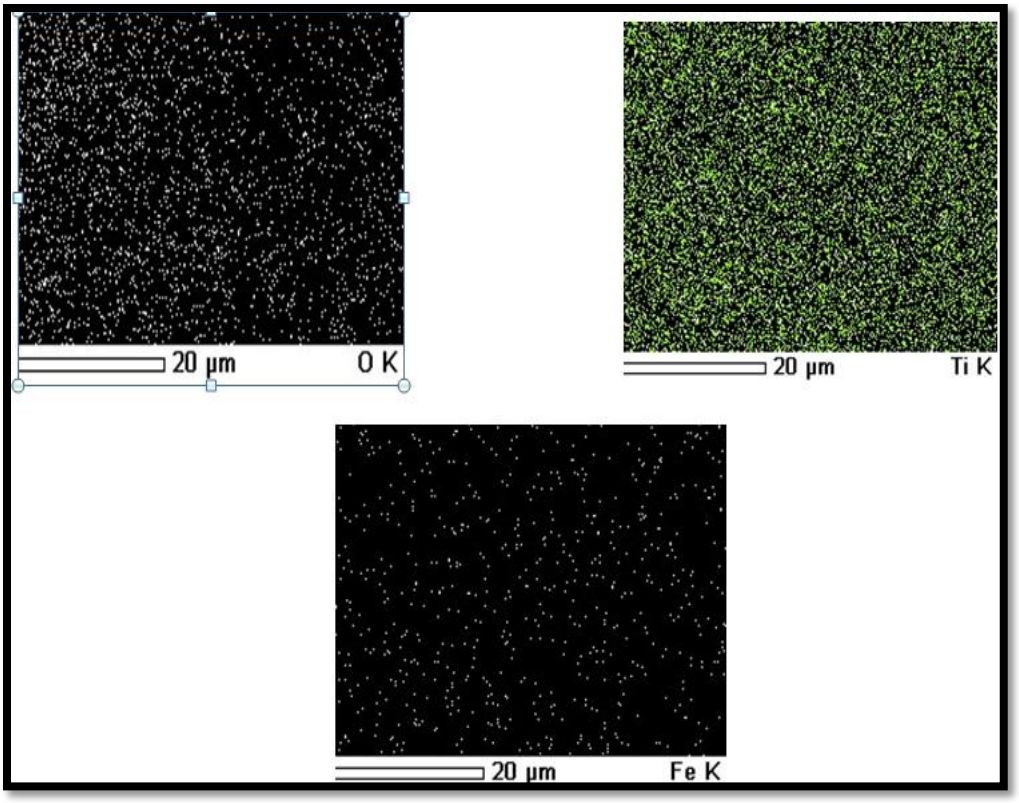


Figure 4.9: Distribution of O_2, Ti and Fe atoms

From the figure 4.9 it can be clearly concluded that the doped metal has been evenly distributed with no clustering.

2.1.5 BET Analysis

Figure 4.10 shows the quantity of liquid Nitrogen adsorbed with the relative pressure. This quantity directly corresponds to the surface area of Titania Nanotubes. Figure shows that at relative pressure of 0.25 the liquid Nitrogen adsorbed on the surface of Titania Nanotubes is almost 94 cm^3/g at Standard Temperature & Pressure (STP). This refers to the surface area 314 m^2/g of pure Titania Nanotubes which is in conformity with the earlier work (Fanchun *et al.*, 2011 and Lee *et al.*, 2009).

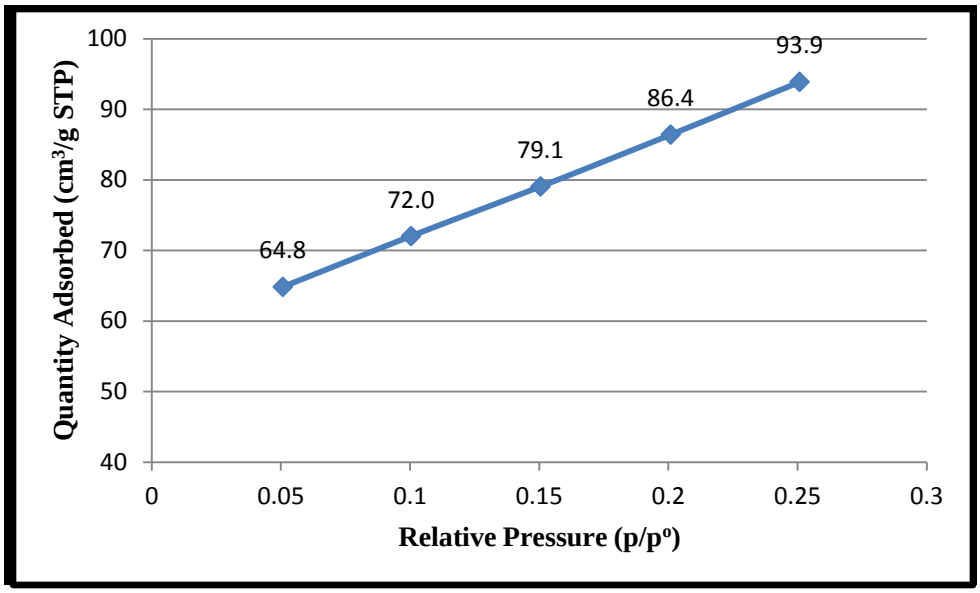


Figure 4.10: Isotherm Plot for N_2 Adsorption of Pure Nanotubes at 77k

1% Fe doped TNTs revealed that at a relative pressure of 0.25 the maximum quantity of liquid Nitrogen adsorbed is almost 72 cm^3/g that corresponds to the area of 241m^2/g. This reveals that there is a reduction in surface area, due to the incorporation of iron within the nanotube structure (Fanchun *et al.*, 2011).

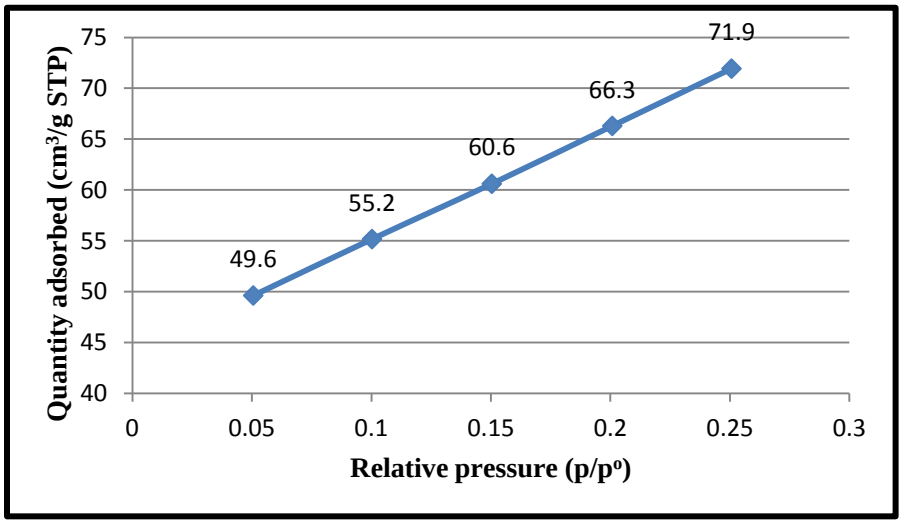


Figure 4.11: Isotherm Plot for N_2 Adsorption of Doped Nanotubes at 77k

4.1.6 Band Gap Calculation

Bandgap of anatase form of Titania can be determined from the direct band gap transition method in which graph is plotted between $(\alpha E_{phot})^2$ and E_{phot} where:

α = Absorption coefficient

E_{phot} (photon energy) = $(1239/\lambda)$ eV, λ is the wavelength in nanometers

Wavelengths between 300 and 500 were used to calculate E_{phot} value of each, for *x-axis*. For *y-axis* square of this value is taken and multiplied with the absorption coefficient. Following graph was obtained which is showing directly the band gap for pure Titania Nanotubes.

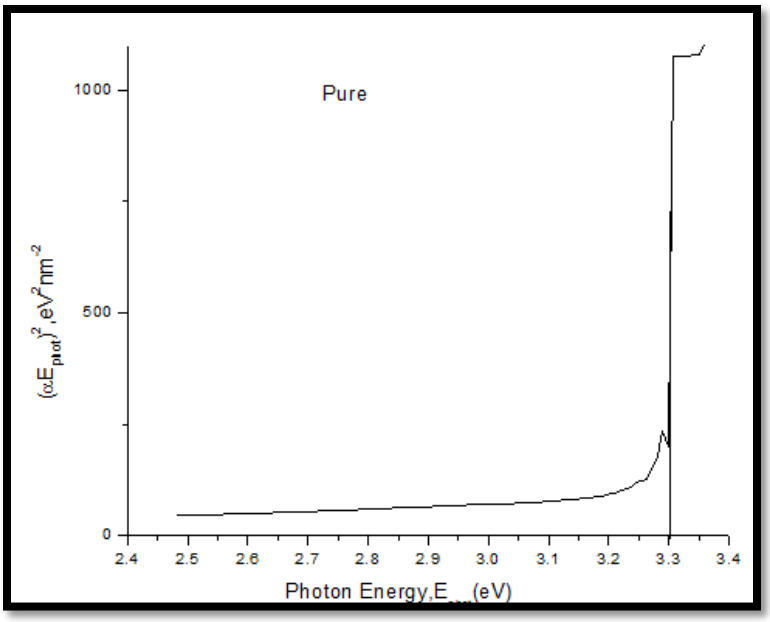


Figure 4.12: Band gap of Pure Titania Nanotubes

Figure 4.12 shows that the band gap of pure Titania Nanotubes is exactly 3.3eV which is very close to the band gap of Titania reported by literature i.e. 3.2eV (Madhusudan *et al.*, 2003).

Same calculations were done for 1% doped Titania Nanotubes and following graph was obtained:

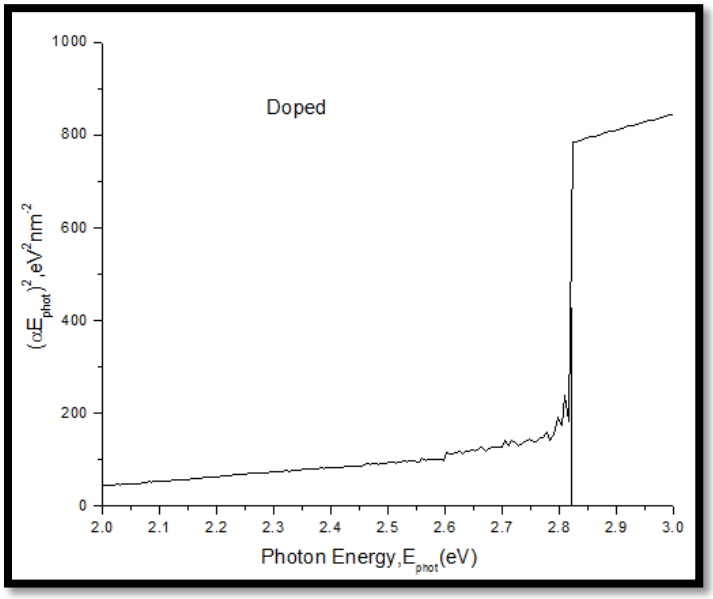


Figure 4.13: Band gap of 1% Doped Titania Nanotubes

Results show that doping has significantly reduced the band gap energy for Titania Nanotubes to 2.8eV. It can be inferred from the results that doped Titania Nanotubes will be efficient even under the fluorescent light as compared to pure Titania Nanotubes that require higher band gap energy (under UV range) for photoexcitation.

2.2 ANALYSIS & RESULTS

This section will discuss the results of LEVO degradation using different analytical techniques.

2.2.1 λ_{max} of LEVO

It was important to first find out the λ_{max} of LEVO i.e. the wavelength at which it gives the maximum absorbance of the light. Every compound has its specific λ_{max}. This helps in comparing the absorbance reading at same wavelength before and

after the degradation, from which we can easily conclude the percentage degradation. For this purpose the absorbance was measured at each wavelength between 200 to 400 nm.

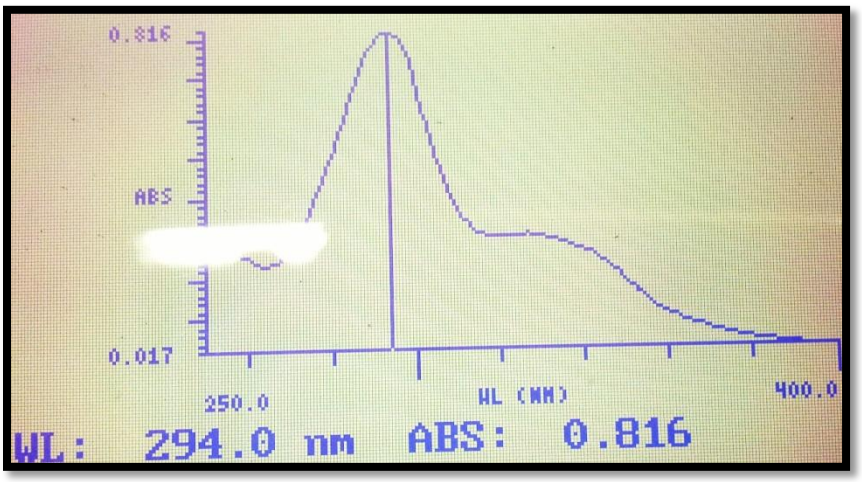


Figure 4.14 : λ_{max} of LEVO

Figure 4.14 shows that the peak formed at 294 nm. This λ_{max} is also supported by literature as (Nasuhoglu *et al.*, 2012 and Wang *et al.*, 2012).

2.2.2 Beer's Law Curve

Different dilutions were made from the stock solution in order to develop a calibration curve which depicted the dependence of absorbance on the concentration of the solution.

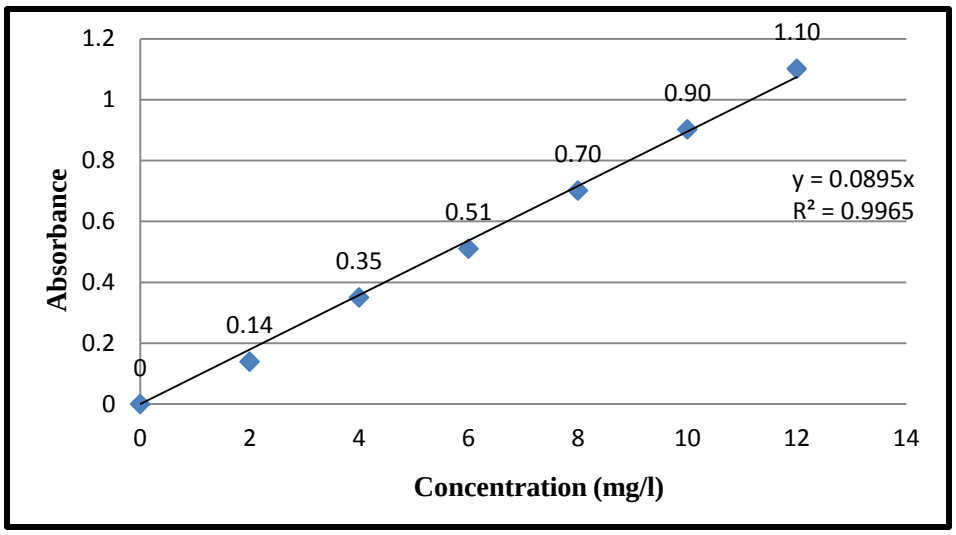


Figure 4.15: Calibration Curve

From the figure 4.15 it may also be concluded that for further experimentation 10ppm solution is suitable as it showed absorbance near one.

2.2.3 Degradation Vs Irradiation Time

The degradation efficiency was also recorded with respect to time ranges from 30 to 120 mins. The results are shown below:

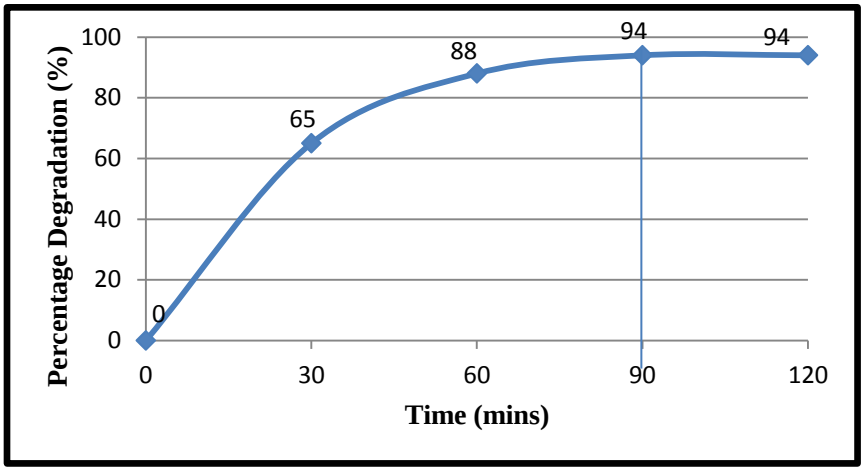


Figure 4.16: Irradiation Time Optimization

Figure 4.16 shows that the maximum degradation with 50 mg of Titania Nanotubes powder has been achieved at 90 min. No further degradation was observed

after that. It may be inferred through the results that reaction time should be limited to 90mins instead of two hours, in case of Titania Nanotubes.

2.2.4 pH Effect

Degradation reaction is highly dependent on the pH factor so in order to find the best pH for maximum degradation, experiments were run at three different pH ranges. The highest activity was observed at pH 2 i.e. an acidic range where as degradation was very low at the basic pH. The fact is also supported by Costa and Prado, (2009) in their work.

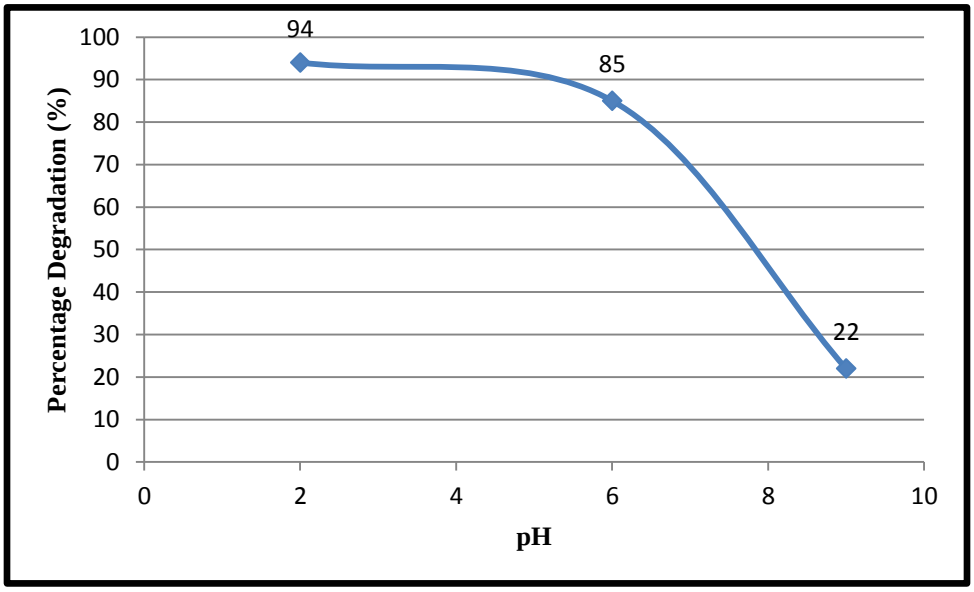


Figure 4.17: Effect of pH on Degradation Efficiency

2.2.5 Degradation vs Titania Nanotubes amount

Different amount of Titana Nanotubes were weighed and mixed with 50 ml of 10ppm solution and placed under UV light, in order to optimize the amount of Titania Nanotubes which shows the maximum or satisfactory degradation. The results are shown below:

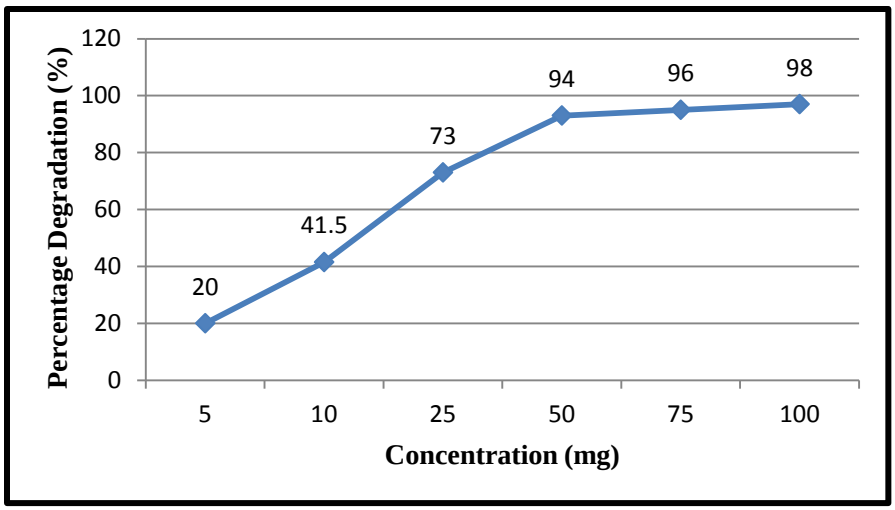


Figure 4.18: Titania Nanotubes Concentration Optimization

Figure 4.18 shows that increase in the amount of Titania Nanotubes powder results in the increase of percentage degradation of up to 94% at 50 mg of powder and then stabilizes. This was therefore selected for further use.

2.2.6 Nanotubes vs Nanoparticles

First of all experiments were performed to get the evidence about efficiency of nanotubes compared to nanoparticles. The absorbance of 10 ppm LEVO solution was taken before experiment by spectrophotometer. 50 mg of Titania Nanotubes powder was then mixed in the 50 ml of 10 ppm LEVO solution in a beaker and placed under the UV light for 120 mins. Same was done for the nanoparticles for 120mins. The results obtained are shown below.

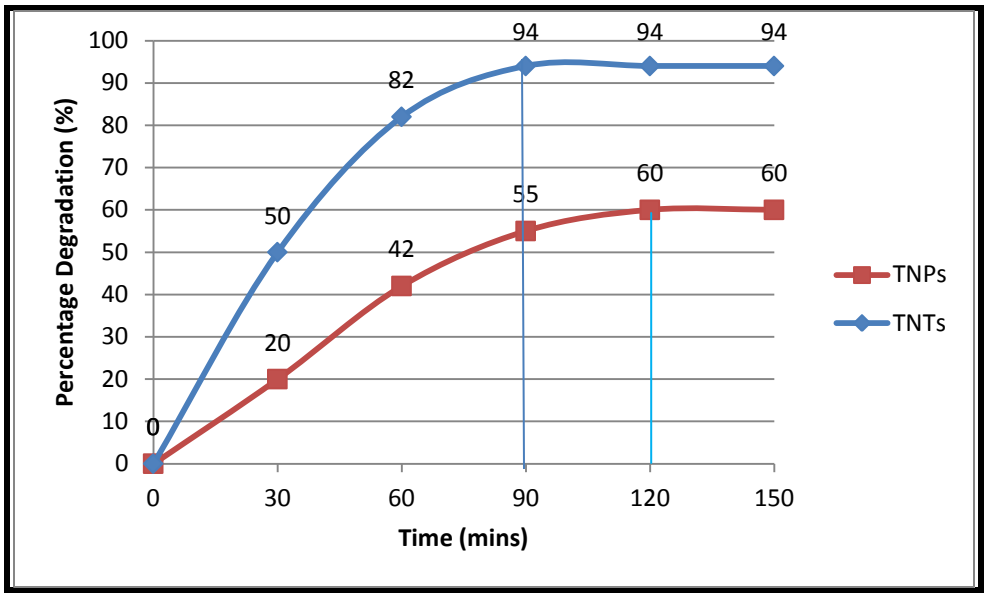


Figure 4.19: Titana Nanoparticles vs Titania Nanotubes

Figure 4.19 above shows that in case of Titania Nanoparticles, they achieve their maximum degradation in 120 minutes whereas Titania Nanotubes attain within only 90 minutes. As expected results are completely in the favor of Titania Nanotubes due to their higher surface area compared to Titania Nanoparticles

2.2.7 Regeneration of Titania Nanotubes

Regeneration efficiency of Titania Nanotubes has also been analyzed and results were highly favorable. There is only 2% decrease in degradation efficiency with each cycle. Titania Nanotubes powder was separated out from the solution by simple filtration and then reused without any treatment. They maintained 80% of their efficiency even after 7 cycles where as Costa and Prado and (2009) have mentioned in their work that Titania anataze can maintain degradation efficiency goes down to 18% after 7th cycle.

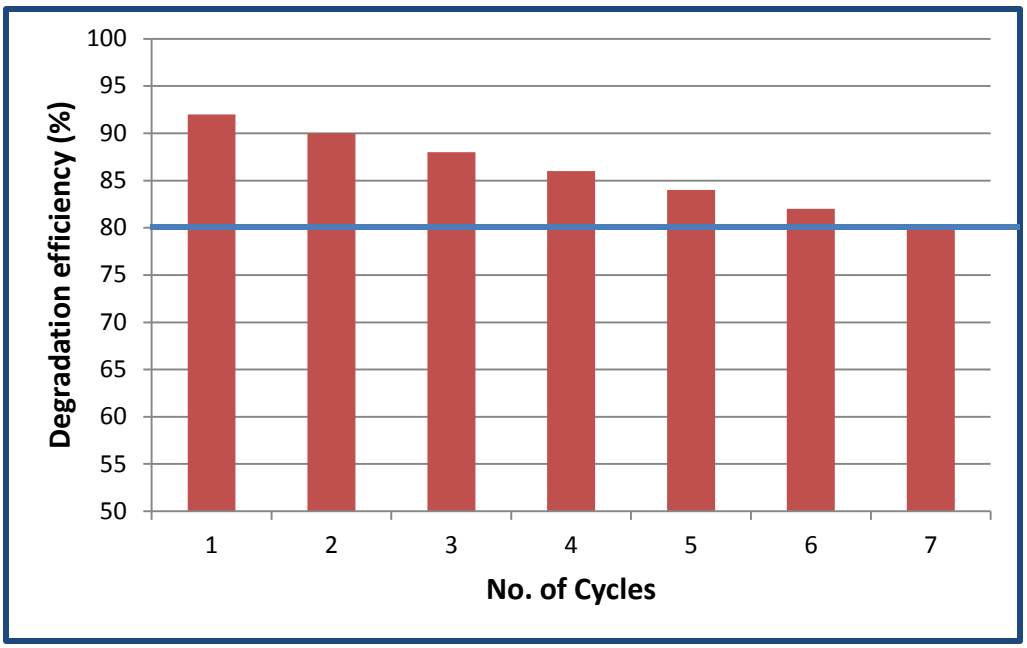


Figure 4.20: Degradation Efficiency of Titania Nanotubes with Each Cycle

Titania Nanotubes are more efficient in reuse compared to anatase nanoparticles because it is difficult to separate nanoparticles by just simple filtration. Nanoparticles have high stability of their hydrocolloids and remain suspended in water, which makes them difficult to separate from solution (Costa and Prado, 2009). Titania Nanotubes however behave in different way. Titania Nanotubes does not remain suspended in the solution due to large volume, so it can easily be separated.

(A) **(B)**

Figure 4.21: Nanoparticles (A) and Nanotubes (B) in Solution

2.2.8 Doped vs Pure

Titania Nanotubes were doped with 1% of iron as described in chapter 3. In order to assess the validity of doping the reaction was conducted under the same conditions (under UV/ 90 min) for doped Titania Nanotubes.

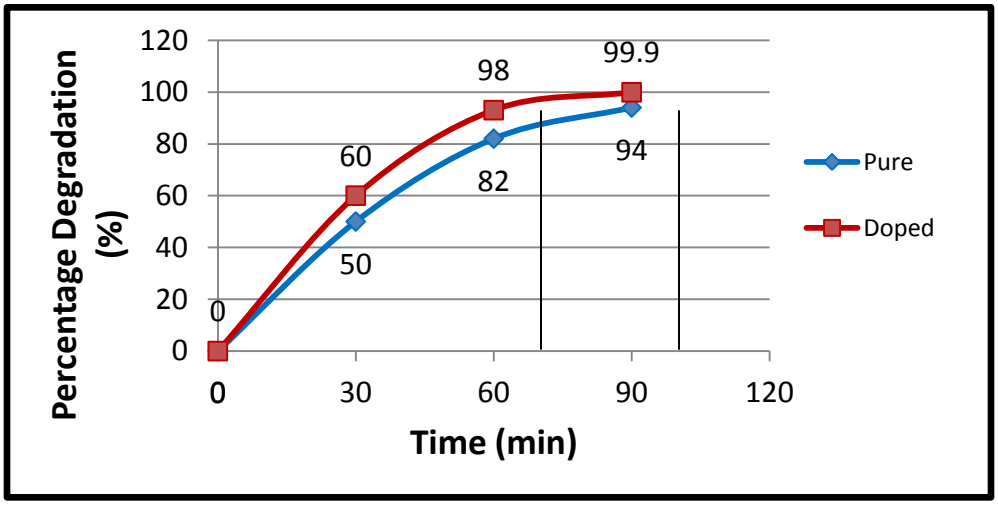
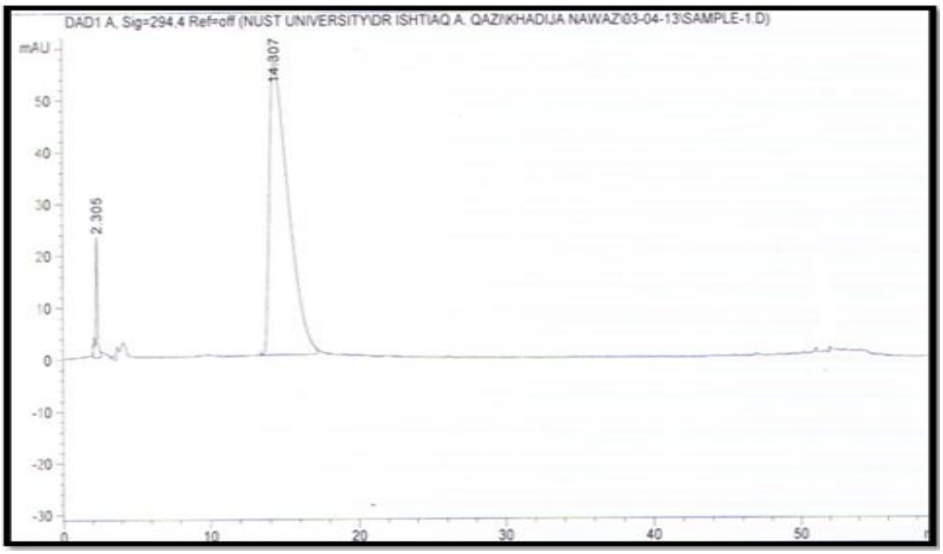


Figure 4.22: Comparison of Pure and Doped Nanotubes under UV

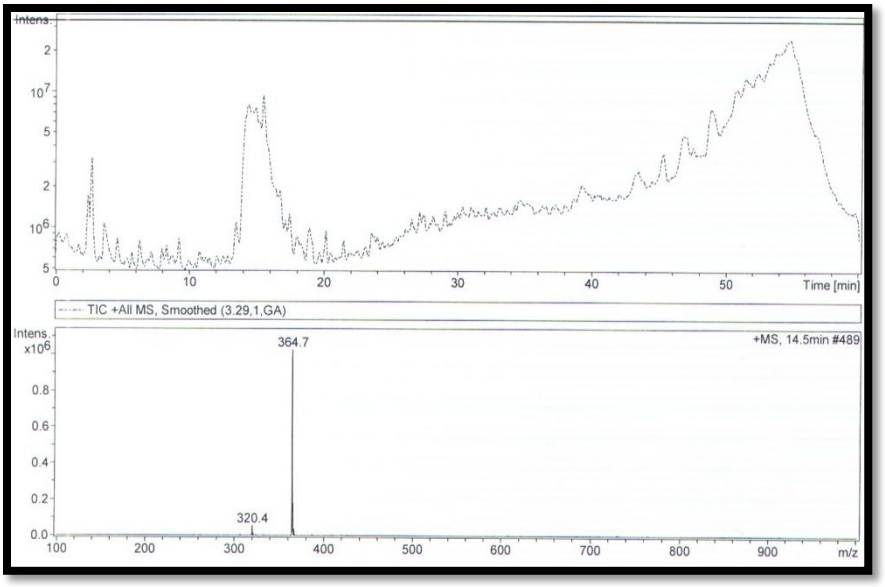
Results show that in case of doped nanotubes almost complete degradation was achieved at 60 min. In case of pure nanotubes it is limited to 94% even after 90min. This shows that doping results in acceleration of degradation (Fu *et al.*, 2013).

2.2.9 Degradation Products/ LCMS

Spectrophotometer is limited to detect only the degradation of original parent compound and cannot determine whether it is transformed to some other compound or degraded completely. In order to assess the transformation of the parent compound the samples treated with pure and doped Titania Nanotubes under UV light were run through Liquid Chromatography Mass Spectrometry, the results of which are shown below.



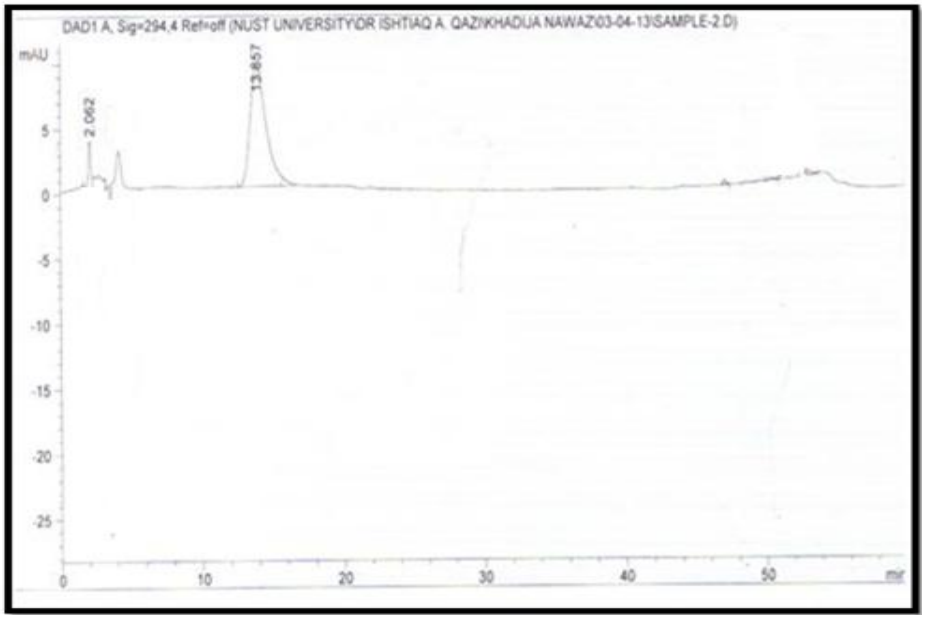
(A)



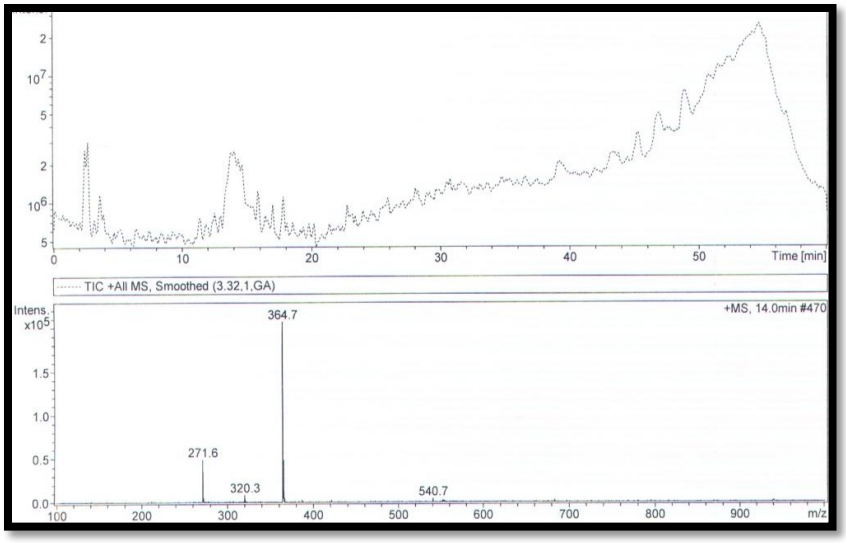
(B)

Figure 4.23: Chromatogram of parent compound (A) and its Mass Spectra (B) before treatment

Figure 4.23 is showing the peak for parent compound and its mass spectra before exposure to UV and Titania Nanotubes**.**



(A)



(B)

Figure 4.24: Chromatogram of Sample (A) and its Mass Spectra (B) treated with pure nanotubes

Figure 4.24 shows the chromatogram after the treatment with pure Titania Nanotubes under UV. This shows the clear decrease in the peak area of Parent compound where as no additional peak for transformed compounds was observed.

Figure 4.25: Chromatogram of Sample Treated with Doped Titania Nanotubes

In figure 4.25 it can be seen that peak for parent compound has completely disappeared with no additional peak. These results confirm that the parent compound degraded and only metabolites formed were below the detection limit. In other words there was complete mineralization of the parent compound. In order to validate the results of LCMS, Total Organic Carbon (TOC) test was performed.

2.2.10 TOC Results

Total organic carbon was monitored using TOC analyzer. This degree of mineralization was detected for both doped and undoped nanotubes and results completely favor the findings of LCMS test. There was 94 and 100% mineralization recorded in pure and doped Nanotubes respectively.

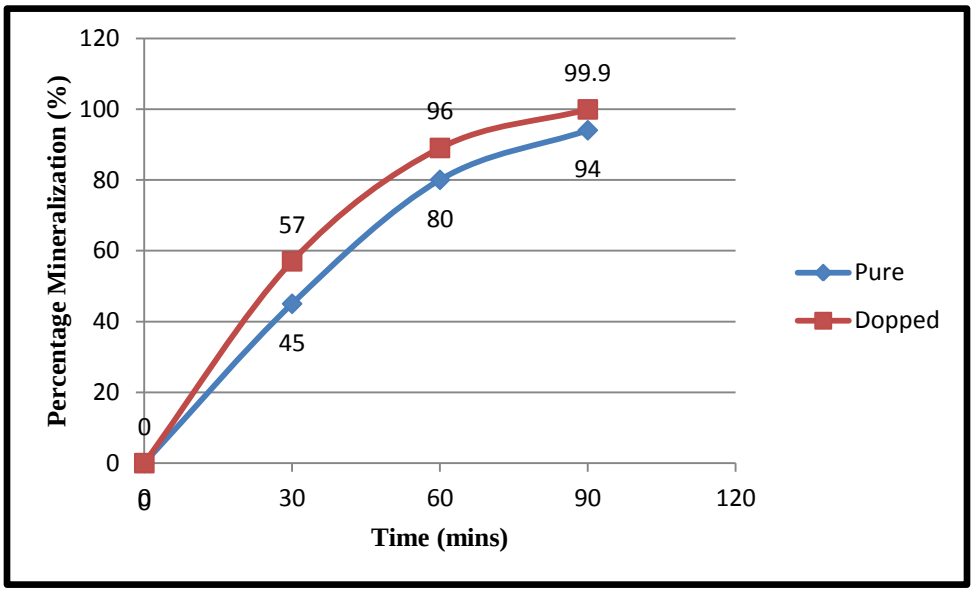


Figure 4.26: Degree of Mineralization with Pure and Doped Titania Nanotubes

Figure 4.26 shows the percentage mineralization for both pure and doped Titania Nanotubes at different time intervals.

2.2.11 Ultraviolet vs Fluorescent Light

Here a very important factor to consider is that when these experiments were conducted under the fluorescent light in order to make comparison between UV and fluorescent, the results were surprising. Fluorescent light showed more or less same or sometimes even slightly higher percentage degradation both in the case of pure and doped Titania Nanotubes, which is usually not expected.

	Pure
UV	94%
Fluorescent	96%

Table 4.3: Comparison of UV & Fluorescent Light for Pure Titania Nanotubes

Extensive study of literature regarding the degradation of LEVO under visible light range was carried out and hence concluded that LEVO can be degraded well under visible light due to an interesting phenomenon. This is because of the complexation of fluoroquinolones with Titania surface which lead towards the red shift in absorption spectrum and photoexcitation could be done by visible light. It was also observed by Paul *et al.*, (2007) that the degradation efficiency reduces when the adsorption of fluoroquinolones is inhibited, from which it can be inferred that more the surface area of Titania nanostructures more will be the adsorption and ultimately the formation of this complex. For further understanding we need to look at the structure of LEVO very closely as shown below:

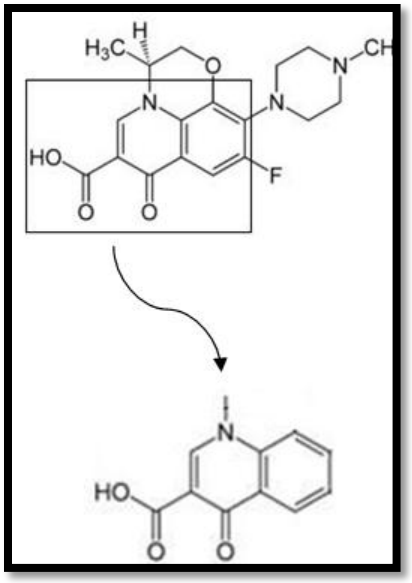
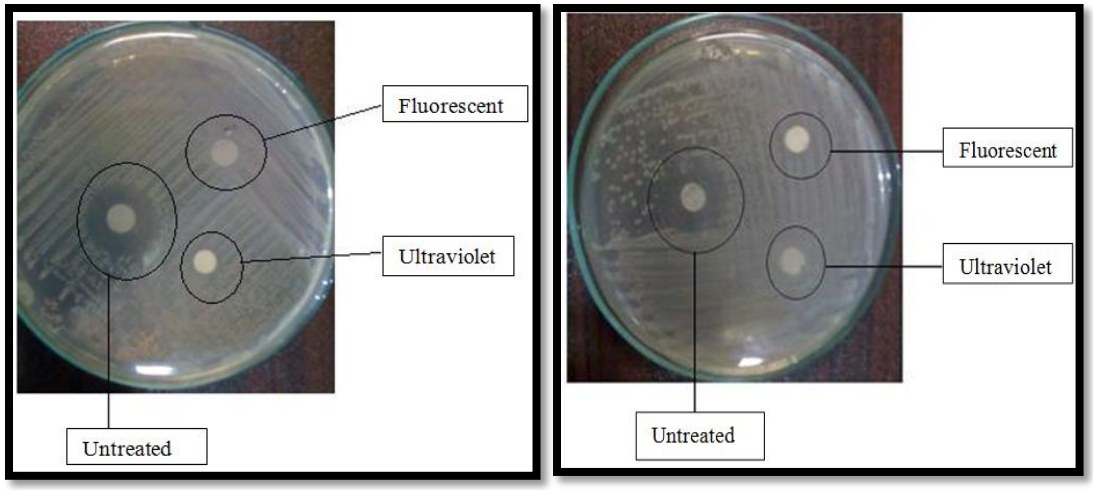


Figure 4.27: LEVO Structure. Box contains Core Oxo-quinoline carboxylic Acid Substructure

It has been reported that the compounds having only the portion of LEVO structure shown in the box show degradation even under the visible light whereas if any compound lack this core structure but contains the rest, it will not show any degradation (Paul *et al.,* 2007).

2.2.12 Zone of Inhibition/ Kirby- Bauer Antibiotic Testing

In order to validate the degradation of LEVO and complete mineralization of pharmaceutically active byproducts, Zone of inhibition or Kirby – Bauer antibiotic test was performed which supported the above mentioned results. Results are shown below:



(A) **(B)**

Figure 4.29: Kirby – Bauer Antibiotic Test for Sample Treated with Pure (A) & Doped (B) Titania Nanotubes

In case of untreated drug clear zone of almost 2 cm is visible after 24h incubation. Test was performed on two species of bacteria i.e. *Escherichia Coli* and *Pseudomonas Aeruginosa* and both showed the same response.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The conclusions of this research work are:

- Hydrothermal method is relatively simple and effective for synthesis of high surface area nanotubes
- Titania Nanotubes show maximum degradation of Levofloxacin, a pharmaceutical compoundin acidic range
- Nanotubes are more effective than Nanoparticles
- Degradation of LEVO can also be achieved under fluorescent light which is the unusual advantage in making the technique more cost effective
- Regeneration of Nanotubes powder proved to be an efficient technique by simply filtration, whereas nanoparticles remain suspended which make their separation difficult

5.2 Recommendations

- At IESE degradation of second and third generation of fluoroquinolones has been investigated. It would be interesting to apply the same degradation technique to the 4th generation of Fluoroquinolones like Travofloxacin
- In case of both Ciprofloxacin and LEVO the possibility of red shift due to the charge transfer complexation existed. Degradation of a pharmaceutical product devoid of this property could yield useful results
- Lab scale reactor may be developed for degradation of real pharmaceutical wastewater for practical utilization in pharmaceutical industry

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