

Study of the degradation of the antibiotic Sulfamethoxazole and Trimethoprim(ST) from light-mediated CuO and ZnO nanoparticles



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ABSTRACT

The discovery of natural antibiotics was considered a revolution for medicine at the time, capable of easily curing bacterial diseases that had no cure. However, the inappropriate use and disposal of these drugs ended up causing an accelerated development of bacteria, causing a natural selection of strains capable of resisting these antibiotics. To try to avoid this evolution, it is necessary to degrade these drugs before disposal. One way to cause this

degradation is to apply ultraviolet radiation on the molecular structure of the antibiotic, providing energy for the breakdown and degradation of the structure, in a photocatalysis process. The application of certain nanoparticles can accelerate the degradation process, such as copper oxide (CuO) and zinc oxide (ZnO), which are used as photocatalysts, as they absorb at low energy (visible light) and at higher energy (UV), and also for being able to recover them after use. The results of the UV-Vis absorption spectroscopy show that the use of nanoparticles was responsible for accelerating the antibiotic degradation process by 1.68x when compared to the use of only UV radiation.

Keywords: Antibiotics, Degradation, Photocatalysis, Spectroscopy.

1 INTRODUCTION

The first antibiotic, dating back to 1940, was a revolution in medicine at the time of its discovery, being able to cure several diseases that until that moment caused thousands of deaths all over the world. It was believed at the time that, with the creation of penicillin, problems with bacterial diseases had come to a definitive end ^[1]. This turned out not to be the reality, as bacteria evolved to develop resistance to these antibiotics.

The misuse and improper disposal of antibiotics in nature are a major factor in the evolution of these microorganisms. Residues of various types of drugs can be found in our raw sewage and even wastewater due to incomplete treatment and removal in Sewage Treatment Plants (WWTP) ^[2]. Bacteria come into contact with these antibiotics, which are in small amounts in the environment, and end up creating natural defenses to fight them, reducing or eliminating the effectiveness of the drugs ^[3]. To try to avoid this natural selection of bacteria, it is necessary that these drugs have their active ingredients degraded before being discarded into the environment.

Degrading a potentially harmful material means breaking its molecular structure into smaller fragments through chemical reactions caused by the absorption of ultraviolet (UV), visible (Vis) and infrared (IR) radiation ^[4]. "The degradation of drugs results in different by-products, which may



present greater or lesser toxicity compared to the original drug" (MELO et al, 2009) [5]. This degradation can be enhanced by using certain nanoparticles as catalysts for this reaction.

Copper oxide (CuO) and zinc oxide (ZnO) nanoparticles are used as photocatalysts because they absorb both at low energy, when visible light is used, and in systems with higher energy such as UV radiation, and these catalysts can be recovered and reused in future processes [6].

2 JUSTIFICATION

The use of antibiotics has been growing worldwide due to their biocidal properties, however their indiscriminate use has caused great environmental impact. In this sense, the study of mechanisms for degradation of such compounds becomes important. It is possible to accelerate this degradation process by using catalyst nanoparticles in the presence of UV radiation and visible light.

3 OBJECTIVE

The general objective of this work is to contribute to the study of the degradation processes of antibiotic compounds, from ceramic CuO and ZnO nanoparticles and ultraviolet (UV) radiation. It is intended to determine the necessary conditions for the photocatalytic degradation of antibiotics, as well as to evaluate the influence of nanoparticles on this degradation.

4 LITERATURE REVIEW

4.1 NANOPARTICLES

Nanoparticles are objects whose dimensions range from 1 to 100 nanometers (nm). The area of science focused on the use of nanoparticles covers a wide variety of market and technology branches, from the production of one of the most promising materials in science today, known as graphene, to the fight against cancer. As quoted by Dr. L. Assis: "Nanotechnology is focused on characterization, fabrication, manipulation, and application of biological and non-biological structures at the nanometer scale." (SAHOO et al., 2007 apud ASSIS et al., 2012) [7].

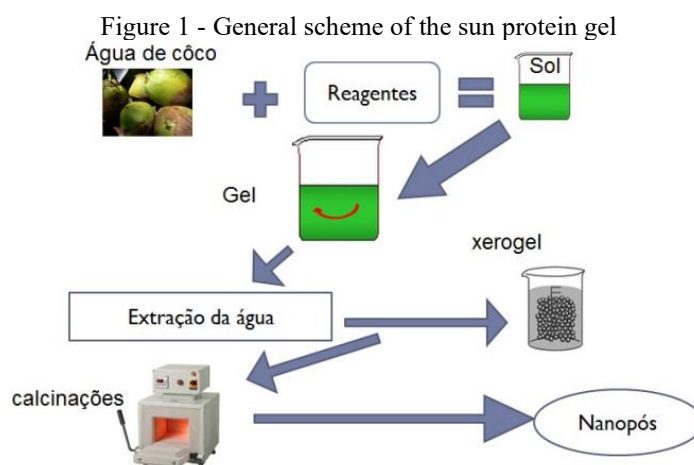
Degradation of substances using nanoparticles has been reported for some time in the area of environmental protection, among them we can mention the work conducted by Magdalena (2021) from the Faculty of Sciences/UNESP in Bauru with the photodegradation of a toxic dye, rhodamine B, using two types of oxides and with a removal efficiency of more than 90% [8].

4.2 SUN PROTEIN GEL

The Sol gel method was developed in 1846, gaining greater attention in the 1980s for the production of rechargeable lithium batteries. It is a technique used to synthesize inorganic oxides in aqueous medium or alcohol. The sol protein gel appeared in 1998 replacing the aqueous medium with



a solvent with a high protein content, and coconut water is usually used ^[9]. The production process of the nanomaterials is illustrated in Figure 1, using coconut water as a solvent. Gelatin can also be used as a substitute for coconut water due to its high protein content, a condition adopted in this study.



Source: GOMES (2013). Available at: https://ri.ufs.br/bitstream/riufs/5367/1/MANASSES_ALMEIDA_GOMES.pdf ^[9]. Accessed on: 31 May 2023

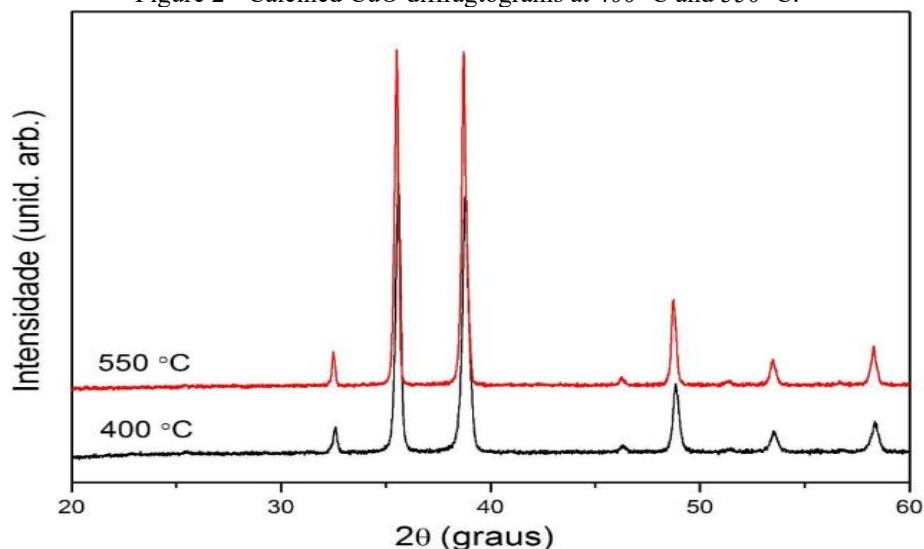
It should be noted that this technique, although simple and low-cost, enables the synthesis of materials with great homogeneity, good crystallinity, making use of sustainable chemistry, in addition to the possibility of synthesizing nanoparticles with different morphologies.

4.3 X-RAY DIFFRACTOGRAM (XRD)

X-ray diffractometry is a widely used technique for the identification of crystalline structures. In this technique, an X-ray beam interacts with the material and provides information about the position, intensity and profile of the Bragg reflections^[10, 11]. Each of these parameters contains information about the crystalline structure of the material, lattice parameters, atomic positions, among others, in addition to allowing comparison with the material's database, in order to enable quality control in the industry. The diffractogram of a material available in the ICSD (Inorganic Crystal Structure Database) is often used as a reference for the determination of structures, since similar materials have very similar diffractograms. As an example, the diffractogram of CuO is shown in Figure 2, the compounds were calcined at temperatures of 400 °C and 550 °C. Depending on the diffractogram available in the ICSD database, it is verified that the material has a single phase for copper II oxide with a monoclinic structure (ICSD n° 16025).



Figure 2 - Calcined CuO diffractograms at 400 °C and 550 °C.



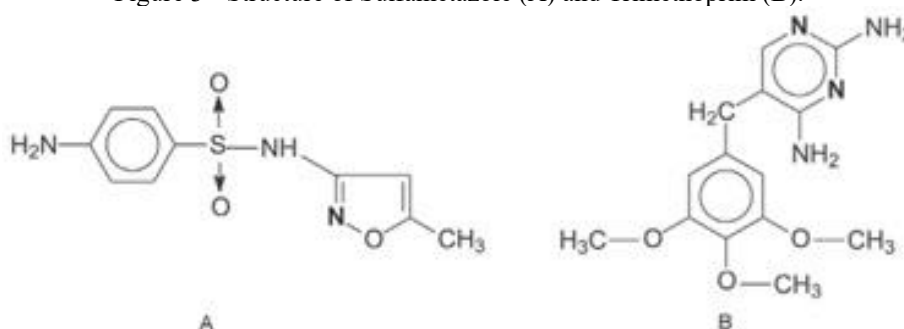
Source: CHIMIDT (2019) [12].

In this diffractogram, it is possible to observe that as the temperature increases, there is a slight increase in diffraction peaks, as well as a slight narrowing of the width at half height, which would indicate an improvement in the crystallinity of the material [12].

4.4 ANTIBIOTIC - SULFAMETHOXAZOLE AND TRIMETHOPRIM(ST)

Sulfonamides, or sulfa drugs, were first tested in the 1930s as an antibiotic element. One of the sulfa drugs used in medicine is sulfataxol which, along with trimethoprim, is used to treat urinary tract infections and for HIV-positive patients who have infections [3]. Their molecular structures are shown in Figure 3.

Figure 3 - Structure of Sulfametazole (A) and Trimethoprim (B).



Source: CORDEIRO (2008). Available at: <http://dx.doi.org/10.1590/s0100-40422008000200012> [13]. Accessed on 05 jun. 2023

The molecular structure of sulfametazole is: 4-amino-N-(5-methyl-3-isoxazolyl)benzenesulfonamide, and the molecular composition of the structure of Trimethoprim is: 5-[(3,4,5-trimethoxyphenyl)methyl]-2,4-pyrimidinediamine [13].



5 METHODOLOGY

5.1 PREPARATION OF NANOPARTICLES

The CuO and ZnO nanoparticles were produced by the Sol-gel protein method, in a very simple process as described below.

In a becker, 300 mL of water was heated to an average temperature of 45 °C. With the help of a magnetic bar, to keep the liquid always moving, 20 g of gelatin was slowly added until it was completely dissolved, then 20 g of Copper Nitrate II - $\text{Cu}(\text{NO}_3)_2$ - was added to the solution. After 15 minutes of dissolving, the temperature was changed to an average of 150°C. The mixture was boiled for another 15 minutes, as shown in figure 4, until it was transferred to the greenhouse where it remained for 5 days so that it lost all possible moisture.

Figure 4 - Heated solution of copper nitrate and gelatin.



Source: Prepared by the author

The material resulting from this drying was macerated using a mortar and pestle. It was then taken to a muffle furnace and placed at a temperature of 200°C for a pre-firing of the material for about 20 minutes, resulting in a black powder shown in Figure 5. The material was placed in the oven again at a temperature of 550°C for 4 hours for the process of calcination and formation of the desired phase. In this work, CuO and ZnO nanoparticles were used to study the antibiotic degradation process.

For the production of ZnO, the same methodology was used, however, the copper salt was replaced by the zinc salt (zinc nitrate hexahydrate).



Figure 5 - Result of copper pre-firing.



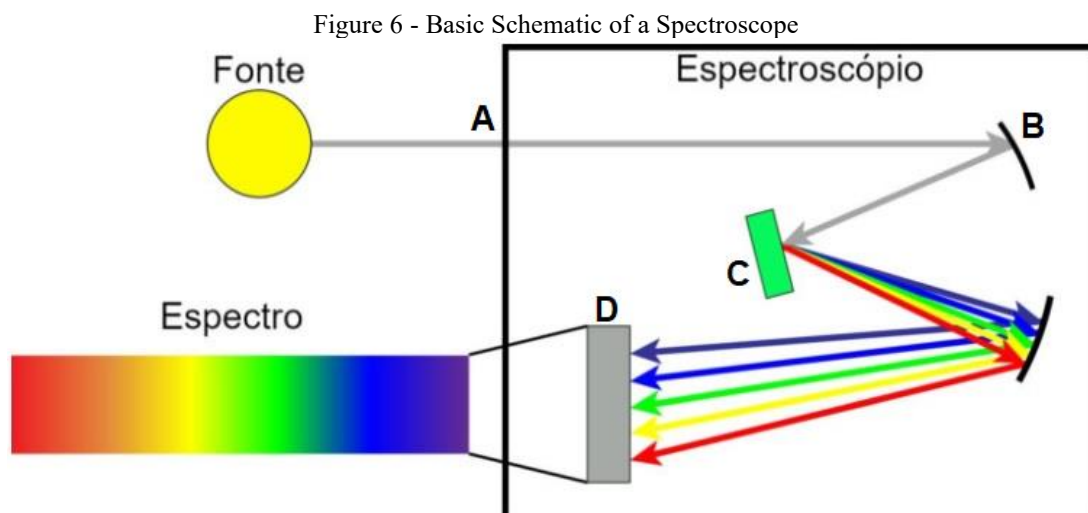
Source: Prepared by the author

The oxides used in this study were analyzed by X-ray diffraction and compared to the diffractograms of the Inorganic Crystal Structure Database (ICSD).

5.2 UV-VIS ABSORPTION SPECTROMETRY

By means of UV and visible absorption spectroscopy (UV-Vis), it is possible to evaluate the intensity of light absorption of materials as a function of wavelength. This technique is used to identify the electronic structure of a material.

As illustrated in Figure 6, spectroscopy is performed by causing light to be collimated through a small hole (A) and hit a focusing lens (B) that directs the light to a prism or diffraction grating in order to promote scattering of the light (C). Such light falls on the sample (D) and the intensity of the transmitted light is compared to a beam of light passing through a reference sample^[14]. Through this method, it is possible to measure the intensity of light absorbed by a sample as a function of the wavelength of the incident light.



Source: SANTIAGO (2012). Available at: <https://infoescola.com/fisica/espectroscopia>.^[14] Accessed on June 10, 2023.



To perform the UV-Vis spectroscopy studies in this work, a 500mL volumetric flask with an aqueous solution at a concentration of 0.1 gL^{-1} ST was prepared and the SPECORD 50 spectrophotometer was used in the range between 190 and 400 nm. Less concentrated solutions of the antibiotic were prepared in order to determine the optimal concentration where it would be possible to visualize the full spectrum of drug absorption. The concentration of 0.03 gL^{-1} was chosen due to the measurable values within the device's reading scale.

5.3 DEGRADATION OF ST BY UV RADIATION IN THE PRESENCE OF NANOPARTICLES

An aqueous solution at 0.03 gL^{-1} of ST was prepared, with a part reserved in a beaker in the absence of nanoparticles to be used as a reference. A second part received 0.1 gL^{-1} of CuO. Both solutions were placed in a drying and sterilization oven under a mercury vapor lamp for exposure to UV radiation (Ultra-Vitalux 300W), as shown in Figure 7. The same procedure was performed for ZnO.

Figure 7 - Solutions under UV light radiation.



Source: Prepared by the author

Samples of the solutions inside the greenhouse were collected over time (Figure 8) to identify the antibiotic.



Figure 8 - Samples of the solutions taken over time.



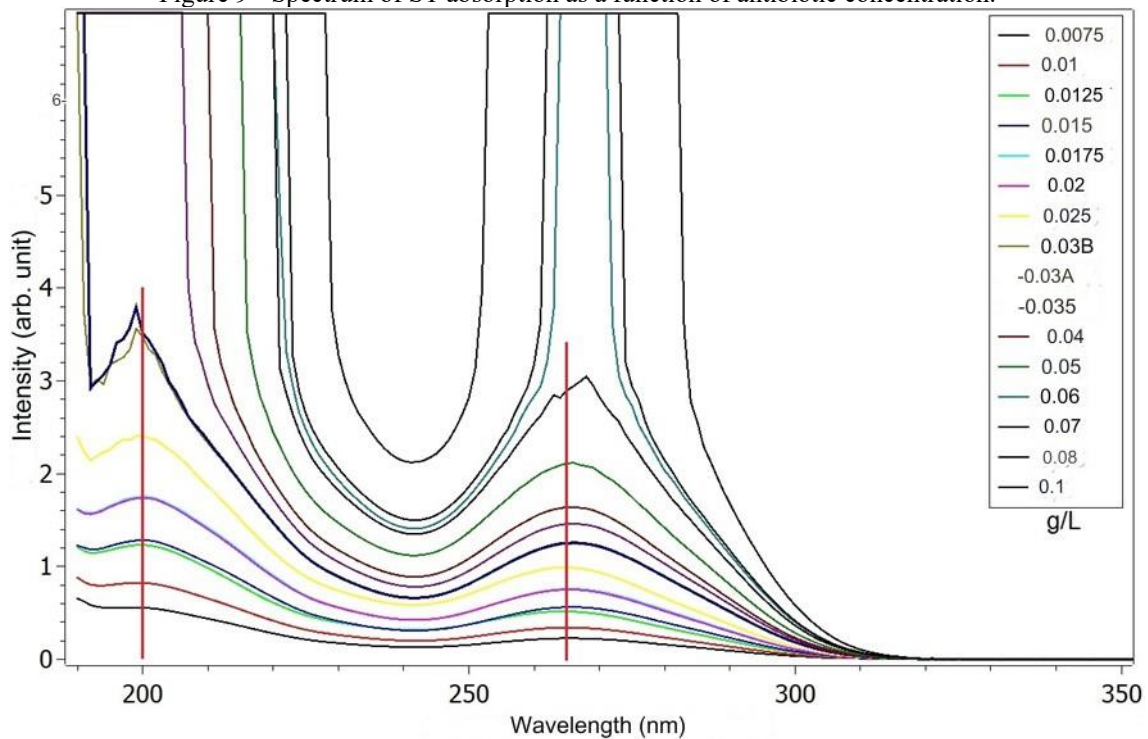
Source: Prepared by the author

6 RESULTS AND DISCUSSIONS

6.1 ANALYSIS OF ST ANTIBIOTIC SOLUTION

The first analysis of the aqueous solution containing ST in various concentrations is shown in Figure 9.

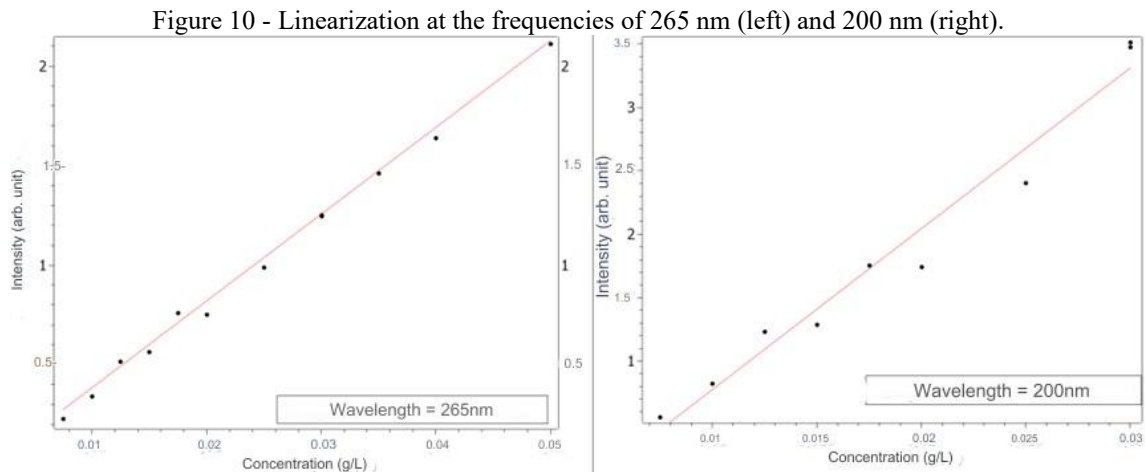
Figure 9 - Spectrum of ST absorption as a function of antibiotic concentration.



Source: Prepared by the author



It is possible to observe that the intensity does not change at wavelengths greater than 320 nm, and two absorption peaks are noted at 200 nm and 265 nm (vertical red lines). In order to determine whether there is a linearity between concentration and intensity at the 200 nm and 265 nm peaks, a calibration curve of the absorption intensity at these wavelengths was constructed as a function of the antibiotic concentration, as can be seen in Figure 10.



Source: Prepared by the author

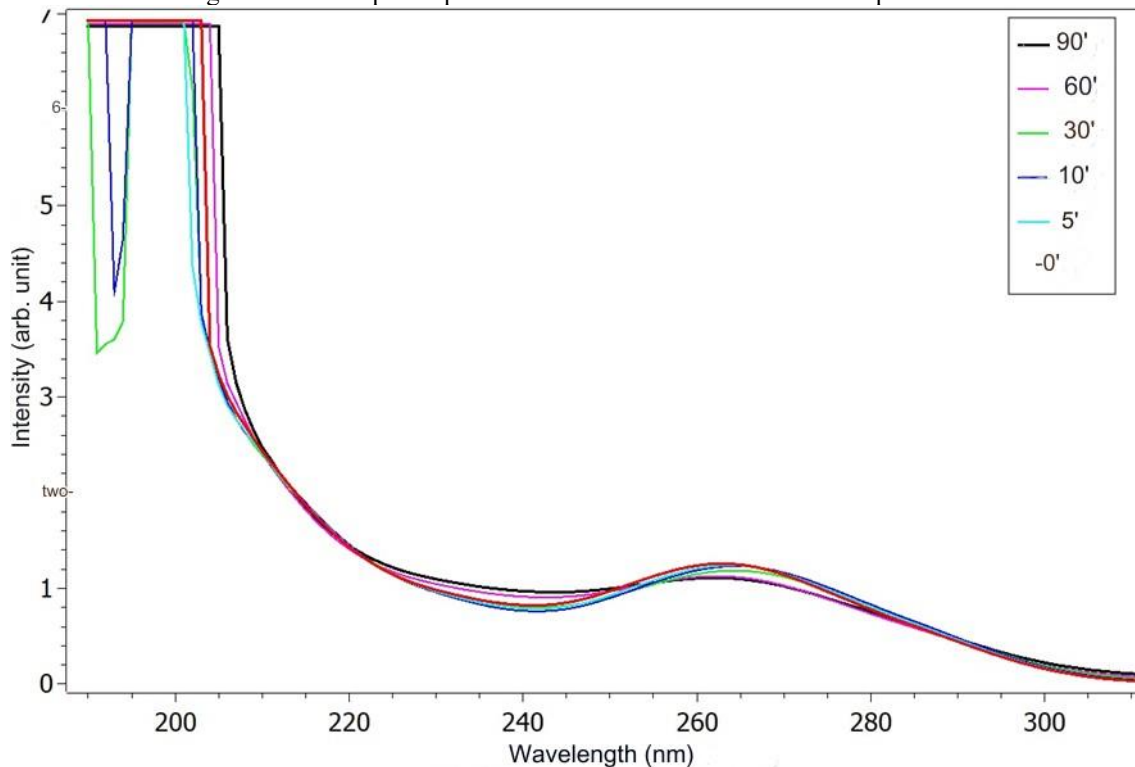
There is a linear increase in the intensity of absorption as a function of the concentration of the antibiotic in the solution. This result is important to illustrate the final concentration of the antibiotic solution after the irradiation process and thus determine how much of the drug was degraded in the process of exposure to UV radiation.

6.2 PHOTODEGRADATION TEST OF THE SOLUTION IN THE ABSENCE OF NANOPARTICLE

To serve as a basis for comparison between the solutions with the addition of nanoparticles, an antibiotic solution with a concentration of 0.03 gL⁻¹ in the absence of nanoparticles was exposed to UV radiation. The absorption spectrum in the range of interest was then collected, and the result is shown in Figure 11.



Figure 11 - Absorption spectrum of the ST solution without nanoparticles.



Source: Prepared by the author

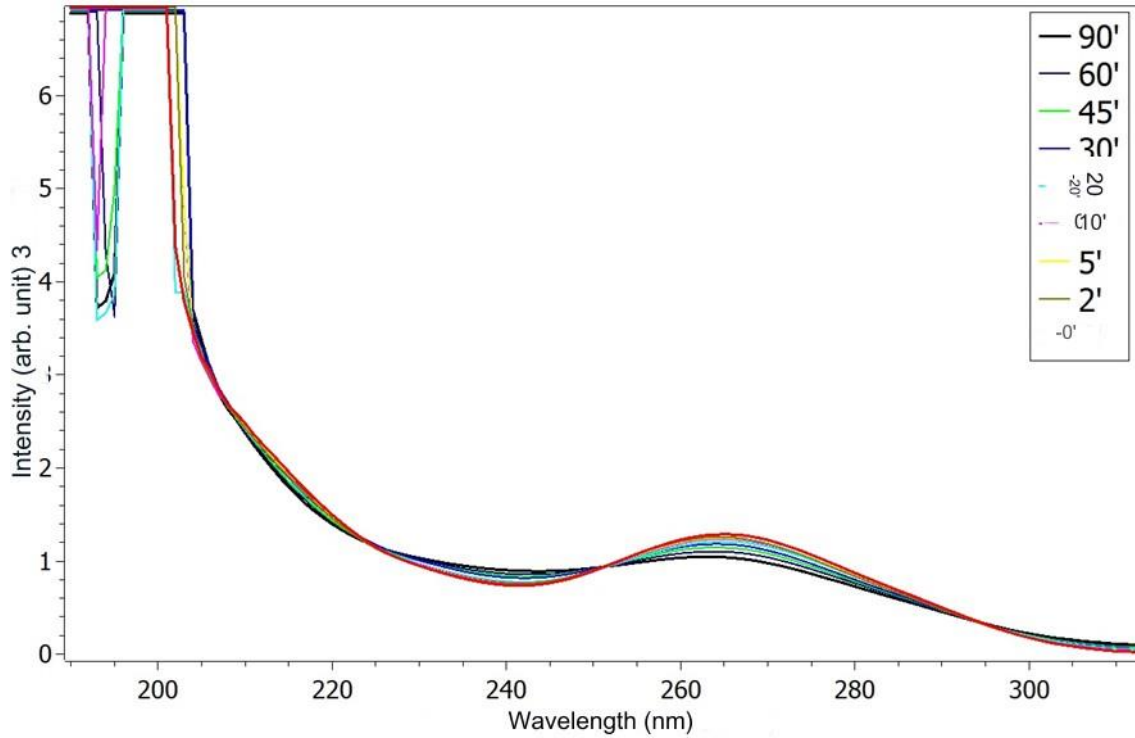
A slight degradation of the drug is observed as a function of the time of exposure to UV radiation. A degradation of about 12% is noted when compared to the non-irradiated antibiotic, considering the peak at 265 nm.

6.3 PHOTODEGRADATION TEST IN THE PRESENCE OF CuO NANOPARTICLES

Together with the aqueous solution of 0.03 gL⁻¹ of antibiotic, CuO nanoparticles were incorporated until a solution with a concentration of 0.1 gL⁻¹ of CuO nanoparticle was reached in relation to the antibiotic solution. This procedure aimed to evaluate the interaction of nanoparticles and the antibiotic during exposure to UV radiation. The results obtained are shown in Figure 12.



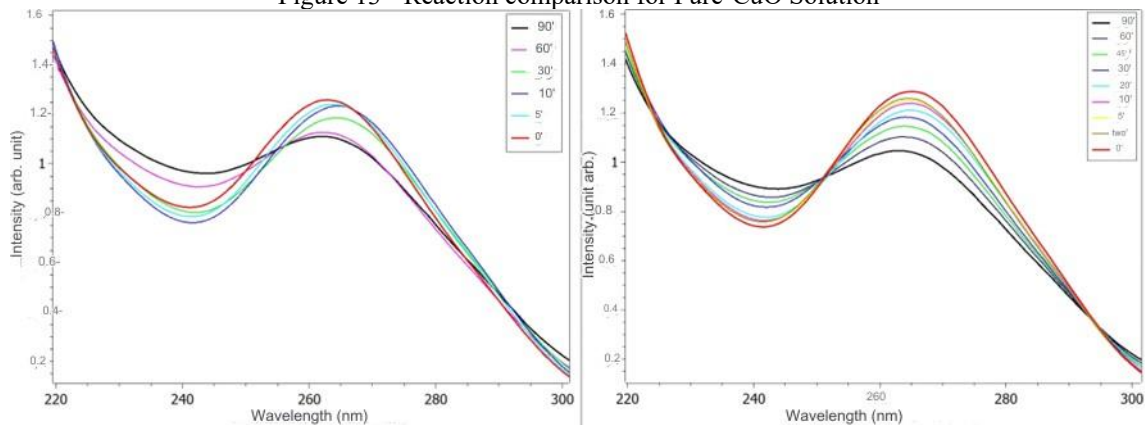
Figure 12 - Absorption spectrum of the ST solution containing CuO nanoparticles.



Source: Prepared by the author

Figures 11 and 12 show that the presence of nanoparticles slightly accelerated the degradation process provided by UV radiation. This observation is better illustrated in Figures 13 and 14, where a widening of the curves is presented, comparing the ST solution in the absence and presence of CuO nanoparticles (0.1 gL⁻¹).

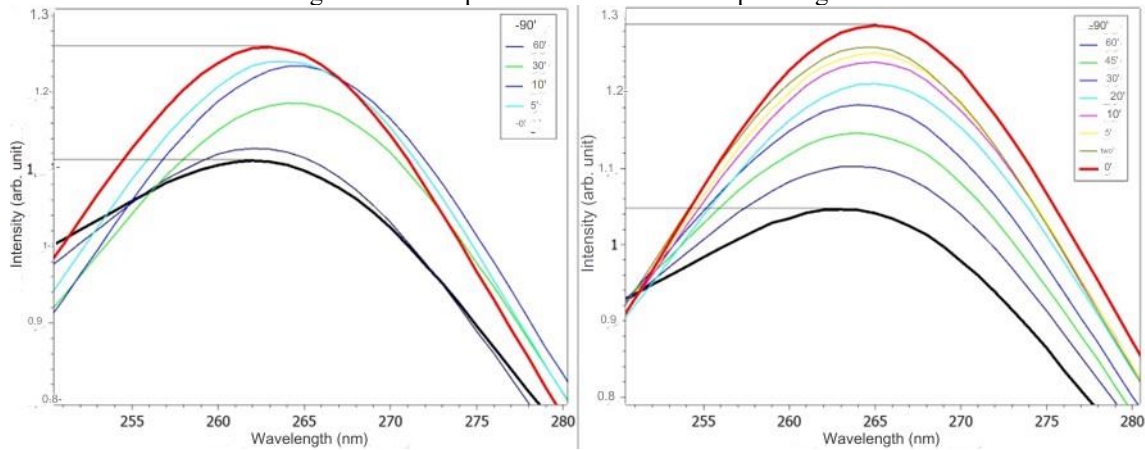
Figure 13 - Reaction comparison for Pure-CuO Solution



Source: Prepared by the author



Figure 14 - Comparison of reaction in the peak region.

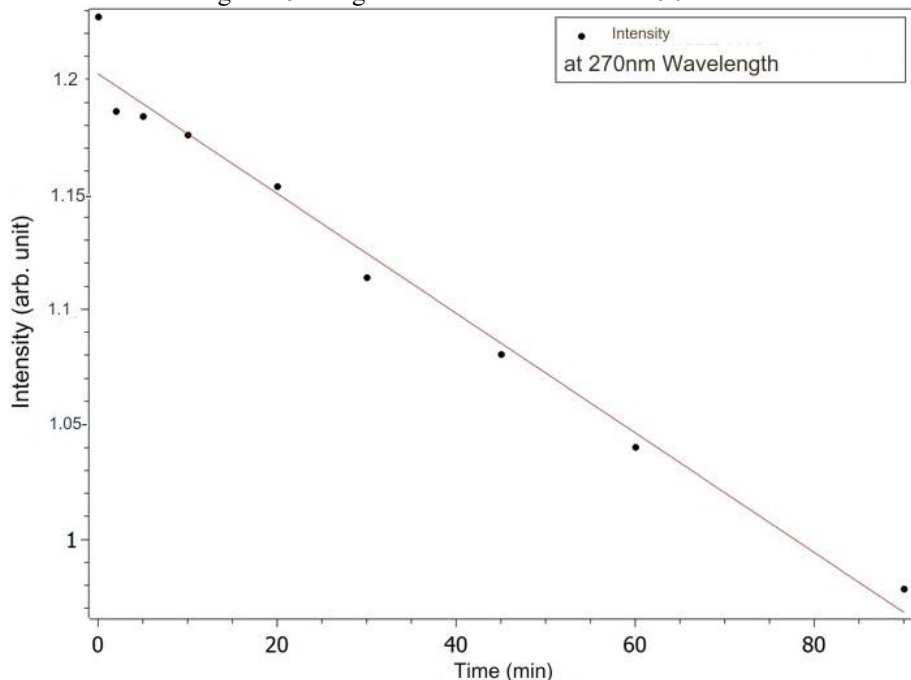


Source: Prepared by the author

It is possible to observe that during the 90 min of exposure to UV radiation, the antibiotic solution containing diluted CuO nanoparticles degraded approximately 20.3% of the compound, 1.68 times higher than the solution in the absence of nanoparticles. In this process, it was found that the use of CuO nanoparticles accelerated the degradation process by 68%.

It is noteworthy that the peak degradation at 265 nm in the reference solution shifted to shorter wavelengths. This phenomenon can be associated with the breakdown of the antibiotic structure and the formation of new smaller structures with shorter conjugation length and larger gaps. This same phenomenon occurs at the point of the valley of the curves, at 242 nm, where the intensity increases over time, with the emergence of smaller molecular structures. The degradation can be best visualized in Figure 15 of the 270 nm peak intensity with respect to exposure time.

Figure 15 - Degradation of ST + CuO over 90 min.

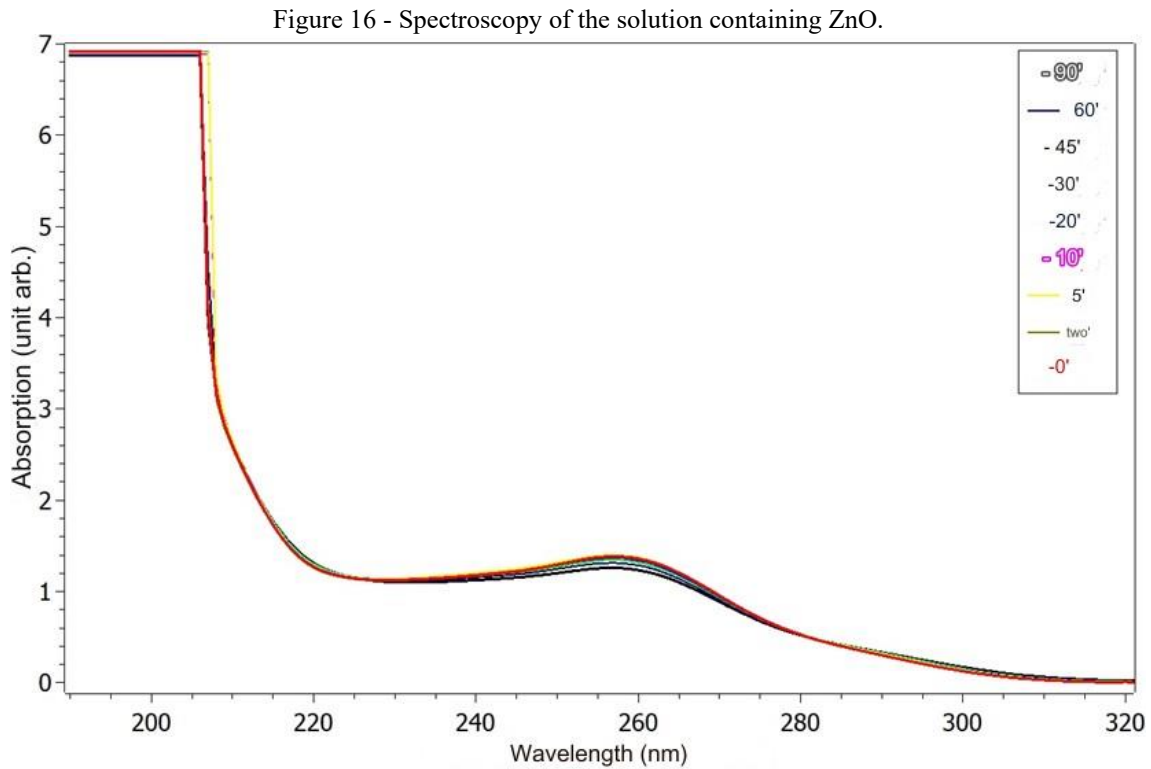


Source: Prepared by the author



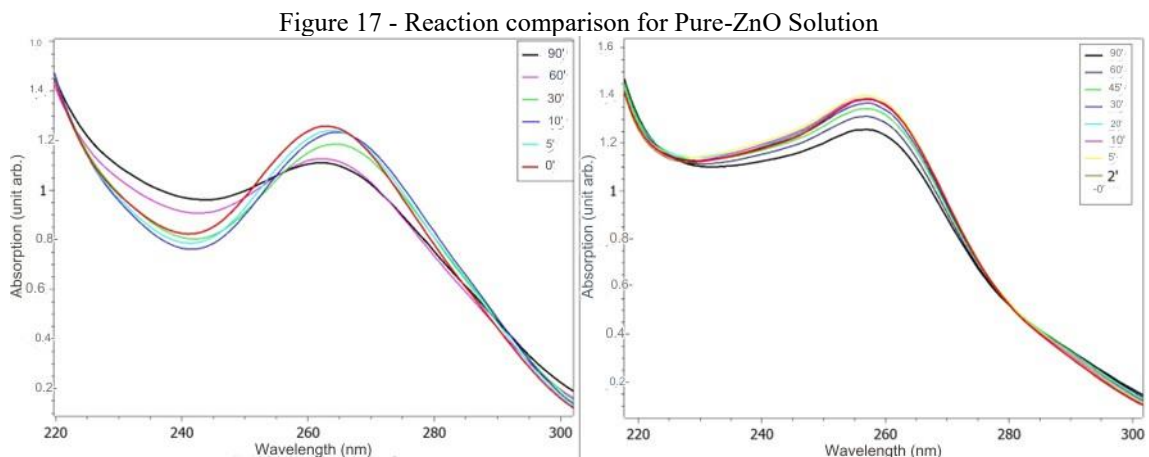
6.4 PHOTODEGRADATION TEST IN THE PRESENCE OF ZnO NANOPARTICLES

Similarly to the CuO assay, ZnO nanoparticles were incorporated to obtain a solution with a concentration of 0.1 gL⁻¹ of nanoparticles in relation to the antibiotic solution. The absorption spectrum of the ZnO nanoparticle degradation assay is shown in Figure 16.



Source: Prepared by the author

By comparing the data presented in Figures 11 and 16, it is possible to observe that the antibiotic solution containing the ZnO nanoparticles demonstrates a lower degradation efficiency when compared to the pure solution. For better visualization, Figure 17 presents a comparative and magnified image of the region around 260 nm.



Source: Prepared by the author



It is observed that during the 90 min of exposure to UV light, the solution containing diluted ZnO presents degradation of 9.2%, compared to the degradation of 12.0% of the solution without nanoparticle, i.e., this result implies a decrease in the degradation rate by about 0.77 times. This result suggests that the nanoparticle absorbed part of the energy of the UV radiation, protecting the antibiotic, a process similar to that observed in modern sunscreens that use ZnO nanoparticles to enhance the protective effect against the sun's ultraviolet rays.

According to the Food & Drug Administration (FDA), zinc oxide is listed as a nutrient considered safe for use and trade as a component of sunscreens thanks to its ability to absorb UV radiation from the sun ^[15]. This ability may be responsible for slowing down the rate of ST degradation, creating a kind of shielding of the antibiotic.

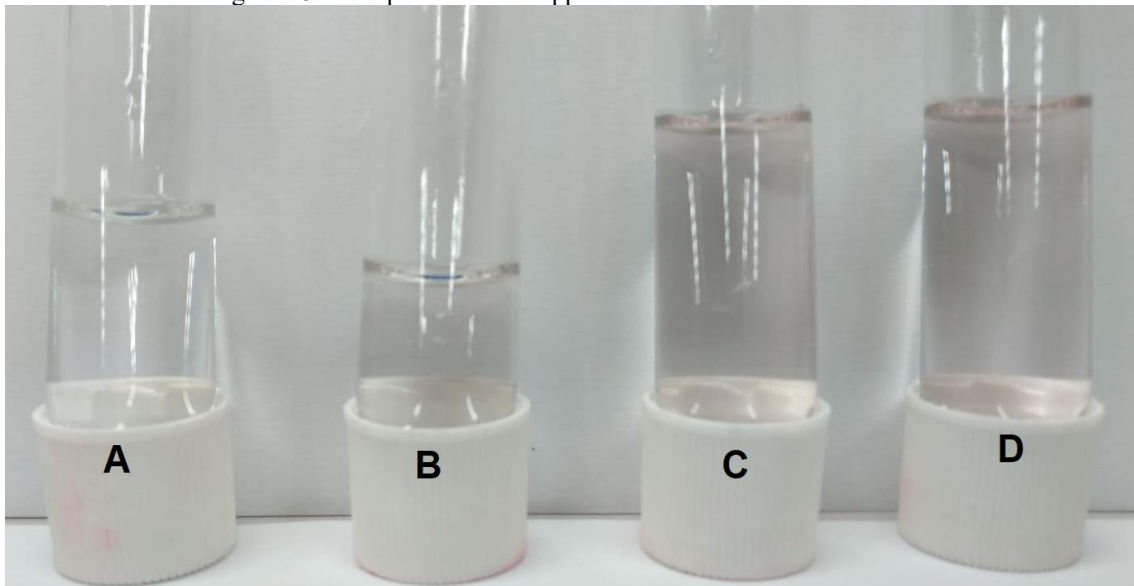
One explanation for the process could be linked to the non-transfer of energy between the nanoparticle and the drug. Previous work has shown that a possible mechanism for the degradation would be the transfer of energy between the nanoparticle and the molecule to be degraded from an "antenna effect", a process that comes from the resonance between the HOMO and LUMO states of the materials. Such charge transfer would be responsible for destabilizing the molecule to be degraded and favoring its breakdown. On the contrary, if there is no resonance between the HOMO and LUMO states of the participants, there would be a drastic limitation in the transfer of charge and, as the shock section of the nanoparticles is much larger than that of the material to be degraded, much of the radiation would end up being absorbed by the nanoparticle, causing the shielding effect, as observed between the ZnO nanoparticle and the ST antibiotic.

6.5 VISUAL CHANGE OF THE SOLUTION

A visual phenomenon occurred in the antibiotic solution with absence of nanoparticles during the photodegradation process. In general, it is noted that the solution, initially transparent, has a slightly reddish color after the irradiation process, as shown in Figure 18. Such a phenomenon occurred for the pure solution and for the solution with the presence of CuO nanoparticles. It is observed that test tube A presents the solution just before being exposed to UV light (minute 0), being totally transparent, while the next tubes (in order: 30, 60 and 90 minutes) showed a subtle reddish coloration.



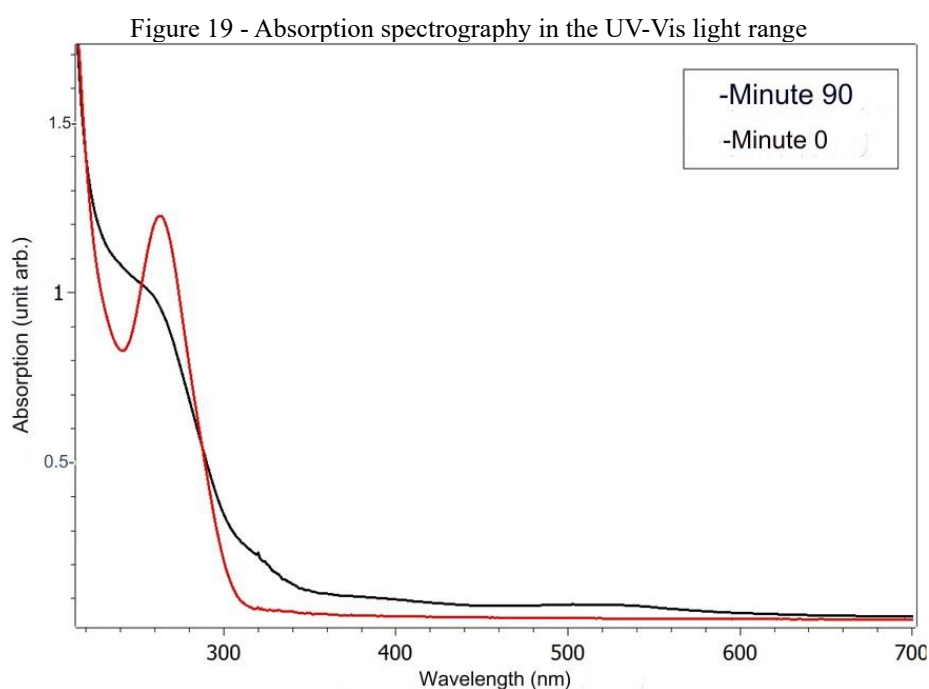
Figure 18 - Comparison of the appearance of the solution over time.



Source: Prepared by the author

This phenomenon suggests that the breakdown of the antibiotic in structures that absorb in the region of 600-650 nm (wavelength range of red light) would be occurring. In view of this observation, UV-Vis absorption spectroscopy was performed again within the wavelength of 190 to 700 nm (spectrum containing the visible region of 400 - 700 nm). It is observed that the absorption spectrum of the drug showed a slight increase in absorption in the visible light region, as shown in Figure 19.

This result clearly illustrates the evolution of the ST antibiotic absorption peaks, indicating a significant decrease in the 260 nm region, as well as a slight increase in the visible region (400 to 700 nm).



Source: Prepared by the author



7 CONCLUSIONS

It is concluded that it was possible to use simple and low-cost techniques to synthesize materials in a single phase, with good crystallinity at a nanometric scale, as performed with CuO and ZnO from the sol-gel protein method.

The results show that the use of CuO accelerates the degradation rate of Sulfamethoxazole + Trimethoprim by 1.68 times, contributing to a more efficient decomposition of its structure. On the other hand, the application of the ZnO nanoparticle seems to cause a shielding effect on the degradation of the antibiotic with UV radiation.

Although there is no complete degradation of the antibiotic during the proposed time, this process has low complexity and low laboratory cost, and can be used in numerous industrial processes for the disposal of this and other drugs or similar structures.



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