DEGRADATION AND INACTIVATION OF CIPROFLOXACIN BY PHOTOCATALYSIS USING TIO_2NANOPARTICLES

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ABSTRACT

To compare Ciprofloxacin attenuation efficiency, Ciprofloxacin solutions mixed with TiO_2nanoparticles 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_2. In contrast, prominent Ciprofloxacin degradation was detected in the presence of 0.01 mg/ml of TiO2. 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_2 nanoparticles, irradiation by UV lamp sources was also significant. The antibactacterial 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_2, 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-1 to mg l-1 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).

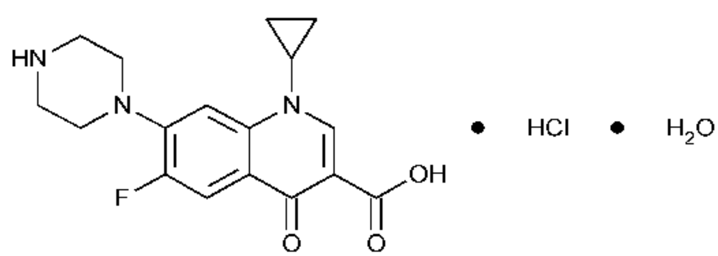
Ciprofloxacin and levofloxacin account for 65% of the total fluoroquinolone use and represent 3.3 billion dollars in global sales (Datamonitor Strategic Report, 2004). Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class Ciprofloxacin has 69 % bioavailability, 4 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_2 (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 ($OH^{\cdot}$) and the superoxide radical anions ($O_2^{\cdot -}$), which are the primary materials of the oxidative degradation. TiO_2 suspension in aqueous environment under irradiation causes an oxidative degradation of many pollutants and mineralizes organic materials into their building blocks i.e. CO_2, H_2O, 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_2 looks to be mediated by a number of reactions initiated by these primary oxidizing radicals, mostly $OH^{\cdot}$ radicals (Ilisz *et al*., 2002; Kaneco *et al*., 2004; Chen *et al*., 2005; Bizani *et al*., 2006). The photocatalytic efficiency of the UV/TiO_2 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_2 plays vital role in the photocatalytic degradation (Schmelling *et al*., 1997; Grzechulska and Morawski, 2002; Uyguner and Bekbölet, 2004).

In this article, Pure TiO_2 and Ag doped TiO_2 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_2 and Ag doped TiO_2 (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 TiO_2 (GPR, BDH Chemicals Ltd. Poole England) was used as TiO_2 source for preparing the nanoparticles, and $AgNO_3$ (GR, Merck, Germany) Ciprofloxacin HCl (GR, Merck, Germany) was used as the target compounds for degradation in this study. 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^{o}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- TiO_2 Nanoparticles

Ag-TiO_2 nanoparticles were prepared by the Liquid Impregnation Method (Sahoo, *et al*., 2005). For **1% Ag- TiO_2** : 50 gm of TiO_2 was poured into 100 ml dionized water in a 500 ml Pyrex beaker. 1.03 gm of $AgNO_3$ 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^{o}C for 12 hr to evaporate the remaining moisture. The solid material resulting from this step was calcined, at 500^{o}C for 3 hr in a furnace. This resulted in fine particles of silver doped TiO_2, herein after referred to as Ag-TiO_2 1%.

For **2%, 3%,4% & 5% Ag- TiO_2** . 50 gm of 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 $AgNO_3$ 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^{o}C for 12 hr to evaporate the remaining moisture. The solid material resulting from this step was calcined, at 500^{o}C for 3 hr in a furnace. This resulted in fine particles of silver doped TiO_2, herein after referred to as Ag-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 TiO_2, (b) TiO_2 and (c) Ag-TiO_2 (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_2 nanoparticles or Ag-TiO_2 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 similer.

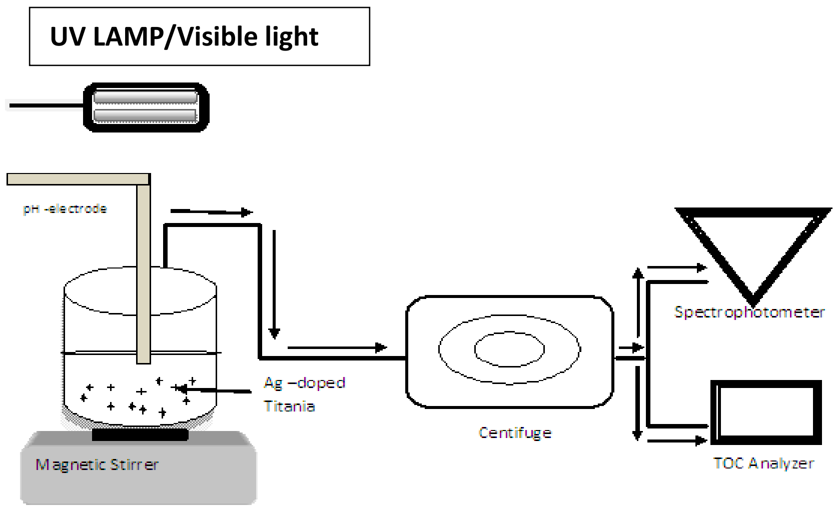


Figure 2: Experimental Setup for Photocatalysis under UV light/ Visible light

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 37C, the inhibition was noted.

3. Results & Discussions

3.1 Ciprofloxacin degradation with TiO_2 nanoparticles

As seen in chart below, a very low ciprofloxascin degradation was observed when it was illuminated in the absence of TiO_2.

In photocatalytic experiments in presence of 0.01 mg/ml , a maximum degradation was obtained under UV lamp illuminations when using Ag-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.

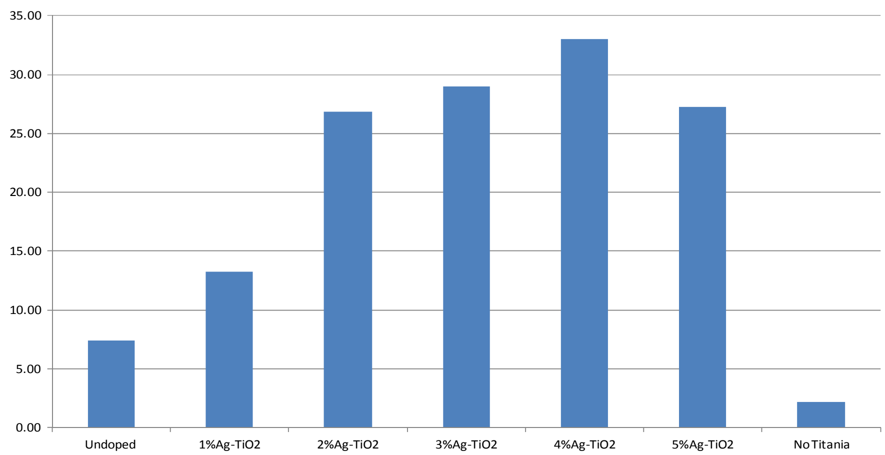


Figure 2: Degradtion profile of Ciprofloxacin irradiated by presence and absence of TiO_2nanoparticles and Ag-TiO_2 nanoparticles

Significant degradation was observed when ciprofloxacin was treated with the TiO_2 nanoparticles with the UV lamp but less as compared to the Ag-TiO_2 in the presence of UV lamp. It was also observed that by increasing the percentages of silver in Ag-TiO_2 i.e from 1% onward, better degradation was noted. Our results encouraged the use of Ag doped TiO_2 for the degadation of Ciprofloxacin.

Table 1: *Showing the level of degradation*

Lamp	Nanoparticles	Degradtion of Ciprofloxacin
Visible	No TiO_2 nanoparticels	Insignificant degradation
UV	No TiO_2 nanoparticels	Significant degradation
Visbible light	With TiO_2 nanoparticles	Major degradation
UV	With Ag-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_2 nanoparticles show better results as compared to pure TiO_2 nano particles.

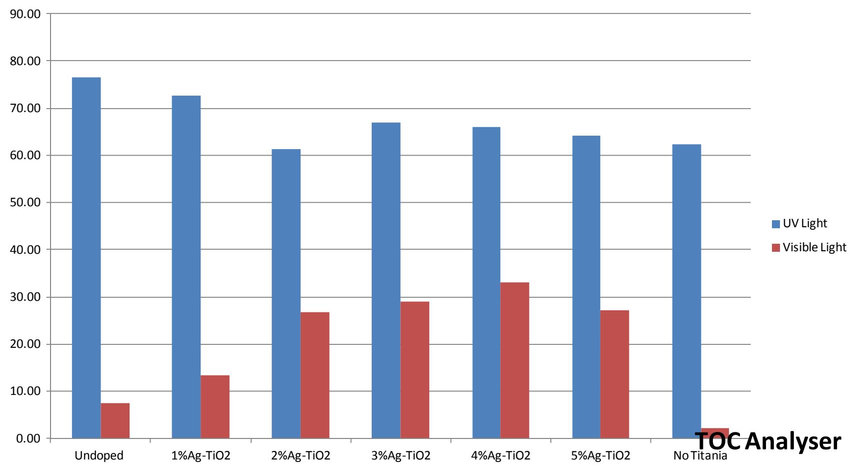


Figure3: Comparitive photocatalytic degradation of Ciprofloxacin with pure and Ag doped Titania Nanoparticles.

Similarly, results by HPlC clearly indicate that better degradation was observed with silver doped titania as compared to only Titanai nanoparticles. Compartive descriptive charts below show the detail of results.Same patern 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+TiO2	7	12.986	641290	25502	88.561
Ciproflox+UV+1%Ag-TiO2	10	12.428	46939	2036	28.258

Ciproflox+UV+2%Ag-TiO2	8	12.123	45979	1669	24.430
Ciproflox+UV+3%Ag-TiO2	3	5.024	10046	415	16.212
Ciproflox+UV+4%Ag-TiO2	5	12.614	122159	4855	30.530
Ciproflox+UV+5%Ag-TiO2	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+TiO2+Light	6	12.964	205614	8312	61.363
Cipro+1%Ag-TiO2+light	12	12.257	21785	916	10.565
Cipro+2%Ag-TiO2+light	4	12.111	142516	4906	53.085
Cipro+3%Ag-TiO2+light	6	12.653	139568	5526	46.236
Cipro+4%Ag-TiO2+light	6	12.593	132630	5298	38.172
Cipro+5%Ag-TiO2+light	3	12.596	17431	722	17.818
Cipro+light only	3	12.706	52657	1314	17.982

3.2 Effect of the TiO_2 photocatalysis on the antibacterial activity

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

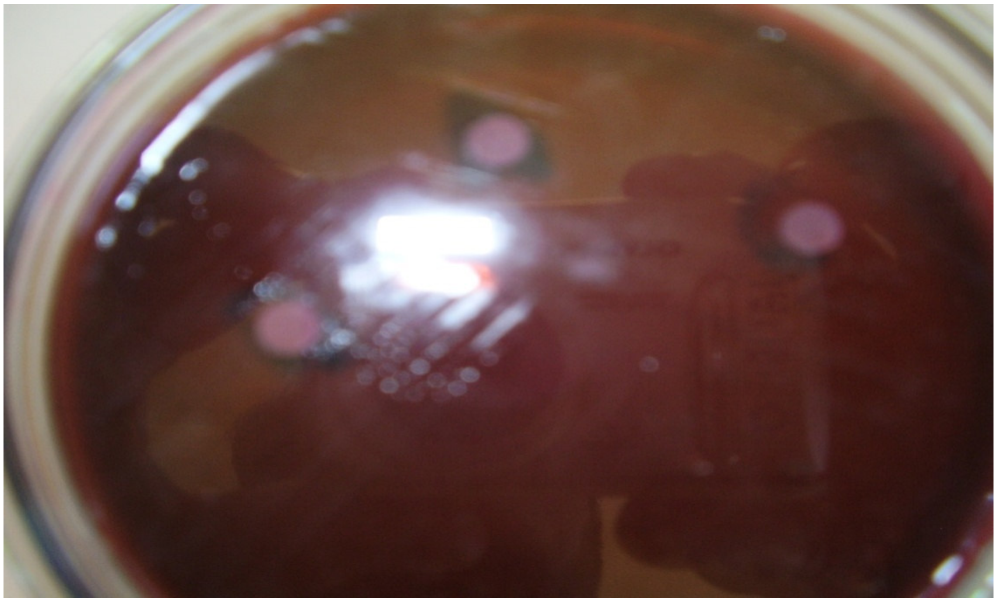
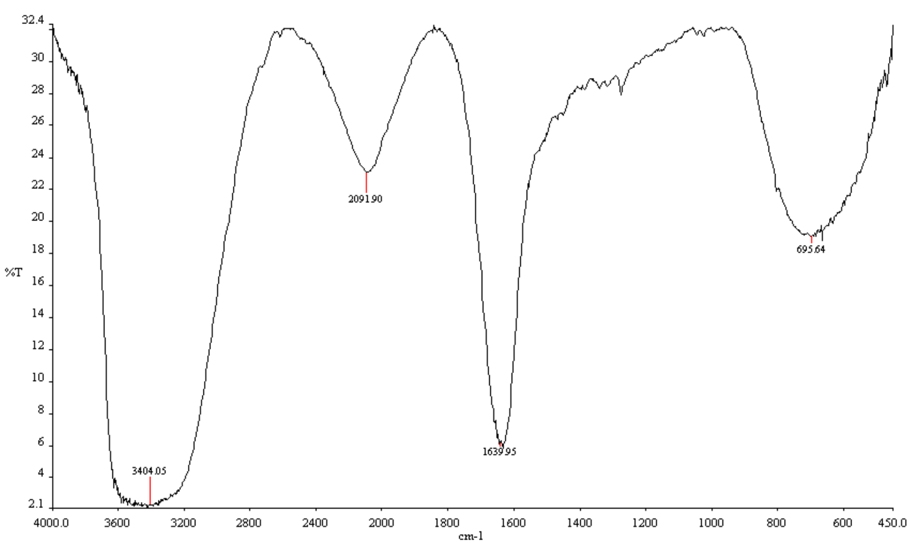


Figure3: Zone of inhibition of ciprofloxacin drug

The effect on biological activity for the irradiated sapmes 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 TiO_2 nanoparticles was used no kind of zone of inhition was noted. And the same with Ag-TiO_2, mean no zone of inhihibition was noted when ciprofloxacin solution treated with 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_2 nanoparticles

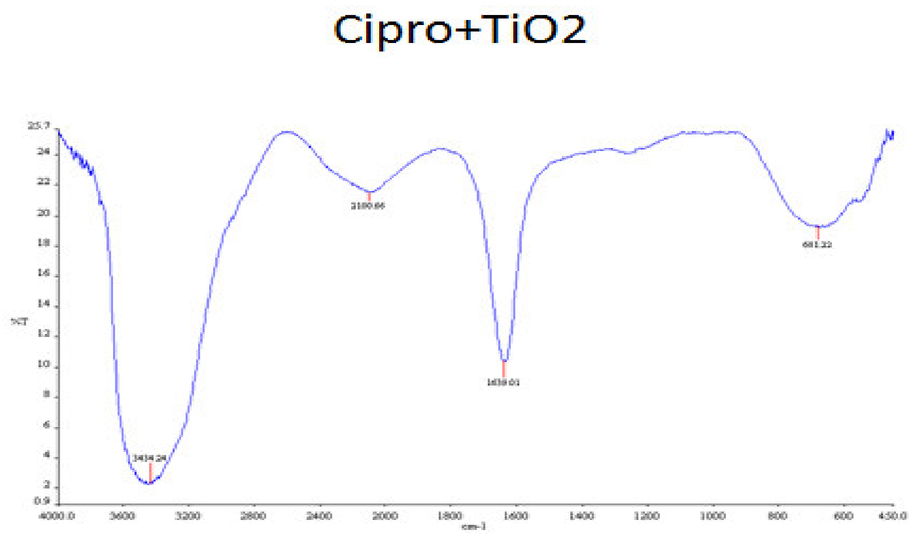


Figure5: FTIR results of Ciprofloxacin with TiO_2 nanoparticles

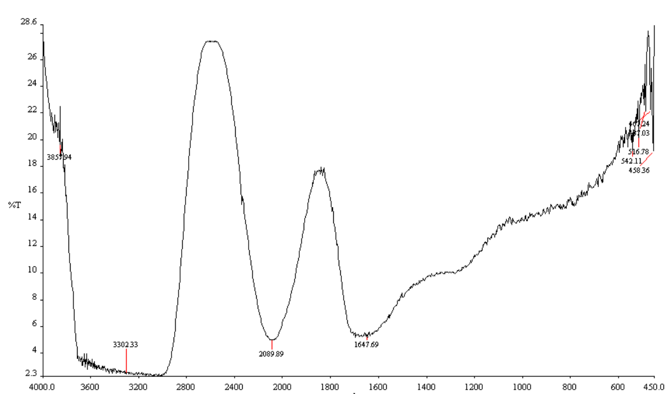


Figure 6: FTIR results of Ciprofloxacin with Ag-TiO_2 nanoparticles

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_2 photocatalysis using different kinds of light sources and TiO_2 nanoparticles. More Oxidation was noted when UV lamp along with Ag-TiO_2 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_2, Ag-TiO_2 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_2-based photocatalysis is feasible way to inactivate the ciprofloxacin drug, as a pretreatment prior to further biological treatments.

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