

## DEGRADATION AND INACTIVATION OF CIPROFLOXACIN BY PHOTOCATALYSIS USING TiO<sub>2</sub> NANOPARTICLES

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### ABSTRACT

To compare Ciprofloxacin attenuation efficiency, Ciprofloxacin solutions mixed with TiO<sub>2</sub> nanoparticles were irradiated with two different light sources: a UV lamp and ordinary electric bulb. Insignificant degradation was witnessed when irradiations were made in absence of TiO<sub>2</sub>. In contrast, prominent Ciprofloxacin degradation was detected in the presence of 0.01 mg/ml of TiO<sub>2</sub>. Close to 90 % and 70 % of its original concentration was eradicated in 120 minutes when the irradiation basis used was a UV lamp and Ordinary electric bulb respectively. Without the use of TiO<sub>2</sub> nanoparticles, irradiation by UV lamp sources was also significant. The antibacterial activity of chosen microorganisms was radically inhibited when exposed to Ciprofloxacin solution treated with photocatalyst for the short periods of irradiation.

**Keywords:** Antibiotics, Ciprofloxacin, Bacterial activity, Photocatalysis, TiO<sub>2</sub>, Nanoparticles.

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### 1. INTRODUCTION

Potential ecological impacts of pharmaceuticals on environment have been detected by recent advancement in analytical chemistry. (Halling-Sørensen et al., 1998). Antibiotics reach the environment through intentional disposal of surplus drugs to sewage, release to sewage through urine and feces, leaching from landfills and discharges from sewage treatment plants or confined animal farming operations (Daughton, 2000).

Ciprofloxacin is frequently detected class of antibiotics in the environment. It inhibit both Gram-positive (GP) and Gram-negative (GN) bacteria, and commonly used to treat tuberculosis, digestive and urinary tract infections and anthrax. Environmental risk studies have estimated environmental loadings of ciprofloxacin from European sewage treatment plants to be as high as 186.2 tones per year in 1999 (Halling-Sorensen, 2000). Monitoring surveys have detected antibiotics in aquatic ecosystems ranging from ng l<sup>-1</sup> to mg l<sup>-1</sup> concentrations (Giger et al., 2003). Several studies on ciprofloxacin sorption to clays and minerals have been published (Seremet and MacKay, 2003; Gu and Karthikeyan, 2005).

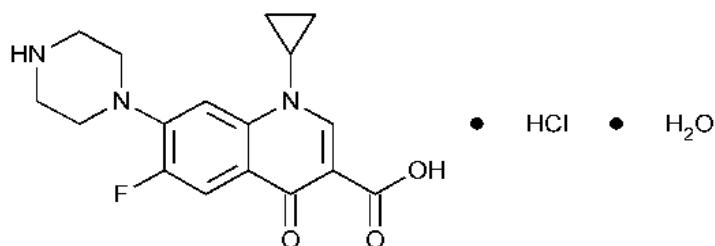
Ciprofloxacin and levofloxacin account for 65% of the total fluoroquinolone use and represent 3.3 billion dollars in global sales (Datamonitor Strategic Report, 2004). Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class Ciprofloxacin has 69 % bioavailability, 4 hours half life and renal excretion.

Heterogeneous photocatalysis is an Advanced Oxidation Process(AOPs), can be utilized to remove many different of pollutants in different phases like liquid and gas (Hoffmann *et al.*, 1995; Stafford *et al.*, 1996; Hager *et al.*, 2000; Deng *et al.*, 2002; Dunlop *et al.*, 2002; Balasubramanian *et al.*, 2004). The TiO<sub>2</sub> (commonly known as titania) is preferentially used for the photocatalytic degradation of organic material due to its different suitable properties such as non-toxicity, low-cost and photochemical stability. The principle of photocatalysis is the formation of nonselective and highly reactive radicals (i.e. hydroxyl radicals (OH<sup>•</sup>) and the superoxide radical anions (O<sub>2</sub><sup>•-</sup>), which are the primary materials of the oxidative degradation. TiO<sub>2</sub> suspension in aqueous environment under irradiation causes an oxidative degradation of many pollutants and mineralizes organic materials into their building blocks i.e. CO<sub>2</sub>, H<sub>2</sub>O, and other related inorganic components (Kim *et al.*, 2002; Carp *et al.*, 2004; Florêncio *et al.*, 2004).

The Ultraviolet (UV) radiation enhances the photocatalytic degradation of organic substrates using TiO<sub>2</sub> looks to be mediated by a number of reactions initiated by these primary oxidizing radicals, mostly OH<sup>•</sup> radicals (Ilisz *et al.*, 2002; Kaneco *et al.*, 2004; Chen *et al.*, 2005; Bizani *et al.*, 2006). The photocatalytic efficiency of the UV/TiO<sub>2</sub> process is based on a number of factors, e.g. the organic substrate,, light intensity, photoreactor design ,solution composition, and the photocatalyst surface composition (Augugliaro *et al.*, 1995; Chhor *et al.*, 2004). Futhermore, the adsorption of organic substrates onto the surface of TiO<sub>2</sub> plays vital role in the photocatalytic degradation (Schmelling *et al.*, 1997; Grzechulska and Morawski, 2002; Uyguner and Bekbölet, 2004).

In this article, Pure TiO<sub>2</sub> and Ag doped TiO<sub>2</sub> nanoparticles were prepared by Liquid Impregnation technique. (Sahoo *et al.*, 2005).These nano particles were then studied for the Ciprofloxacin as a model drug and the photocatalytic degradation of the drug by Pure TiO<sub>2</sub> and Ag doped TiO<sub>2</sub> (i.e 1%, 2%, 3%, 4% & 5%) nanoparticles were examined by UV Spectrophotometer, HPLC and Microbiologically.

Fig 1: Chemical structure of Ciprofloxacin HCl



## 2. MATERIAL & METHODS

### 2.1 Material

In this study  $\text{TiO}_2$  (GPR, BDH Chemicals Ltd. Poole England) was used as  $\text{TiO}_2$  source for preparing the nanoparticles, and  $\text{AgNO}_3$  (GR, Merck, Germany) Ciprofloxacin HCl (GR, Merck, Germany) was used as the target compounds for degradation in this study.  $\text{TiO}_2$  of Riedel-de-Haen Sigms-Aldrich Lab was purchased.

### 2.2 Nanoparticles synthesis

#### 2.2.1 Pure titania nanoparticles

For pure titania nanoparticles, titania (GPR) was taken in a clean china dish. It was placed in a furnace to be calcined at  $500^\circ\text{C}$  for 3 hr. The calcined titania was then allowed to cool down slowly so as to attain the nanosized crystal structure.

#### 2.2.2 Ag- $\text{TiO}_2$ Nanoparticles

Ag- $\text{TiO}_2$  nanoparticles were prepared by the Liquid Impregnation Method (Sahoo, *et al.*, 2005). For **1% Ag-  $\text{TiO}_2$**  : 50 gm of  $\text{TiO}_2$  was poured into 100 ml dionized water in a 500 ml Pyrex beaker. 1.03 gm of  $\text{AgNO}_3$  was also added to the suspension for 1% silver doping(molar ratio). Vigorous stirring was used mix the resulting slurry thoroughly and allowed to settle, at room temperature, over night. The obtained liquid was dried in an oven at  $100^\circ\text{C}$  for 12 hr to evaporate the remaining moisture. The solid material resulting from this step was calcined, at  $500^\circ\text{C}$  for 3 hr in a furnace. This resulted in fine particles of silver doped  $\text{TiO}_2$ , herein after referred to as Ag- $\text{TiO}_2$  1%.

For **2%, 3%,4% & 5% Ag-  $\text{TiO}_2$**  . 50 gm of  $\text{TiO}_2$  was poured into 100 ml dionized water in a 500 ml Pyrex beaker. 2.06 g, 3.09 g, 4.12 g & 5.15 g of  $\text{AgNO}_3$  was also added to the suspension for 2%,3%,4% & 5% silver doping(molar ratio) respectively . Vigorous stirring was used mix the resulting slurry thoroughly and allowed to settle, at room temperature, over night. The obtained liquid was dried in an oven at  $100^\circ\text{C}$  for 12 hr to evaporate the remaining moisture. The solid material resulting from this step was calcined, at  $500^\circ\text{C}$  for 3 hr in a furnace. This resulted in fine particles of silver doped  $\text{TiO}_2$ , herein after referred to as Ag- $\text{TiO}_2$  2%,3%,4% & 5% respectively.

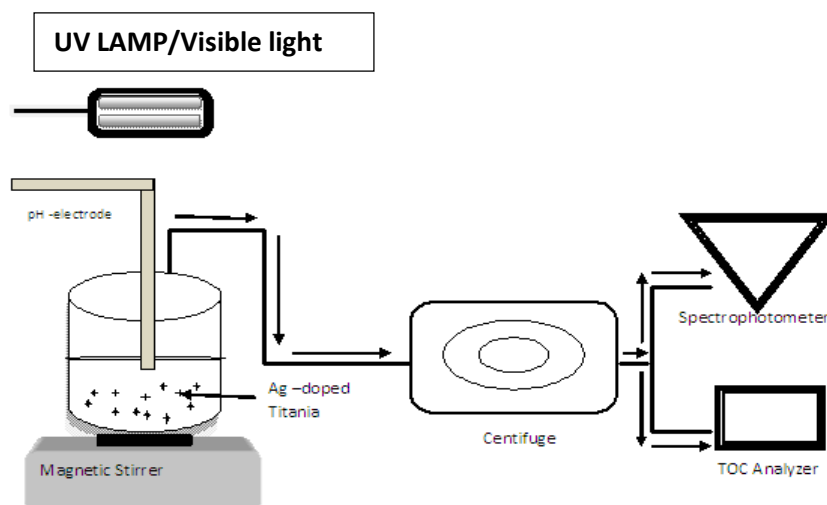
### 2.3 The Photocatalysis Experiment

The schematic diagram of the experimental setup of photocatalysis experiment is shown in Figure-1. 500mg of high purity *Ciprofloxacin HCl powder* was dissolved in 100 ml of 0.1 N HCl in a 500 ml beaker and transferred to a 500mL analytical flask. The volume was made up of, with 0.1 N HCl to 500mL to obtain a stock solution of 0.01mg /ml Ciprofloxacin HCl

The absorbance reading of this solution was taken by a UV visible spectrophotometer (HACH DR 2400). The absorbance so obtained served as the reference value for determining the proportionate reduction in the concentration of the Ciprofloxacin, after exposing the solution to UV light and visible light under the conditions of (a) no  $\text{TiO}_2$ , (b)

TiO<sub>2</sub> and (c) Ag-TiO<sub>2</sub> (1%,2%,3%,4% and 5%).

To 250 ml of the stock solution of *Ciprofloxacin HCl*, in a china dish, 0.05 g of pure TiO<sub>2</sub> nanoparticles or Ag-TiO<sub>2</sub> nanoparticles(1%,2%,3%,4% and 5%) were added. The china dish was placed under a UV lamp and allowed to stay exposed to the UV radiation for 2 hours. Its absorbance was then measured at 278 nm on spectrophotometer as described above, while taking into account any dilution effects. In view of the literature reported, experiments were carried out at a pH of 5.8 with UV lamp distance of 5cm from the target surface of the solution in the china dish. Similar experiments were repeated under visible light keeping all other conditions similar.



**Figure 2:** Experimental Setup for Photocatalysis under UV light/ Visible light

## 2.4. Chemical Analysis

Samples were taken and absorbance measurements were performed in a Shimadzu UV 1603 spectrophotometer. The ciprofloxacin concentrations was also determined by HPLC using Column 18. Acetonitrile was used as eluent. The ciprofloxacin absorbance was recorded at 278 nm. Total organic carbon was measured in Shimadzu 5000TOC analyzer.

## 2.5. Antibacterial activity of treated solutions

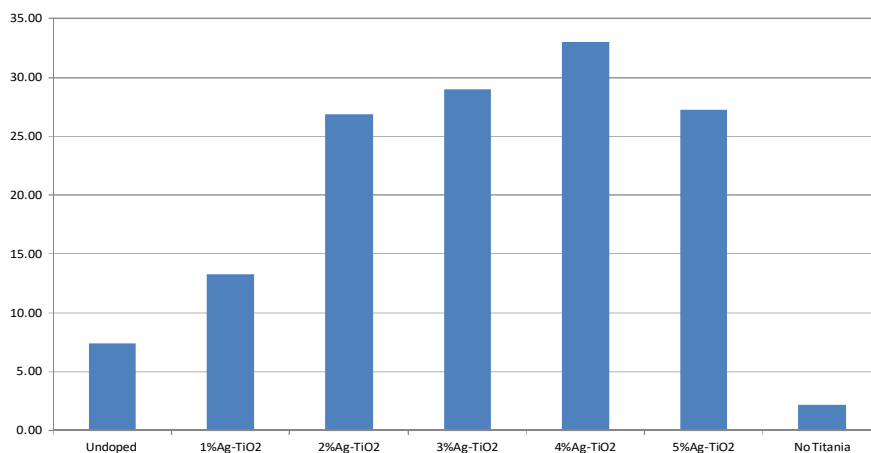
Microbiologic assays with irradiated samples were carried out and *S.Aureus* was used as an assay microorganism. Tripticase agar plates were inoculated with Irradiated Ciprofloxacin samples. After incubation for 24 hours at 37°C, the inhibition was noted.

## 3. Results & Discussions

### 3.1 Ciprofloxacin degradation with TiO<sub>2</sub> nanoparticles

As seen in chart below, a very low ciprofloxacin degradation was observed when it was illuminated in the absence of  $\text{TiO}_2$ .

In photocatalytic experiments in presence of 0.01 mg/ml, a maximum degradation was obtained under UV lamp illuminations when using Ag- $\text{TiO}_2$  nanoparticles. This could be because of the positive effect of silver on the photoactivity of Titania at degradation of Ciprofloxacin that can be clarified by its ability to trap electrons, therefore reducing the recombination of light generated electron-hole pairs at Titania surface.



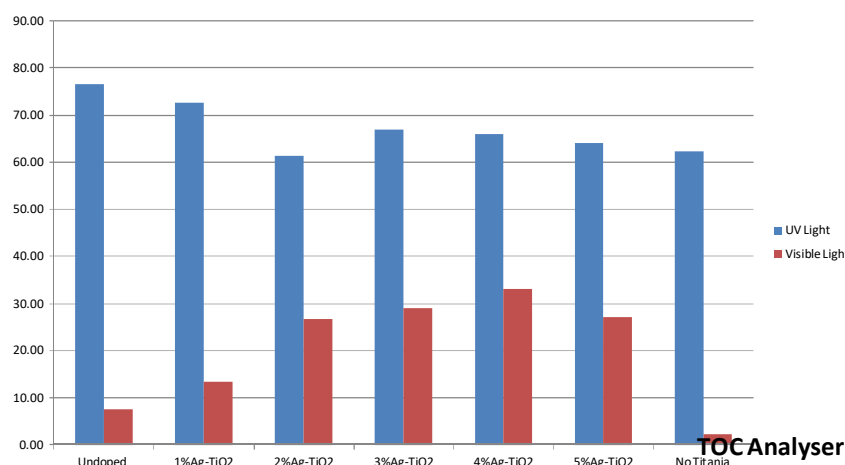
**Figure 2:** Degradtion profile of Ciprofloxacin irradiated by presence and absence of  $\text{TiO}_2$ nanoparticles and Ag- $\text{TiO}_2$  nanoparticles

Significant degradation was observed when ciprofloxacin was treated with the  $\text{TiO}_2$  nanoparticles with the UV lamp but less as compared to the Ag- $\text{TiO}_2$  in the presence of UV lamp. It was also observed that by increasing the percentages of silver in Ag- $\text{TiO}_2$  i.e from 1% onward, better degradation was noted. Our results encouraged the use of Ag doped  $\text{TiO}_2$  for the degradation of Ciprofloxacin.

**Table 1:** Showing the level of degradation

| Lamp           | Nanoparticles                     | Degradtion of Ciprofloxacin |
|----------------|-----------------------------------|-----------------------------|
| Visible        | No $\text{TiO}_2$ nanoparticels   | Insignificant degradation   |
| UV             | No $\text{TiO}_2$ nanoparticels   | Significant degradation     |
| Visbible light | With $\text{TiO}_2$ nanoparticles | Major degradation           |
| UV             | With Ag- $\text{TiO}_2$           | Maximum Degradation         |

Comparative photocatalytic degradation of Ciprofloxacin was also investigated in the presence of UV light. As it is clear from the chart below, Ag-TiO<sub>2</sub> nanoparticles show better results as compared to pure TiO<sub>2</sub> nano particles.



**Figure3:** Comparative photocatalytic degradation of Ciprofloxacin with pure and Ag doped Titania Nanoparticles.

Similarly, results by HPLC clearly indicate that better degradation was observed with silver doped titania as compared to only Titanai nanoparticles. Compative descriptive charts below show the detail of results. Same pattern confirmed by FTIR results in Figures 7,8 and 9.

**Table 2:** Degradation of 0.01 ppm Ciprofloxacin by 1-5% Ag doped titania & undoped titania under Uvlight determined by HPLC

| Name                               | # of Peak | Ret. Time | Area   | Height | Area % |
|------------------------------------|-----------|-----------|--------|--------|--------|
| Ciprofloxacin Stock                | 1         | 12.868    | 625824 | 24846  | 100    |
| Ciprofloxacin+UV+TiO <sub>2</sub>  | 7         | 12.986    | 641290 | 25502  | 88.561 |
| Ciproflox+UV+1%Ag-TiO <sub>2</sub> | 10        | 12.428    | 46939  | 2036   | 28.258 |
| Ciproflox+UV+2%Ag-TiO <sub>2</sub> | 8         | 12.123    | 45979  | 1669   | 24.430 |

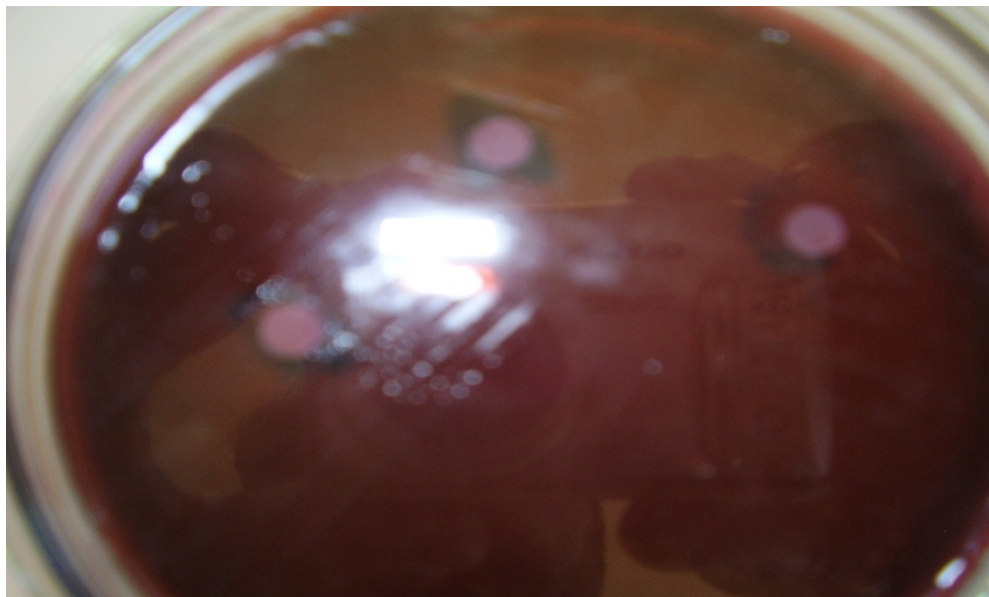
|                                    |   |        |        |      |        |
|------------------------------------|---|--------|--------|------|--------|
| Ciproflox+UV+3%Ag-TiO <sub>2</sub> | 3 | 5.024  | 10046  | 415  | 16.212 |
| Ciproflox+UV+4%Ag-TiO <sub>2</sub> | 5 | 12.614 | 122159 | 4855 | 30.530 |
| Ciproflox+UV+5%Ag-TiO <sub>2</sub> | 6 | 12.613 | 145364 | 5756 | 52.792 |
| Ciproflox+UV only                  | 6 | 12.572 | 123721 | 9934 | 22     |

**Table3:** Degradation of 0.01 ppm Ciprofloxacin by 1-5% Ag doped titania & undoped titania under Visible light determined by HPLC

| Name                               | # of Peak | Ret.Time | Area   | Height | Area % |
|------------------------------------|-----------|----------|--------|--------|--------|
| Ciproflox                          | 1         | 12.868   | 625824 | 24846  | 100    |
| Cipro+TiO <sub>2</sub> +Light      | 6         | 12.964   | 205614 | 8312   | 61.363 |
| Cipro+1%Ag-TiO <sub>2</sub> +light | 12        | 12.257   | 21785  | 916    | 10.565 |
| Cipro+2%Ag-TiO <sub>2</sub> +light | 4         | 12.111   | 142516 | 4906   | 53.085 |
| Cipro+3%Ag-TiO <sub>2</sub> +light | 6         | 12.653   | 139568 | 5526   | 46.236 |
| Cipro+4%Ag-TiO <sub>2</sub> +light | 6         | 12.593   | 132630 | 5298   | 38.172 |
| Cipro+5%Ag-TiO <sub>2</sub> +light | 3         | 12.596   | 17431  | 722    | 17.818 |
| Cipro+light only                   | 3         | 12.706   | 52657  | 1314   | 17.982 |

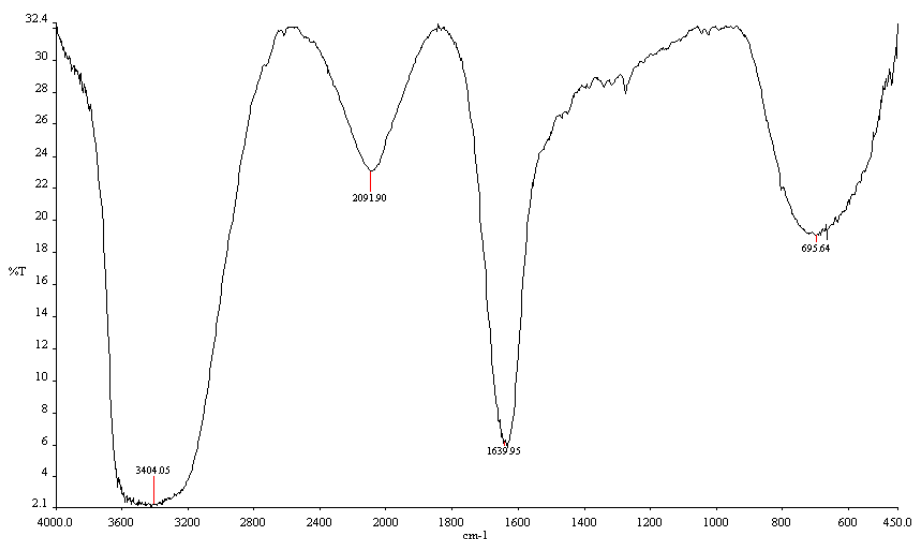
### 3.2 Effect of the TiO<sub>2</sub> photocatalysis on the antibacterial activity

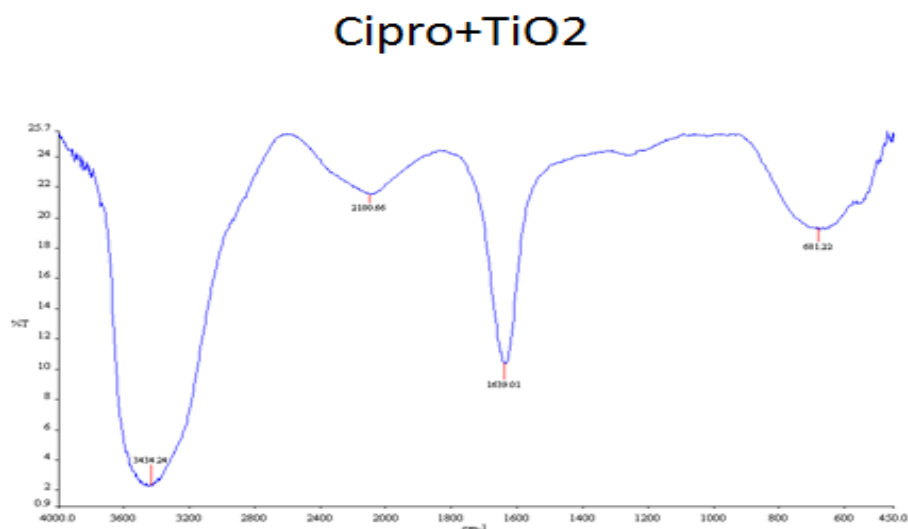
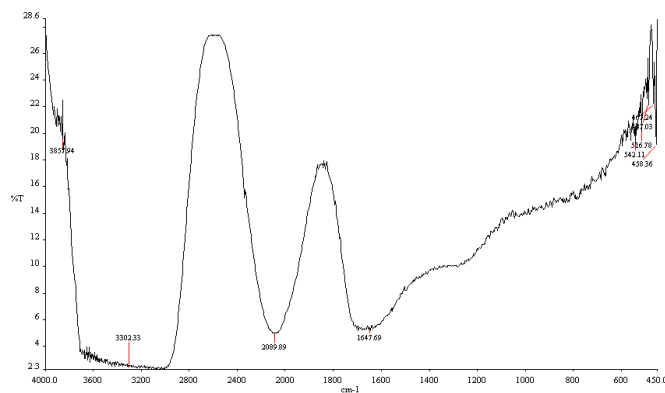
Experiments with selected bacteria (*S.aureus*) were performed to determine the antibacterial activity of ciprofloxacin during photocatalysis. Assays were done on bacterial inoculated agar plates.



**Figure3:** Zone of inhibition of ciprofloxacin drug

The effect on biological activity for the irradiated samples was observed by noting the inhibition halo formed around the drop seed on the agar plate. It was observed that when untreated ciprofloxacin solution was used, inhibition zone of 5.5 cm was observed. But when ciprofloxacin solution treated with  $\text{TiO}_2$  nanoparticles was used no kind of zone of inhibition was noted. And the same with  $\text{Ag-TiO}_2$ , mean no zone of inhibition was noted when ciprofloxacin solution treated with  $\text{Ag-TiO}_2$  was used. This could be explained that original drug ciprofloxacin has been modified and degraded.



**Figur4:** :FTIR results of Ciprofloxacin with no TiO<sub>2</sub> nanoparticles**Figure5:** FTIR results of Ciprofloxacin with TiO<sub>2</sub> nanoparticles**Figure 6:** FTIR results of Ciprofloxacin with Ag-TiO<sub>2</sub> nanoparticles

It is also important to note that even on UV light treated ciprofloxacin solution where minimum amount of drug was degraded, did not show any kind of zone of inhibition. This support the concept that the intermediated product of photocatalytic process do not represent any antibacterial activity.

#### 4. CONCLUSION

The Ciprofloxacin structure was effectively degraded by TiO<sub>2</sub> photocatalysis using different kinds of light sources and TiO<sub>2</sub> nanoparticles. More Oxidation was noted when UV lamp along with Ag-TiO<sub>2</sub> nanoparticles were used. This could be because of the positive effect of silver on the photoactivity of Titania at degradation of Ciprofloxacin that can be clarified by its ability to trap electrons, therefore reducing the recombination of light generated electron-hole pairs at Titania surface.

The most remarkable conclusion is that as a result of treatment of ciprofloxacin with TiO<sub>2</sub>, Ag-TiO<sub>2</sub> nanoparticles and UV light, degraded ciprofloxacin and its products do not present antibacterial activity against S.aureus. Successful degradation of the ciprofloxacin antibiotics was found in this studies. Therefore, TiO<sub>2</sub>-based photocatalysis is feasible way to inactivate the ciprofloxacin drug, as a pretreatment prior to further biological treatments.

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