

## Treatment of pharmaceutical wastewater containing antibiotic with oxidation processes by metallic catalysts

Kambiz Tahvildari<sup>1,\*</sup>, Tania Bigdeli<sup>1</sup>

<sup>1</sup>Department of Chemistry, Islamic Azad University, Tehran North Branch, Tehran, Iran.

\*corresponding author e-mail address: [k\\_tahvildari@iau-tnb.ac.ir](mailto:k_tahvildari@iau-tnb.ac.ir)

### ABSTRACT

Pharmaceuticals have turned in to a major class of contaminants due to their widespread usage and known biological effects. Rifampin is known as an antibiotic with a strong red color. It is used in the treatment of many infectious, especially tuberculosis. If it is used in sub-therapeutic concentrations, it can lead to drug resistance in the microbial population. Thus, it is essential to remove its low concentrations from the wastewater to inhibit resistance in a pathogenic microorganism and save its effectiveness against tuberculosis. In the present research, the "Oxidation process by metallic catalysts" method, which consisted of zinc and copper, was employed for the first time in order to remove Rifampin from pharmaceutical wastewater. Variables including the total amount of zinc and copper catalysts, the ratio of the catalysts, duration of the reaction, rotation rate, temperature, and the pH considered and evaluated. It revealed that 0.154 mL of the total amount of the mixture of zinc and copper at the ratio of 70:30 in 100 ml of 40-ppm Rifampin solution, in the neutral pH and at ambient temperature (around 25°C) and rotation rate, could decrease the concentration of Rifampin to 1 ppm after 120 minutes. The catalysts could be used many times in order to remove the Rifampin from fresh wastewater. It was also shown that 96.4% of Rifampin could be removed from handmade Rifampin solutions and actual wastewater with 70% zinc and 30% copper in 600 rpm, the neutral pH, and the ambient temperature for 120 minutes. The catalysts could be used repeatedly to remove the Rifampin from fresh wastewater.

**Keywords:** *Copper and zinc, Metallic catalysts, Pharmaceutical wastewater, Rifampin, treatment.*

### 1. INTRODUCTION

There has been growing emphasis in recent years on the effect of drug compounds on the health of plants, animals, humans, and the environment [1-6]. For the most part of these pollutants are released into water from pharmaceutical companies; other sources include the direct disposal of excess and expired drugs in households and hospitals, water treatments in fish farms and in feces, and the urine of humans and animals following the use of drugs[7]. It has been found that trace levels of antibiotics in treated wastewater effluents can pose a human health risk owing to the rise of antibacterial activity in the downstream environments [8]. It has been well recognized that there is a connection between wastewater treatment plant effluent discharge and the presence of antibiotic-resistant microorganisms in the environment. These trends have been shown to be in downstream of an antibiotic manufacturer discharge, facilities treating hospital wastewater [9-10] and pharmaceutical manufacturing wastewater [11], conventional WWTPs [12-16] and a wastewater treatment plant (WWTP) using the tertiary treatment [17-18]. Wastewater produced from drugs is extremely hazardous and toxic; imposing serious side effects on humans and other organisms [19-22].

The possible fates of pharmaceuticals, when they enter the aquatic environment, are as follows:

- The contaminants are, eventually decomposed and converted to carbon dioxide and water.
- The lipophilic contaminants are partially retained in the sedimentation sludge.

The contaminants are metabolized to a more hydrophilic molecule, remaining in wastewater; consequently, hydrophilic molecules bring the polluted water into plants. These compounds show the highest persistence in the environment [23]. Several studies have

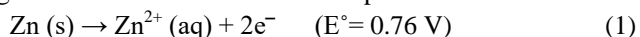
also shown the implications for water reuse. For instance, soil irrigated with the treated wastewater effluent exhibits a higher presence and a broader spectrum of antibacterial resistance in comparison to the soil watered with groundwater [24]. Another study has demonstrated that plants irrigated with recycled water can absorb some of the antibiotics [25]. Some antibiotics which are present in reclaimed water are used for irrigation; they are accumulated in soils, persisting for months after the irrigation season [26]. Overall, the previous findings show that antibiotic-resistant human pathogens can be transferred to food crops, with the resulting health benefits and the decreased acceptance of water reuse practices for crop irrigation [18]. Antibiotics are a class of drugs first produced in the early 1940s [27]. The average annual consumption of antibiotics has been reported to be 100,000-200,000 tons per year [28]. In the last decade, they have been regarded as environmental contaminants [29-37]. Due to the high solubility of most antibiotics, as compared to other pharmaceuticals, oxidative or other transformative processes (e.g., photolysis), instead of sorption-based processes, they have been regarded as the most promising technology. [18]. While the concentration of antibiotics in the environment is low; they can be highly dangerous because of their continuous input and high stability in the aquatic ecosystem. Furthermore, it should be noted that their low concentration can lead to drug resistance in bacterial populations, causing ineffectiveness in the near future [38-40]. Antibiotic-resistant bacteria can be a mark of ecological change. There have been several processes employed to degrade or remove antibiotics from aquatic ecosystems. For instance, we can refer to biological processes, filtration, coagulation, flocculation, and sedimentation, advanced oxidation processes (AOPs) adsorption,

advanced oxidation, with all these serving as an effective treatment technology for transforming trace antibiotics in wastewater effluents; however, the residual or newly generated antibacterial properties of transformation products remain a big challenge. Membrane processes are employed to remove antibiotics from wastewater [41-44]. The growth of antibacterial resistance in the environment can be very problematic due to trace antibiotics in WWTP effluents; this has encouraged scientists to evaluate the potential treatment technologies [45-46]. Rifampin (Molecular formula:  $C_{43}H_{58}N_4O_{12}$ ) is a semi-synthetic antibiotic in the form of a red-brown crystalline powder; it is used in the form of capsules, injections, and drops to treat tuberculosis. This antibiotic is often utilized with isoniazid and pyrazinamide. Rifampin is slightly soluble in water; freely soluble in chloroform, and soluble in ethyl acetate and methyl alcohol. This antibiotic is also sensitive to temperatures above 40 °C and light. It can also be oxidized in the presence of oxygen [31]. Rifampin can be environmentally hazardous in different ways:

- Rifampin is an antibiotic, so it has the potential to destroy the beneficial bacteria too;
- Rifampin has a very strong red color, thereby changing the color of waste to red;
- Degradation and removals of antibiotics in nature by biological and oxidation methods can be so slow;
- Although it is present at vestigial levels, Rifampin and other antibiotics can result in drug resistance in bacterial populations; so it is likely that in the near future, they may be ineffective in the treatment of tuberculosis [22-28].

According to the aforementioned problems, it is essential to use physical and chemical methods for the removal of this drug.

KDF<sup>®</sup> Process Media are known as high- purity, granulated copper and zinc-based alloys that treat water by means of a process based on the principle of redox (Oxidation-Reduction Process). Originally, KDF was the shorthand representing Kinetic Degradation Fluxion. redox can be represented as:

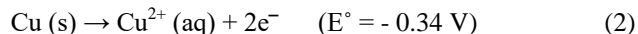


## 2. MATERIALS AND METHODS

### 2.1. Materials.

All chemicals used were of analytical grade from Merck or Fluka unless otherwise stated. Copper and zinc manufactured in industrial scales were used as a powder with less than 20 µm diameters. Standard and stock solutions were prepared using double distilled water. Rifampin was supplied by India - SUN company. All the solutions were protected from light and were analyzed on the day of preparations. Equipments used in the project included the following: a Spectrometer (Optizen 3220UV), a centrifuge (Hettich EBA20), a Ultrasonic (Wisd.23 Wise clean ), a Scale (AND HR-200), a Steirer Heater ( Heidolph MR Hei-End, 1400 rpm, 300 °C), a Mixer(IKA RW20.n, a pH meter (Metrohm 827 pH lab, filter paper (Slow42, Whatman), sintered glass, and High-performance liquid chromatography (HPLC) (Younglin).

### 2.2. Methods.



KDF Fluid Treatment is known as a unique combination of copper and zinc, creating an electrochemical reaction. By this reaction, electrons are transferred between molecules and as a result, new products are created. Some harmful contaminants are converted into harmless components. Free chlorine, as an example, is changed into benign, water-soluble chloride; then it is carried harmlessly through the water supply. In the same way, some heavy metals, such as copper, zinc, lead, and mercury, can react to plate out onto the medium's surface, leading to the effective removal from the water supply.

It has been shown that redox media can remove up to 98% of water-soluble cation (positively- charged ions) including lead, mercury, copper, zinc, nickel, chromium, and other dissolved metals. This water treatment decreases bacteria and other microorganisms by disrupting electron transport, causing cellular damage.

KDF<sup>®</sup> Media is recyclable and its lifetime is more than six years. The pH of the medium is also in the range ~ 6.5- 8.5.

Sulfur compounds present in wastewater can create a bad smell. This compound reacts with the copper, removing the odor of the effluent. Mechanism acting in copper and zinc to remove the odor is to one of destroying hydrogen sulfide.



Indeed, hydrogen sulfide reacts with copper, producing the insoluble copper.

KDF Process Media can be beneficial in many ways:

- Cooper and zinc are cheap and recyclable;
- They can effectively remove chlorine and heavy metals and control microorganism contamination;
- They are also effective in removing the odor of the effluent owing to the reaction with sulfur compounds [46].

In this study, for the first time, the "Oxidation by metallic catalysts" method was employed in order to remove Rifampin from the pharmaceutical wastewater.

**2.2.1. Preparation of Standard Stock Solution.** The standard stock solution containing Rifampin was prepared by dissolving 100 mg of Rifampin in 20 ml of deionized water. It was then sonicated for 10 minutes and the final volume of the solution was made up to 100 ml to get a stock solution containing 1000 µg/ ml of Rifampin in 100 ml volumetric flasks.

**2.2.2. Procedure for determination of the wavelength for analysis.** Rifampin is a colored material and many π bonds can be seen in its structure so it probably has an absorbance in UV and visible area (Figure 1). By appropriate dilution of the standard stock, solutions containing 20µg/ml of Rifampin was scanned in the range of 400-200 nm to determine the wavelength of maximum absorption ( $\lambda_{max}$ ). This scan has three maximum absorptions in 236, 252, and 332 nm as shown in figure 2. Wavelength 332 nm due to the sharpness of peak and less interaction with other materials have selected as  $\lambda_{max}$ .

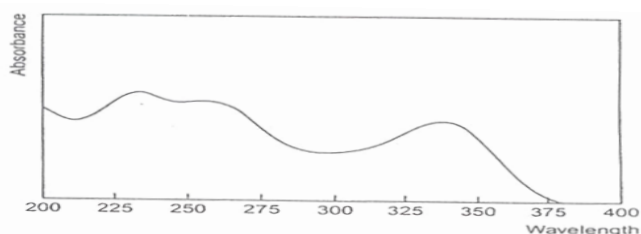


Figure 1. Rifampin scan in the UV range.

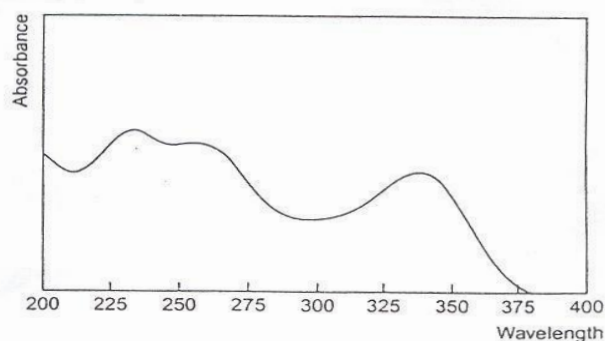


Figure 2. The absorption spectrum of Rifampin (20 µg/ml) showing  $\lambda_{\max}$  332.

**2.2.3. Procedure for the plotting calibration curve.** Rifampin showed linearity with absorbance in the range of 3–40 ppm at its wavelength maxima, which was validated by least square regression method. The coefficient of correlation was found to be 0.9944 for Rifampin. For estimating of Rifampin, a series of linearity solutions were prepared by diluting an appropriate volume of standard stock solution. Then 3, 4, 5, 10, 20, 30, and 40 ppm solutions were prepared and absorption of all solutions measured at  $\lambda_{\max}$ . Each measurement was repeated three times and each dilution was measured three times; therefore, nine data groups were obtained for each solution and a calibration curve was drawn by all 18 data as seen in Figure 3. The following equation was used to calculate the concentration of the unknown solution of Rifampin:

$$Y = 0.0327x - 0.0072 \quad (1)$$

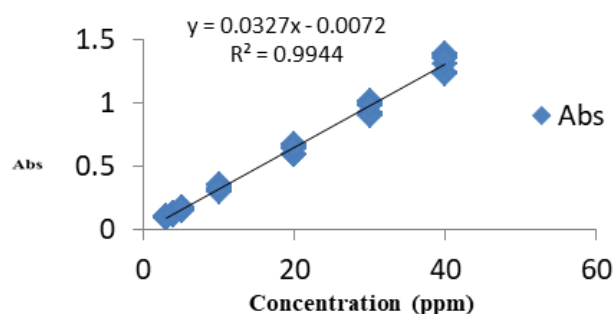


Figure 3. Calibration curve of Rifampin (3–40 ppm).

**2.2.4. Analytical procedure for Rifampin removal studies.** Batch experiments were conducted by varying contact time, stirring speed, light against, pH, catalyst amount, temperature, And analysis of the real samples. Adsorption experiments were performed by shaking appropriate amount of the metallic catalyst with 100 ml of the 40 ppm Rifampin solution in a thermostatic shaker bath adjusted at  $25 \pm 1$  °C. The pH of each solution was adjusted to values ranging from 5.0 to 9.0 with dilute solutions of sodium hydroxide and nitric acid. To determine the appropriate ratio of copper and zinc for Rifampin removal, a different Ratio of Cu/Zn was tested. To find the best duration of reaction between copper/ zinc catalyzer and Rifampin, the reaction was continued for several time ranges. To find the best rotation rate, a mixture of zinc and copper in 100 ml of 40 ppm Rifampin solution in 600 and 1400 rpm by the mixer and 600 rpm by magnetic stirrer were studied for 120 minutes in this experiment.

**2.2.5. Reuse of copper and zinc.** To understand whether the catalyst is recyclable or not, the solution of Rifampin was prepared and it was tested with zinc and copper in neutral pH at 25 °C. Then the catalyst was separated from the solution through filter paper. The isolated catalyst was mixed with 40 ppm of fresh Rifampin solution in similar circumstances.

**2.2.6. High-performance liquid chromatography (HPLC).** In this test, the USP method was used to prepare a 2, 5, 10, 20, and 40 ppm Rifampin solution. Then each solution was passed through filter paper 0.45 µm and 20 µL of each solution was injected to the HPLC apparatus.

### 3. RESULTS

The UV spectrophotometric method for the estimation of Rifampin in sample solutions was found to be simple. Beer's law was obeyed in the concentration range of 5–40 ppm. The correlation coefficient was found to be 0.9944 which shows the good linear relationship for Rifampin.

#### 3.1. Optimization of the Cu/Zn ratio.

Determination of the appropriate ratio of copper and zinc to remove Rifampin from wastewater was one of the purposes of this study. Different Ratio of Cu/Zn was tested as shown in Table 1. 40 ppm solution of Rifampin has prepared and 100 mL of solution was mixed with Different Ratio of Cu/Zn. The results are shown in Table 2.

Table 1. Different ratio of copper and zinc to remove Rifampin.

Experiment	Zn%	Cu%
1	0%	100%
2	10%	90%
3	20%	80%
4	30%	70%
5	40%	60%
6	50%	50%
7	60%	40%
8	70%	30%
9	80%	20%
10	90%	10%
11	100%	0%

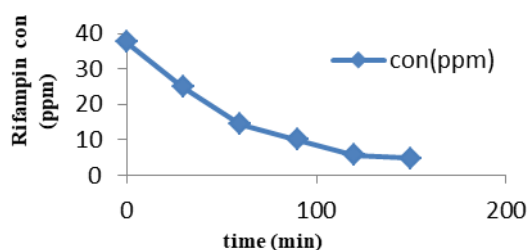
**Table 2.** elimination of Rifampin in the reuse of Cu/Zn.

Time(min)	Concentration (ppm)
120 (1)*	2.055
120 (2)	2.360
120 (3)	3.186
120 (4)	4.838
120 (5)	7.345
120 (6)	7.620
120 (7)	8.293
120 (8)	8.966
120 (9)	13.737
120 (10)	14.685
120 (11)	15.663

\* This Numbers refer to the Different ratio of copper and zinc to remove Rifampin (shows on table 1)

### 3.2. Duration of reaction.

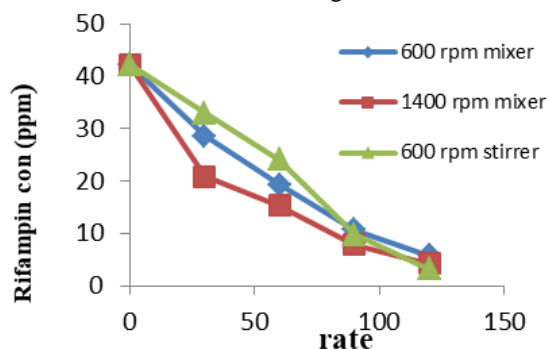
To find the best duration of reaction between copper/ zinc catalyzer and Rifampin, the reaction was continued for several time ranges, including 30, 60, 90, 120, and 150 minutes. The results are shown in Figure 4.

**Figure 4.** Duration of reaction

Based on these results, the slope of degradation curve by time is very low, so 120 minutes duration of the reaction was selected as the best reaction time.

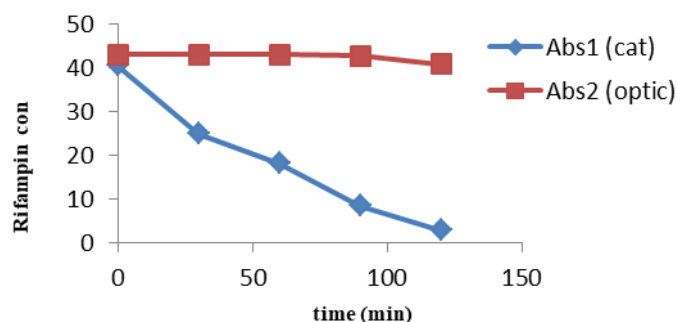
### 3.3. Effect of stirring speed on the removal of Rifampin.

To find the best rotation rate, the 0.154-mole total amount of mixture of zinc and copper at the ratio of 70:30 in 100 ml of 40 ppm Rifampin solution in 600 and 1400 rpm by the mixer and 600 rpm by magnetic stirrer were studied for 120 minutes in this experiment. The results are shown in Figure 5.

**Figure 5.** Comparison of the effect of stirring speed on the removal of Rifampin.

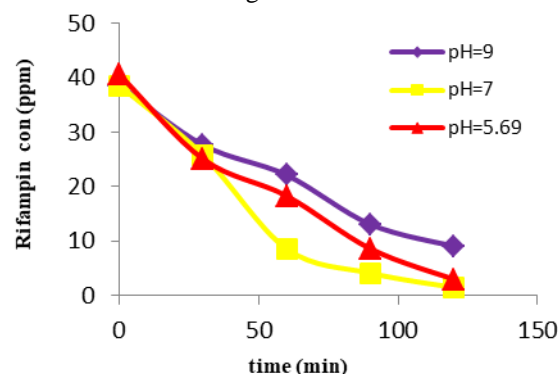
### 3.4. Removal of Rifampin against light.

Rifampin is sensitive to light so 100 ml of 40 ppm solution of Rifampin was placed exposed to light for 120 minutes. Then its wavelength changes were compared to the reaction between Rifampin and Cu/Zn in similar circumstances, the results are shown in Figure 6.

**Figure 6.** Comparison of removal of Rifampin against light and the catalyst.

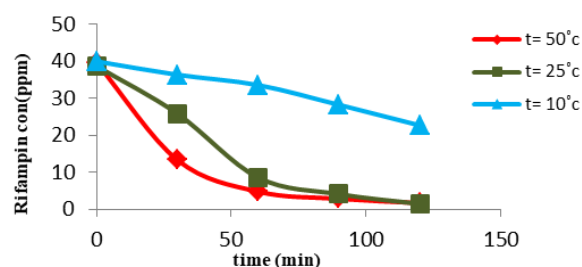
### 3.5. Effect of pH on the removal of Rifampin.

To find the best pH, three 100 mL of 40 ppm solution of Rifampin and deionized water were prepared and were tested with 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in different pH (acidic=5.7, alkaline=9, and neutral=7) in 600 rpm for 120 minutes. It should be noted that the pH of Rifampin solution was acidic so this experiment was done in pH 7 and 9. The results are shown in Figure 7.

**Figure 7.** Comparison of different pH in the elimination of Rifampin.

### 3.6. Effect of temperature on the removal of Rifampin.

To find the best temperature, three 100 mL of 40 ppm solution of Rifampin and deionized water were prepared in neutral pH and test them with 0.154 mole of total amount of Zinc and copper by the ratio of 70:30 respectively in different temperature (10, 25, and 50 °C) in 600 rpm for 120 minutes. Figure 8 shows a comparison of the results.

**Figure 8.** Comparison of different temperature in the elimination of Rifampin.

### 3.7. Study of adsorption of copper and zinc.

To find out what kind of reaction exists, 100 mL of 40 ppm solution of Rifampin was prepared and tested with 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in neutral pH at 25 °C in 600 rpm for 120 minutes. Then the catalyst was separated from the solution through filter paper. The isolated catalyst was mixed with purified water at 50° C for 120 minutes. Then the absorption of purified water was measured at a



wavelength of 332 nm. The concentration of Rifampin in purified water was 2.2 ppm.

### 3.8. Reuse of copper and zinc.

To understand whether the catalyst is recyclable or not, 100 mL of 40 ppm solution of Rifampin was prepared and it was tested with 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in neutral pH at 25 °C in 600 rpm for 120 minutes. Then the catalyst was separated from the solution through filter paper. The isolated catalyst was mixed with 40 ppm of fresh Rifampin solution in similar circumstances. This cycle was repeated 12 times. The results are shown in Table 2.

### 3.9. Use of copper and zinc for the removal of Rifampin from actual wastewater.

In this experiment, a sample of actual wastewater which was infected with Rifampin was prepared. Then it was tested with 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in 600 rpm for 120 minutes. At first, wastewater had stench but after 60 minutes there was no odor since Cu/Zn are effective in destroying the odor of the effluent due to reaction with sulfur compounds. In addition, the catalyst could reduce the concentration of Rifampin and other contaminants that had an absorbance in Rifampin wavelength. It should be noted that the results were qualitative.

### 3.10. Used the less catalyst to remove Rifampin.

In this test, 10% of the total amount of the catalyst that was used in other experiments in optimal ratio and similar circumstances were used. In this situation, the concentration of Rifampin reached 15 ppm after 120 minutes but in other experiments, the concentration of Rifampin reached 15 ppm after 60 minutes.

### 3.11. Determined Rifampin concentrations by HPLC.

In this test, the USP method was used to prepare a 2, 5, 10, 20, and 40 ppm Rifampin solution. Then each solution was passed through filter paper 0.45 μ and 20 μL of each solution was injected to the HPLC apparatus. The results are shown in Figure 9.

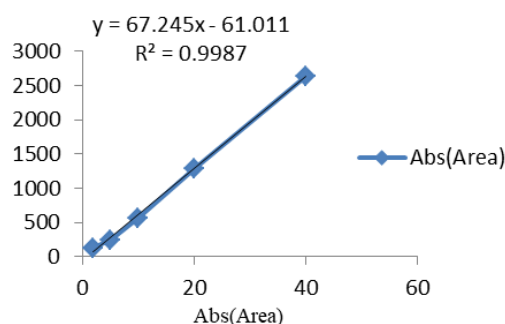


Figure 9. Calibration curve by HPLC.

## 4. CONCLUSIONS

In this study, 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in 100 mL of 40 ppm Rifampin solution was selected as an optimum ratio of Cu/Zn for the removal of Rifampin. The best duration of the reaction was 120 minutes because absorptions after 120 minutes did not obey the Beer-Lambert law. In the stirrer speed experiment, the end point of three velocities was close to each other and 600 rpm speed of the mixer was selected as the optimum speed because of lower energy consumption. In addition, using a mixer in the industry was more possible than a magnetic stirrer. In the experiment of "effect of

Use the following equation (2) to obtain the concentration of the unknown solution of Rifampin was used:

$$Y = 67.245x - 61.011 \quad (2)$$

40 ppm of Rifampin solution was prepared in neutral pH as a test solution and 20 μL of it was injected to HPLC apparatus. Then the test solution was mixed with 0.154 mL of the total amount of zinc and copper at the ratio of 70:30 in the ambient temperature in 600 rpm for 120 minutes. The test solution was passed through filter paper 0.45 μ and 20 μL of it was injected to the HPLC apparatus Chromatogram (Figure 10). Table 3 shows a comparison of the results.

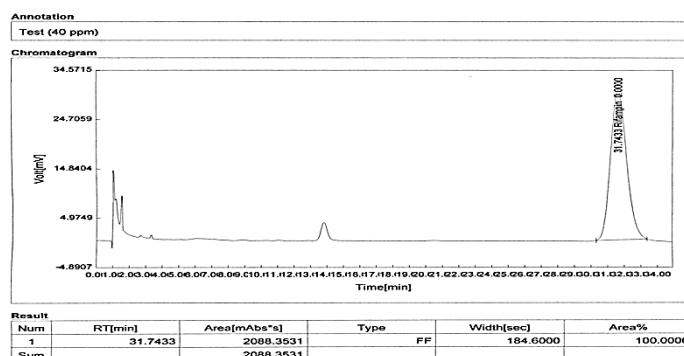


Figure 10. Chromatogram of 40 ppm Rifampin test solution.

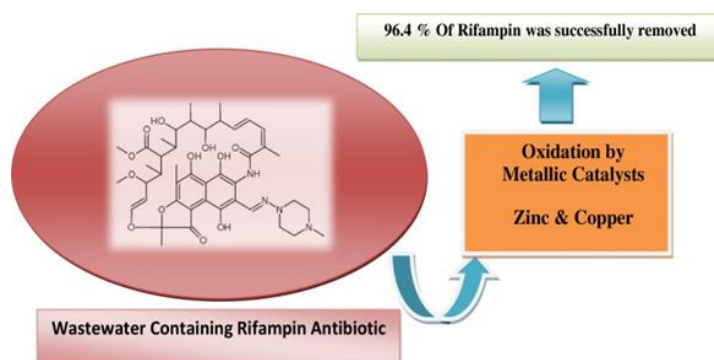


Figure 11. Schematic of Rifampin antibiotic with oxidation by metallic catalysts.

Table 3. The concentration of test solution of Rifampin; before and after the reaction.

	Time (min)	Area (mAbs*s)	Concentration (ppm)
Test solution before reaction	31.7433	2088.3531	31.963
Test solution after reaction	-	2088.3531	-

light on Rifampin", the concentration of Rifampin against light did not show significant changes during the reaction. Thus, the effect of light on Rifampin was not significant. In the experiment of "effect of pH on the removal of Rifampin", according to Figure 7, neutral pH was selected as optimum pH. In the experiment of "effect of temperature on the removal of Rifampin", according to Figure 8, the ambient temperature was selected as the optimum temperature. It should be noted that endpoints of 25 and 50 °C were close to each other but more energy was consumed at 50 °C. Finally, Rifampin was removed by the process of oxidation and

adsorption did not occur. The mixture of zinc and copper is also capable of removing contaminants in further times but its efficiency is lower than the first time. In actual wastewater, Cu/Zn is effective in destroying the stench and reducing the concentration of Rifampin and other contaminants that have an absorbance at its wavelength. Fewer amounts of the catalyst can remove Rifampin but it needs more time and as Figures 10 and 11 show, there was

not any peak for Rifampin after the reaction. Therefore, this contaminant was removed from wastewater. Finally, in this study, 96.4% of Rifampin was successfully removed from handmade Rifampin solutions and actual wastewater with 70% zinc and 30% copper in 600 rpm, neutral pH and ambient temperature for 120 minutes. The catalysts can be used several times to remove the Rifampin from fresh wastewater.

## 5. REFERENCES

- Chen, Y.L.A.; Hsien, Y.T.; Khaja, L.S. Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. *J. Hazard. Mater.* **2009**, *167*, 1163-1169, <https://doi.org/10.1016/j.jhazmat.2009.01.108>.
- Lemus, D.; Martin, A.; Montoro, E.; Portals, F.; Palomino, J. Rapid alternative methods for detection of rifampicin resistance in Mycobacterium tuberculosis. *J. Antimicrob. Chemother.* **2004**, *54*, 130-133, <https://doi.org/10.1093/jac/dkh320>.
- Wacławek, S.; V.Lutze, H.; Grübel, K.; Padil, V.V.T.; Černík, M.; Dionysiou, D.D.; Chemistry of persulfates in water and wastewater treatment: A review. *Chem. Eng. J.* **2017**, *330*, 44-62. <https://doi.org/10.1016/j.cej.2017.07.132>
- Ahmad, S.; Mokaddas, E. Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis. *Respir. Med.* **2009**, *103*, 1777-1790, <https://doi.org/10.1016/j.rmed.2009.07.010>.
- Zhang, Y.; Marrs, C.F.; Simon, C.; Xi, C. Wastewater treatment contributes to the selective increase of antibiotic resistance among Acinetobacter spp. *Sci. Total Environ.* **2009**, *407*, 3702-3706. <https://doi.org/10.1016/j.scitotenv.2009.02.013>.
- Bull, R.J.; Crook, J.; Whittaker, M.; Cotruvo, J.A. Therapeutic dose as the point of departure in assessing potential health hazards from drugs in drinking water and recycled municipal wastewater. *Regul. Toxicol. Pharm.* **2011**, *60*, 1-19, <https://doi.org/10.1016/j.yrtph.2009.12.010>.
- Swarnalakshmi, K.S.; Chinnaiyan, P.; Nivetha, S.; Nair, A.S.; Use of rice husk ash as an adsorbent to remove contaminants in water and comparison with advanced oxidation process – a study. *Mater Today Proc.* **2018**, *5*, 24248-24257. <https://doi.org/10.1016/j.matpr.2018.10.220>
- Shavi, R.; Hiremath, V.; GilSeo, J. Radical-initiated oxidative conversion of methane to methanol over metallic iron and copper catalysts. *Mol. Catal.* **2018**, *445*, 232-239, <https://doi.org/10.1016/j.mcat.2017.12.001>.
- Yahiat, S.; Fourcade, F.; Brosillon, S.; Amrane, A. Removal of antibiotics by an integrated process coupling photocatalysis and biological treatment – Case of tetracycline and tylosin. *Int. Biodeterior. Biodegrad.* **2011**, *65*, 997-1003, <https://doi.org/10.1016/j.ibiod.2011.07.009>.
- Sires, I.; Brills, E.; Oturan, M.A.; Rodrigo, M.A.; Panizza, M. Electrochemical Advanced Oxidation Processes: Today and Tomorrow. A Review. *Environ Sci Pollut Res.* **2014**, *21*, 8336-8367, <https://doi.org/10.1007/s11356-014-2783-1>.
- Olya, S.; Keen, K.G. Degradation of Antibiotic Activity during UV/H<sub>2</sub>O<sub>2</sub> Advanced Oxidation and Photolysis in Wastewater Effluent. *Environ. Sci. Technol.* **2013**, *47*, 13020-13030, <https://doi.org/10.1021/es402472x>.
- Li, D.; Qi, R.; Yang, M.; Zhang, Y.; Yu, T. Bacterial community characteristics under long-term antibiotic selection pressures. *Water Res.* **2011**, *45*, 6063 - 6073, <https://doi.org/10.1016/j.watres.2011.09.002>.
- Elazab, H.A. The catalytic activity of copper oxide nanoparticles towards carbon monoxide oxidation catalysis: microwave – assisted synthesis approach. *Biointerface Res. Appl. Chem.* **2018**, *8*, 3278 - 3281.
- Nguyen-Huy, C.; Lee, H.; Lee, J.; Kwak, J.H.; An, K. Mesoporous mixed CuCo oxides as robust catalysts for liquid-phase furfural hydrogenation. *Appl. Catal. A. Gen.* **2019**, *571*, 118-126, <https://doi.org/10.1016/j.apcata.2018.12.010>.
- Andersson, D.I.; Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resist Update* **2012**, *15*, 162-172, <https://doi.org/10.1016/j.drug.2012.03.005>.
- D'Costa, V.M.; McGrann, K.M.; Hughes, D.W.; Wright, G.D. Sampling the antibiotic resistance. *Science.* **2006**, *311*, 374-377, <https://doi.org/10.1126/science.1120800>.
- Goni-Urriza, M.; Capdepuy, M.; Arpin, C.; Raymond, N.; Caumette, P.; Quentin, C. Impact of an Urban Effluent on Antibiotic Resistance of Riverine Enterobacteriaceae and Aeromonas spp. *Appl. Environ. Microbiol.* **2000**, *66*, 125-132.
- Qi, W.; Ran, J.; Zhang, Z.; Niu, J.; Zhang, P.; Fu, L.; Hu, B.; Li, Q. Methane combustion reactivity during the metal→metallic oxide transformation of Pd-Pt catalysts: Effect of oxygen pressure. *Appl. Surf. Sci.* **2018**, *435*, 776-785, <https://doi.org/10.1016/j.apsusc.2017.11.178>.
- Pirsaheb, M.; Moradi, S.; Shahlaei, M.; Wang, X.; Farhadian, N. A new composite of nano zero-valent iron encapsulated in carbon dots for oxidative removal of bio-refractory antibiotics from water. *J Clean Prod.* **2018**, *209*, 1523-1532.
- Iwane, T.; Urase, T.; Yamamoto, K. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Water Sci. Technol.* **2001**, *43*, 91-99.
- Watkinson, A.J.; Micalizzi, G.B.; Graham, G.M.; Bates, J.B.; Costanzo, S.D. Antibiotic-Resistant Escherichia coli in Wastewaters, Surface Waters, and Oysters from an Urban Riverine System. *Appl. Environ. Microbiol.* **2007**, *73*, 5667-5670, <https://doi.org/10.1128/AEM.00763-07>.
- Koczura, R.; Mokracka, J.; Taraszewska, A.; Łopacinska, N. Abundance of Class 1 Integron-Integrase and Sulfonamide Resistance Genes in River Water and Sediment Is Affected by Anthropogenic Pressure and Environmental Factors. *Microb Ecol.* **2016**, *72*, 909-916, <https://doi.org/10.1007/s00248-016-0843-4>.
- LaPara, T.M.; Burch, T.R.; McNamara, P.J.; Tan, D.T.; Yan, M.; Eichmiller, J. Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *J. Environ. Sci. Technol.* **2011**, *45*, 9543-9549, <https://doi.org/10.1021/es202775r>.
- Farhadi, S.; Aminzadeh, B.; Torabian, A.; Khatibikamal, V.; Alizadeh Fard, M. Comparison of COD removal from pharmaceutical wastewater by electrocoagulation, photoelectrocoagulation, peroxi-electrocoagulation and peroxi-photoelectrocoagulation processes. *J. Hazard. Mater.* **2012**, *219-220*, 35-42, <https://doi.org/10.1016/j.jhazmat.2012.03.013>.
- Rivas, J.; Gimeno, O.; Encinas, A.; Beltran, F. Ozonation of the pharmaceutical compound ranitidine: reactivity and kinetic aspects. *Chemosphere.* **2009**, *76*, 651-656, <https://doi.org/10.1016/j.chemosphere.2009.04.028>.

26. Zhou, Y.; Liu, X.; Zhao, Y.; Luo, S.; Wang, L.; Yang, Y.; Mu, Y. Structure-based synergistic mechanism for the degradation of typical antibiotics in electro-Fenton process using Pd-Fe<sub>3</sub>O<sub>4</sub> model catalyst: Theoretical and experimental study. *J. Catal.* **2018**, *365*, 184-194. <https://doi.org/10.1016/j.jcat.2018.07.006>.
27. Yang, S.; Carlson, K. Routine monitoring of antibiotics in water and wastewater with a radioimmunoassay technique. *Water Res.* **2004**, *38*, 3155-3166. <https://doi.org/10.1016/j.watres.2004.04.028>.
28. Klavarioti, M.; Mantzavinos, D.; Kassinos, D. Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. *Environ. Int.* **2009**, *35*, 402-417. <https://doi.org/10.1016/j.envint.2008.07.009>.
29. Pan, Z.; Song, Ch.; Li, L.; Wang, H.; Pan, Y.; Wang, Ch.; Li, J.; Wang, T.; Feng, X.; Membrane technology coupled with electrochemical advanced oxidation processes for organic wastewater treatment: Recent advances and future prospects. *Chem. Eng. J.* **2019**, In Press, Corrected Proof. <https://doi.org/10.1016/j.cej.2019.01.188>.
30. Michael-Kordatou, I.; Karaolia, P.; Fatta-Kassinos, D.; The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater: A review. *Water Res.* **2018**, *129*, 208-230. <https://doi.org/10.1016/j.watres.2017.10.007>.
31. Ferella, F.; Optimization of a plant for treatment of industrial waste solutions: Experimental and process analysis. *JECE*. **2018**, *6*, 377-385. <https://doi.org/10.1016/j.jece.2017.12.018>.
32. Boukhchina, S.; Akrou, H.; Berling, D.; Bousselmi, L.; Highly efficient modified lead oxide electrode using a spin coating/electrodeposition mode on titanium for electrochemical treatment of pharmaceutical pollutant. *Chemosphere*, **2019**, *221*, 356-365. <https://doi.org/10.1016/j.chemosphere.2019.01.057>.
33. Martínez, F.; Molina, R.; Rodríguez, I.; Pariente, M.I.; Segura, Y.; Melero, J.A.; Techno-economical assessment of coupling Fenton/biological processes for the treatment of a pharmaceutical wastewater. *JECE*. **2018**, *6*, 485-494. <https://doi.org/10.1016/j.jece.2017.12.008>.
34. Zheng, W.; Wen, X.; Zhang, B.; Qiu, Y.; Selective effect and elimination of antibiotics in membrane bioreactor of urban wastewater treatment plant. *Sci Total Environ.* **2019**, *646*, 1293-1303. <https://doi.org/10.1016/j.scitotenv.2018.07.400>.
35. Miklos, D.B.; Remy, Ch.; Jekel, M.; Linden, K.G.; Drewes, J.E.; Hübner, U.; Evaluation of advanced oxidation processes for water and wastewater treatment – A critical review. *Water Res.* **2018**, *139*, 118-131. <https://doi.org/10.1016/j.watres.2018.03.042>.
36. Serpone, N.; Artemev, Y.M.; Ryabchuk, V.K.; Emeline, A.V.; Horikoshi, S.; Light-driven advanced oxidation processes in the disposal of emerging pharmaceutical contaminants in aqueous media: A brief review. *Curr Opin Green Sustain Chem.* **2017**, *6*, 18-33. <https://doi.org/10.1016/j.cogsc.2017.05.003>.
37. Malik, A.; Aleem, A. Incidence of metal and antibiotic resistance in *Pseudomonas* spp. from the river water, agricultural soil irrigated with wastewater and groundwater. *Environ. Monit. Assess.* **2011**, *178*, 293-308. <https://doi.org/10.1007/s10661-010-1690-2>.
38. Jones-Lepp, T.L.; Sanchez, C.A.; Moy, T.; Kazemi, R. Method development and application to determine potential plant uptake of antibiotics and other drugs in irrigated cropproduction systems. *J. Agric. Food Chem.* **2010**, *58*, 11568-11573. <https://doi.org/10.1021/jf1028152>.
39. Kinney, C.A.; Furlong, E.T.; Werner, S.L.; Cahill, J.D. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ. Toxicol. Chem.* **2006**, *25*, 317-326. <https://doi.org/10.1897/05-187R.1>.
40. Li, S.Z.; Li, X.Y.; Wang, D.Z. Membrane (RO-UF) filtration for antibiotic wastewater treatment and recovery of antibiotics. *Sep. Purif. Technol.* **2004**, *34*, 109-114. [https://doi.org/10.1016/S1383-5866\(03\)00184-9](https://doi.org/10.1016/S1383-5866(03)00184-9).
41. Homem, V.; Santos, L. Degradation and removal methods of antibiotics from aqueous matrices--a review. *J. Environ. Manage.* **2011**, *92*, 2304-2347. <https://doi.org/10.1016/j.jenvman.2011.05.023>.
42. Watkinson, A.J.; Murby, E.J.; Costanzo, S.D. Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. *Water Res.* **2007**, *41*, 4164-4176. <https://doi.org/10.1016/j.watres.2007.04.005>.
43. Moore, J.E.; Rao, J.R.; Moore, P.J.A.; Millar, B.C.; Goldsmith, C.E.; Loughrey, A.; Rooney, P. Determination of total antibiotic resistance in waterborne bacteria in rivers and streams in Northern Ireland: Can antibiotic-resistant bacteria be an indicator of ecological change? *J. Aquat. Ecol.* **2010**, *44*, 349-358. <http://dx.doi.org/10.1007/s10452-009-9294-z>.
44. Ata, R.; Yildiz, T.G. Characterization and removal of antibiotic residues by NFC-doped photocatalytic oxidation from domestic and industrial secondary treated wastewaters in Meric-Ergene Basin and reuse assessment for irrigation. *J. Environ. Manage.* **2019**, *233*, 673-680. <https://doi.org/10.1016/j.jenvman.2018.11.095>.
45. Anthony, C.; Moffat, M.; Osselton, D.; Widdop, B. *Clarke's Analysis of Drugs and Poisons*. Third Edition, Pharmaceutical Press, London, 2004, 2, 1534.
46. The United States Pharmacopeial Convention, *U.S. Pharmacopeia National Formulary (USP 34- NF 29)* Edition 29, 2011, 3-2, 4149.



© 2019 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).