




Development of Biodegradable Polymeric Films Added with CuO-NPS and Evaluation of Their Antifungal Activity

Kassara Évelyn Costa^a, Luciano Luiz Silva^b , Maria Ana Pignatel Marcon Martins^c,
Janaina dos Santos^d, Micheli Zanetti^b, Franciele Dalcanton^b, Alexandra Monego Bugiereck^b,
Gustavo Lopes Colpani^{a,b} , Carolina Riviera Duarte Maluche Baretta^a, Márcio Antônio Fiori^{a,b,e},
Josiane Maria Muneron de Mello^{a,b,*} 

^aUniversidade Comunitária da Região de Chapecó, Pós-graduação em Ciências Ambientais, Chapecó, SC, Brasil.

^bUniversidade Comunitária da Região de Chapecó, Pós-graduação em Tecnologia e Gestão da Inovação, Chapecó, SC, Brasil.

^cUniversidade do Sul de Santa Catarina, Departamento de Química, Tubarão, SC, Brasil.

^dUniversidade Comunitária da Região de Chapecó, Escola Politécnica, Chapecó, SC, Brasil.

^eUniversidade Tecnológica Federal do Paraná, Laboratório de Física, Pato Branco, PR, Brasil.

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This research was aimed to obtain biodegradable polymeric films with antifungal action against *Aspergillus niger*, through the addition of copper oxide nanoparticles (CuO-NPs) in the commercial polymer (Ecoflex®) and to evaluate its mechanical, morphological, biodegradation, and copper release properties. The microbiological results showed that the films with 4 wt% CuO-NPs inhibited 97.57% the growth of the fungus growth of 1×10^5 spores·mL⁻¹ compared to the control. The morphological characterization revealed that the CuO-NPs presented irregular form with particles size of 50 to 400 nm and the XRD showed characteristic peaks of copper oxide. The addition of CuO-NPs to Ecoflex® provided increased maximum and rupture tension, strain, and elasticity modulus the obtained films with 1 wt% CuO-NPs and did not alter the biodegradability of the films. It can be said that, through the addition of CuO-NPs to Ecoflex®, it was possible to add antifungal activity for *Aspergillus niger* without compromising its biodegradability, meeting the safety standards established by legislation regarding the release of copper.

Keywords: CuO-NPs, Ecoflex®, antimicrobial activity, antimicrobial packaging.

1. Introduction

Polymers of fossil origin are the most used in the industry due to characteristics such as non-toxicity, lightness, low cost, durability, the versatility of application due to the distinct physicochemical characteristics, and easy processing^{1,2}. Although such characteristics are of great industrial interest, the residues generated by these polymers, originating from non-renewable sources such as petroleum, arouse concern among researchers regarding their degradation^{3,4}. As they are highly resistant to microbial attack, when deposited in the environment, they take many decades to decompose, accumulating and triggering serious environmental problems^{2,5-7}.

To deal with this problem, researchers and industries have been looking for alternatives to minimize the environmental impacts caused by the improper disposal of polymeric materials. Among the alternatives are the reuse and recycling of these materials, practices that have been increasing over the time, but still not enough to alleviate the environmental problem. Therefore, biodegradable polymers emerge as an alternative due to, mainly to their biodegradation characteristic combined

with technical feasibility, presenting great potential for expansion^{8,9}. These polymeric materials are degraded mainly by the action of naturally occurring microorganisms, such as bacteria, fungi, and algae, in the absence or presence of oxygen, and derive from this process, mainly, carbon dioxide (CO₂), methane (CH₄) and microbial cell components^{10,11}.

Aiming to know the needs of the market, companies already commercialize biodegradable polymers, such as Ecoflex®, produced by the German company BASF, and Luminy®, from the company Corbion, in which the Poly (lactic acid) (PLA) nowadays represents about 10% of the bioplastics market^{3,4}. PLA is one of the more outstanding biodegradable and green polymers, for being commercially available in large-scale production and a wide range of grades, enabling polymer all usual thermoplastic manufacturing processes⁵. In addition, the high resistance to humidity, fat and temperature changes gives this polymer satisfactory quality for applications in food packaging¹². The packaging industry is an important sector for the world economy, and the food segment is the one that most demands innovative technologies aimed at increasing the shelf life of packaged

*e-mail: josimello@unochapeco.edu.br

foods, and the focus of innovations is on active packaging, which can change the conditions of products and packaging media by inserting additives into the polymeric material^{13,14}.

Different additives can be incorporated into biodegradable polymers, which in addition to maintaining their main biodegradation characteristic, also allows adding functionality to it, such as blocking ultraviolet (UV) radiation, gas barrier, and antimicrobial activity¹²⁻¹⁷. The antimicrobial property in polymers is of great industrial interest, mainly in the food sector to produce antimicrobial packaging, as this help to promote food safety and make it possible to increase the shelf life of foods, by inhibiting spores and pathogenic microorganisms that contaminate them^{15,18,19}.

According to the Food and Agriculture Organization of the United Nations (FAO), 25% of food losses occur during logistics and storage²⁰. This fact may be associated with contamination by microorganisms, such as fungi, some of which produce mycotoxins, which are highly toxic secondary metabolites, harmful to the general population²¹⁻²³. Among the fungi most commonly causing food spoilage, *Aspergillus niger* stands out, a filamentous fungus widely found in nature, which causes mold in food²⁴⁻²⁸, and is widely found in tropical and subtropical foods²⁹.

The antimicrobial property can be obtained by incorporating antimicrobial compounds directly into the raw material of polymer packaging²⁵. The mechanism consists of the interaction between the food, the free spaces, and the packaging, making the presence of these compounds in the packages a fundamental characteristic to control the microbial growth of the packaged food^{30,31}. Among the compounds with antimicrobial properties, metallic oxides, mainly zinc oxide (ZnO) and copper oxide (CuO), have been the focus of research because they have proven antimicrobial activity against many bacteria and fungi that cause infections and also to be safe for both humans, animals and the environment at low concentrations^{17,32-35}. Copper (Cu) is widely distributed in nature and can be found in various mineral salts and organic compounds³⁶. It can be considered a good element for the development of antimicrobial materials, as it has a broad spectrum of proven antimicrobial activity against many bacteria that cause infections, as well as different types of viruses and fungi³⁷⁻⁴¹. The effectiveness of this metal has boosted studies in different applications ranging from water purification⁴², to the development of new antimicrobial materials, such as food packaging, paints, coatings, and cosmetics^{13,40,43-46}. In the form of nanoparticles, it has advantages due to the volume/area ratio, ensuring the maintenance of antimicrobial activity even in the presence of a small amount⁴⁷⁻⁴⁹.

Some works have already been developed aiming to incorporate antimicrobial additives in conventional polymeric materials⁵⁰⁻⁵⁵ and biodegradable polymers^{13,17,56-58}. Among them, the work of Capezello et al.¹³ evaluated the action of zinc oxide nanoparticles (ZnO-NPs) added to Ecoflex® film against *Staphylococcus aureus* and *Escherichia coli*, reaching the point of inhibition/death after 24 h of contact with the film, at a concentration of 1×10^4 UFC·mL⁻¹. Ejaz et al.⁵⁸ developed bovine skin gelatin (BSG) composite films incorporated with 2% zinc oxide nanorods (ZnO NRs) and clove essential oil (CEO) Composite films loaded with 50% CEO, especially in

combination with ZnO NRs, showed maximum antibacterial activity against *Listeria monocytogenes* and *Salmonella Typhimurium* inoculated in shrimp during refrigerated storage. Ghorbani et al.⁵⁰ studied the antifungal potential of polyurethane films added with copper oxide particles, obtaining an inhibition zone for the *Penicillium* fungus of up to 8 mm. Polymeric films with antifungal properties are also reported in the work of Grafia et al.⁵¹ who proved the effectiveness of polyethylene films added with natamycin against *Aspergillus niger*, obtaining an inhibition zone of 3 cm.

There are few research works with polymeric materials associated with metal nanoparticles to inhibit the growth of fungi, mainly those that affect food. Faced with the environmental problems caused by the accumulation of plastic materials, and to meet the requirements of the food industry for antimicrobial packaging, this work aimed to obtain biodegradable polymeric films with antifungal properties against the fungus *Aspergillus niger*, apt to their potential application in farinaceous products, cereals (wheat, barley, corn), fruits and oilseeds, using the commercial biodegradable polymer Ecoflex® and different CuO-NPs concentrations. To characterize the obtained films, microbiological, mechanical, biodegradability, and copper release tests were performed.

2. Materials and Methods

2.1. Preparation and characterization of copper oxide nanoparticles

Copper oxide nanoparticles (CuO-NPs) were synthesized by the coprecipitation method⁵⁹. As a percussive agent, 200 mL of copper sulfate pentahydrate (CuSO₄·5H₂O) (Synth, PA standard) 0,2 M were used, heated to 50 °C in a water bath. After reaching the temperature, 200 mL of sodium hydroxide (NaOH) 1 M (Merk, PA standard) solution was added, keeping under mechanical stirring in a propeller stirrer (Fisatom, 710). The pH of the solution was approximately 14. The obtained material was filtered using a vacuum pump (Fisatom, 824) and Buchner funnel, and subsequently dried in an oven (Quimis, Q317M) at 80 °C for 5 h. Afterward, the obtained material was macerated in a mortar until it was in the form of powder.

The obtained CuO-NPs were characterized by Scanning Electron Microscopy (SEM) in an electron microscope with a FEG field emission source (Philips XL-30 FEG), and by X-Ray Diffraction (XRD), in a diffractometer with theta-theta goniometer with Ka radiation in a copper tube under conditions of 40 kV and 40 mA. X-ray spectra were obtained in the 2θ range from 1.5° to 90.0°, with a scanning speed of 5° min⁻¹.

The antifungal activity of CuO-NPs was performed through diffusion assays in a solid medium and the Minimum Inhibitory Concentration (MIC) was determined. The inoculum was previously prepared, following the manufacturer's instructions, using the strain ATCC 16888 of *Aspergillus niger*, initially in powder form, and hydrated in *Brain Heart Infusion* (BHI) (Merk) and incubated at 28 °C in a bacteriological oven (J Prolab, B3). After 24 h, the strain, which showed growth, was transferred to *Sabouraud* culture

medium with chloramphenicol (Merk) and incubated again in an oven at 28 °C for 48 h. After growth, the inoculum concentration was adjusted with sterile saline water (0.9% NaCl) (Synth, PA standard) using a *Neubauer* chamber until 1×10^5 spores·mL⁻¹ were obtained. The diffusion test in a solid medium was carried out according to the methodology proposed by Perez et al.⁶⁰ with adaptations. A 20 µL aliquot of the inoculum at the concentration of 1×10^5 spores·mL⁻¹ of the fungus was added to *Sabouraud* agar (Himedia), homogenized, and placed in a Petri dish. After solidification of the medium, three holes were made in each Petri dish, 5 mm in diameter, where the CuO-NPs were deposited. The plates were incubated in a bacteriological oven at 28 °C for 14 days. In addition to the CuO-NPs, the Ecoflex® pellets were also analyzed, which were deposited in the holes. Assays were performed in triplicate.

The MIC values of the CuO-NPs were determined by diluting them in agar, using the methodology described by the *Clinical and Laboratory Standards Institute*⁶¹ with modifications. The CuO-NPs were weighed on an analytical balance (OHAUS), in proportions corresponding to concentrations from 0.5 wt% to 3 wt% (with 0.5 wt% intervals) about the *Sabouraud* agar medium, and then were homogenized with 20 mL of *Sabouraud* agar and placed in *Petri* dishes for solidification. After solidification of the medium, 20 µL of the spore suspension of concentration 1×10^5 spores·mL⁻¹, sowing on *Drigalski* loop plates. The plates were incubated at 28 °C for 14 days, and the reading was performed by observing the growth of the fungus, in which the MIC was qualitatively defined as the concentration that did not show growth of the fungus on the plate. Assays were performed in triplicate.

2.2. Obtaining Ecoflex® films added with CuO-NPs

The polymeric films added with CuO-NPs were obtained directly by extrusion process using a single screw industrial extruder (Oryzon) containing seven heating zones with screen speed of 150 rpm, with a blow ratio on 2:1 and always keeping the films with equal thicknesses. First, a masterbatch was produced, consisting of Ecoflex® (molar mass of 14.2×10^4 g·mol⁻¹ and melting point of 110-120 °C) with 6 wt% CuO-NPs. The CuO-NPs were manually mixed with the Ecoflex® pellets in a beaker, and then this mixture was processed in the extruder at a temperature of 120 °C and a screw rotation speed of 10 rpm. Afterward, the obtained *masterbatch* was mixed with Ecoflex® to obtain polymeric films with additives, in the proportions of 1 wt%, 1.5 wt%, 2 wt%, and 4 wt% CuO-NPs about polymer mass. The mixtures were processed in extruder at temperatures of 120, 120, 125, 130, 140, and 145 °C, respectively in each heating zone, and a rotation screw rotation speed of 90 rpm. In addition to the polymeric films added with CuO-NPs, a sample of the polymeric film without the additive (pure Ecoflex®) was also produced, designated as a control film.

2.3. Characterization of polymeric obtained films

2.3.1. Evaluation of the antifungal activity of polymeric films

The antifungal action of the control film and the added obtained films against the fungus *Aspergillus niger* was

evaluated according to the methodology described by the Japanese standard JIS Z 2801-10⁶² with adaptations. Samples of 2 cm diameter films were placed in a *Petri* dish. In the center of each film, 100 µL of *Aspergillus niger* was previously prepared with *Sabouraud* agar, at the concentration of 1×10^5 spores·mL⁻¹. Around each sample, 1 mL of 0.9% saline water was added, to maintain the proper humidity for the growth of the fungus. The plates were incubated in an oven at 28±2 °C for 7 days. After this period, the samples were removed from the plates and placed in test tubes containing 10 mL of 0.9% saline water and then homogenized for approximately 1 min in a vortex shaker (Quimis, Q220). Then 100 µL of this solution was transferred to a *Petri* dish and later inoculated by depth (Pour Plate) in *Sabouraud* agar. The plates were incubated in an oven at 28±2 °C for 48 h. After the incubation time had elapsed, the colonies formed and the respective areas of the hyphae were counted using a colony counter. Assays were performed in triplicate.

2.3.2. Evaluation of the physical properties of polymeric films

To evaluate the effect of the addition of CuO-NPs on the mechanical properties of Ecoflex®, the polymeric obtained films with and without additives were submitted to the tensile test according to ASTM D-882⁶³ using the universal mechanical test equipment (EMIC DL2000), with a 20 kN load cell. The tests were carried out with 6 replicates for each sample, and the parameters of modulus of elasticity, yield stress, rupture tension, and deformation presented refer to the arithmetic average of the tests.

The polymeric obtained films were also submitted to the differential scanning calorimetry (DSC) test (Perkin Elmer, 4000) using a nitrogen atmosphere with a gas flow of 19.8 mL·min⁻¹ and heating rate of 10 °C·min⁻¹. To ensure that the entire thermal history arising from the processing of the material was destroyed, only the second heating was considered.

2.3.3. Evaluation of copper release in aqueous media from polymeric films

To verify the release of copper present in polymeric films in an aqueous solution, the metal release test was performed in ultrapure water, following ANVISA's *Collegiate Board Resolution – RDC n° 51*, of November 26, 2010⁶⁴. The samples were immersed in 300 mL of ultrapure water and kept at a temperature of 40 °C for 10 days. Afterward, 100 mL aliquots were collected and acidified with 0.1 M HCl (up to pH 5.5) for copper solubilization. The assay was performed in quadruplicate. The copper concentration of the water samples was quantified using a flame atomic absorption spectrometer (ContrAA 700).

2.3.4. Biodegradation test of obtained polymeric films

The biodegradation test of polymeric films added with 1 wt%, 2 wt% and 4 wt% of CuO-NPs and without the additive (control), in the dimensions of 2x2 cm and thickness of the 0.1 mm, was carried out in compost soil according to ASTM D 5988-18⁶⁵ with adaptations¹³, based on the respirometry test. The compost soil used was provided by

the Unochapecó Forestry Nursery – Chapecó/SC, and had an average granulometry of 2 mm, 23.7% moisture, 7.1% organic matter, 4.13% organic carbon, 70% of water retention capacity and the C/N ratio of 4. The characterization was performed by Epagri-Brazil-SC's Soil Laboratory.

The experiment took place inside glass flasks with a capacity of 3 L, in which each film sample was buried completely in 200 g of compost soil. In each flask, a snap cap glass containing 10 mL of a 0.5 M NaOH solution (carbon dioxide capture solution, CO₂) and another snap cap containing 30 mL of distilled water were added. The flasks were sealed with PVC film (polyvinyl chloride) and incubated in a Biochemical Oxygen Demand (BDO) incubator (NI1705) at 28±2 °C in the absence of light, for 140 days. Glass flasks containing only the capture solution and distilled water were set up as control of CO₂ in the air contained in the flask, and glass flasks containing only the soil were also set up as a blank. Tests were performed in triplicate.

Every 72 h, the glass snap cap bottle containing the NaOH solution was removed from the 3 L bottle for the addition of 1 mL of a 0.5 M barium chloride solution (BaCl₂ - Dynamic) in order to stop the CO₂ capture reaction¹³. After that, the barium carbonate formed was decanted for 30 min and the NaOH that did not react with CO₂ was titrated with a 0.5 M HCl solution, using 1% phenolphthalein as an indicator. The NaOH solution was replaced in the snap cap glasses, and these were again inserted into the 3 L flasks containing the polymeric film sample, closed with PVC film, and taken back to the BOD incubator. Through the stoichiometric relation, the CO₂ produced in the biodegradation process of each sample was quantified, through Equation 1.

$$m_{CO_2} = (V_{HClb} - V_{HCl a}) * N_{HCl} * \left(\frac{M_{CO_2}}{2} \right) * 1000 \quad (1)$$

Where: m_{CO₂}: mass of CO₂ produced in the biodegradation process (mg); N_{HCl}: normality HCl (0.5 M); V_{HClb}: volume of HCl used to titrate the blank (L); V_{HCl a}: volume of HCl used to titrate the sample (L); M_{CO₂}: molecular mass of CO₂ (g·mol⁻¹).

To ensure that soil moisture remained above 60% throughout the experiment, after 30 days 2 mL of distilled water was added to the soil every 6 days, and this procedure was carried out until the end of the experiment.

To evaluate and compare the morphology of the films before and after the biodegradation test, an electron microscopy analysis was performed using a scanning electron microscope with a FEG field emission source (Philips XL-30 FEG) at the LCE - Characterization Laboratory Structural from the Department of Materials Engineering at UFSCar - Federal University of São Carlos – Brazil-SP.

The results of the analysis of the death/growth curve, mechanical analysis, copper release, and biodegradability were submitted to statistical analysis and evaluated by the Tukey test to verify the existence of significant differences between the conditions studied at 95% confidence (p < 0.05). The statistical treatment of the results was carried out using the Statistica® 7.0 software (StatSoft®, USA).

3. Results and Discussions

3.1. Morphological and chemical characterization of CuO-NPs

The Figure 1 shows the image obtained with scanning electron microscopy of CuO-NPs at 80,000 times magnification. It is possible to observe that the formed CuO-NPs have an agglomerated structure, with sizes ranging from 50 nm to 400 nm and irregular shapes. Similar results were also obtained by Soni et al.⁴⁰ and Lasfargues et al.⁶⁶, in which the CuO-NPs obtained also presented in the form of agglomerates and with sizes from 20 nm to 40 nm. Nanoparticles tend to agglomerate during their synthesis due to the high binding tendency of copper nuclei, indicating that the surface energy of nanoparticles is relatively strong, and due to their high surface area^{67,68}. The results obtained in this work are like those obtained by Usman et al.⁶⁷, in which they synthesized copper nanoparticles with CuSO₄ precursor, obtaining nanoparticles with sizes between 50 - 300 nm.

Through Figure 2, which presents the X-ray diffractogram for the obtained CuO- NPs, it can be verified that the synthesized

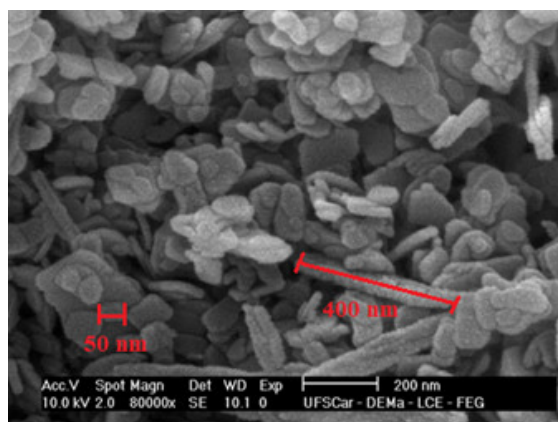


Figure 1. SEM images obtained for CuO-NPs with 80,000 times magnification.

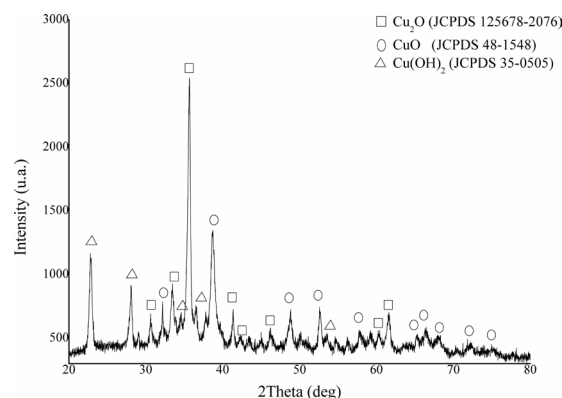


Figure 2. X-ray diffractogram obtained for the CuO-NPs.

nanoparticles have characteristic peaks of CuO according to the JCPDS standard (05-661) and (48-1548), with principal diffraction peaks at angles $2\theta = 32.18^\circ, 38.7^\circ, 48.9^\circ, 52.7^\circ, 57.8^\circ, 66.5^\circ, 66.6^\circ, 68.2^\circ, 72.4^\circ$ and 74.9° corresponding to the crystal planes (110), (111), (-202), (112), (202), (022), (311), (220), (311) and (004), respectively⁶⁹⁻⁷³. The presence of Cu_2O is also evidenced by the principal peaks in $2\theta = 30.8^\circ, 35.5^\circ, 42.4^\circ$ and 61.6° , corresponding to planes (110), (111), (200), (220)^{70,74-76}. The presence of the $\text{Cu}(\text{OH})_2$ from incomplete chemical reaction is evidenced by the presence of principal peaks in 2θ equal to $22.8^\circ, 34.7^\circ, 36.6^\circ$ and 53.5° , which correspond to the crystal planes (021), (002), (111) and (150)^{77,78}. Narrow and intense peaks obtained on XRD suggest that the material has a high degree of crystallinity and purity^{79,80}.

3.2. Antimicrobial characterization of CuO-NPs and Ecoflex® pellets

Figure 3a presents the results of the diffusion assay in the solid medium of CuO-NPs for 1×10^5 spores·mL⁻¹ of the fungus *Aspergillus niger*; Figure 3b result for the control sample, that is, only the pure inoculum (without adding any material) to verify the viability of the inoculum, and Figure 3c the result of the diffusion test for the Ecoflex® pellets.

Figure 3a shows that the obtained CuO-NPs showed a mean inhibition halo of 9.00 ± 0.10 mm, while for pure Ecoflex® polymer pellets, Figure 3c, there was no formation of inhibition halo, as expected the polymer does not have antifungal action, as there was growth throughout the Petri dish, including on the pellets. Thus, it can be said that the additive obtained (CuO-NPs) has antifungal action against the fungus *Aspergillus niger*. Similar results were found by Ramyadevy et al.⁸¹, which evaluated the inhibitory effect of copper nanoparticles, against the fungus *Aspergillus niger*, at the concentration of 1×10^4 spores·mL⁻¹, obtaining inhibition halos of approximately 16 mm. The difference in the results obtained in this work when compared to the results obtained by Ramyadevy et al.⁸¹ is possible due to the difference in the initial concentration of spores, which was lower than that used in the present study (1×10^5 spores·mL⁻¹). Shende et al.⁸² obtained a mean inhibition halo of 12.75 mm for copper nanoparticles against *Aspergillus niger*, at a concentration of 1×10^5 spores·mL⁻¹. The divergence may be due to the inhibition halo having been evaluated after 4 days of the experiment, while in the present work it was

evaluated in 14 days. As for Figure 3b, one can observe the viability of the inoculum used, as there was the growth of the fungus in the analyzed period.

According to Mani et al.³⁸, the antifungal activity of CuO-NPs is found to be low when compared to antibacterial activity and this is because of the cell wall present in the fungi; the rigidity of the fungal cell wall is composed of N-acetylglucosamine, which makes it difficult the entry of metal NPs but the repeated dose for a stipulated period would eventually break the cell wall and burst out the pathogenic fungi. Therefore, the antifungal property of CuO-NPs obtained is possibly due to the mechanism of action of CuO-NPs against filamentous fungi may be related to the fact that CuO-NPs enters the fungal cells and releases the copper ions thereby the copper destructs the structure of DNA leading to cell death⁸³ Or even by the structure of fungi, as reported by Sardella et al.⁸⁴, which related the efficiency of zinc oxide nanoparticles against filamentous fungi to the action of this compound on the structure of fungi, altering cellular function and causing changes in fungal morphology, thus increasing the mortality of hyphae due to the production of reactive species of oxygen.

The results obtained for the minimum inhibitory concentration (MIC) of CuO-NPs at concentrations of 0.5 wt%, 1 wt%, 1.5 wt%, 2 wt%, 2.5 wt%, and 3 wt% and the control, at a fungal concentration of 1×10^5 spores·mL⁻¹ are presented in Table 1. The signs “+” and “-” indicate growth and non-growth of the fungus for the referred concentration of the additive in the plate, respectively. It can be verified, through Table 1, that there was no growth of the fungus *Aspergillus niger* on the surface of the agar in the presence of CuO-NPs in the tested concentrations, showing that the minimum concentration for the nanoparticles to present antifungal action was 0.5 wt%. It is also observed that in the absence of CuO-NPs, the control plate, there was the growth of the fungus, as expected.

3.3. Polymeric obtained films

Figure 4 shows an image of the polymeric films in the concentrations of 1.0 wt%, 1.5 wt%, 2.0 wt% and 4.0 wt% CuO-NPs, as well as an image of the polymeric film in the absence of CuO-NPs (pure Ecoflex®).

As can be observed, from Figure 4, there was a small variation in the coloration of the polymeric films obtained as the concentration of the additive increased. Moreover, as

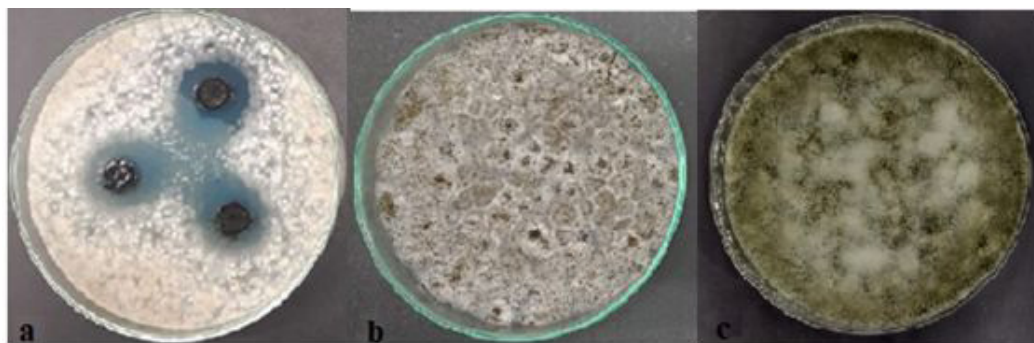


Figure 3. Results of orifice solid diffusion tests for: (a) CuO-NPs; (b) Control; (c) Pure Ecoflex® pellets for *Aspergillus niger*.

Table 1. Result of the MIC assay of CuO-NPs at concentrations of 0.5 wt%; 1 wt%; 1.5 wt%; 2 wt%; 2.5 wt%; 3 wt% and the control, against 1×10^5 spores of *Aspergillus niger* mL⁻¹. (-) there was no growth of the fungus on the plate and (+) there was growth of the fungus on the plate.

Repetition	Percentage (wt%) CuO-NPs						
	Control	0.50	1.00	1.50	2.00	2.50	3.00
1	+	-	-	-	-	-	-
2	+	-	-	-	-	-	-
3	+	-	-	-	-	-	-



Figure 4. Image of the polymeric obtained films with 1 wt%, 1.5 wt%, 2 wt% and 4 wt% CuO-NPs and of the polymeric obtained films in the absence of CuO-NPs (pure Ecoflex®).

expected a large difference when compared to the polymeric film without the additive, since the CuO-NPs present dark coloration due to the presence of copper.

3.4. Antifungal characterization of polymeric films

To prove that the polymeric film of Ecoflex® without the presence of the CuO-NPs does not present antifungal activity and that the agent capable of promoting the inhibition of the fungus is the CuO-NPs, the diffusion test in the solid medium was carried out for the pure Ecoflex® film, and the result is shown in Figure 5a. Figures 5b to the Figures 5e also show the results of the diffusion test in the solid medium of the polymeric films with 1 wt%, 1.5 wt%, 2 wt%, and 4 wt% CuO-NPs, respectively. It is observed that the polymeric film of pure Ecoflex® (control film) did not show antifungal activity against the *Aspergillus niger* fungus, since there was no formation of an inhibition halo, and the fungus grew on the polymer sample placed in the center of the plate. This behavior was already expected because the Ecoflex® polymer does not have antimicrobial additives in its composition. By observing Figures 5b to 5e, one can perceive that there was no formation of the inhibition halo for the added polymeric obtained films. This behavior is possibly because there was no diffusion and/or migration of CuO-NPs from the surface of the films to the external environment. Supposedly this occurred due to the nanoparticles being trapped in the polymer chains, preventing their diffusion¹³. However, it was verified that the hyphae of the fungus *Aspergillus niger* did not develop under and over the polymeric films, which

indicates a possible inhibition of the fungus when this is in direct contact with a surface of film.

Therefore, to confirm the effective antifungal action of the polymeric films at concentrations of 1 wt%, 1.5 wt%, 2 wt%, and 4 wt% of CuO-NPs and in the absence of the CuO-NPs, called control film (Ecoflex®), were evaluated through the microorganism growth/death curve, JIS Z 2801-2010⁶² methodology, and the results shown in Figure 6. And Table 2 shows the values of the number of colonies of fungi formed, the area of colonies of the fungus *Aspergillus niger* formed, as well as the percentage of inhibition for the different polymeric obtained films.

From the results presented in Figure 6 and Table 2, it is possible to confirm that the films added with CuO-NPs have the property of inhibiting the growth of the fungus *Aspergillus niger*, reaching a percentage reduction in relation to the control (pure Ecoflex®) of 97.57% for the obtained film with 4 wt% CuO-NPs. When comparing the films with additives to each other, there is a significant difference between the concentrations of 1 wt% and 1.5 wt% about concentrations of 2 wt% and 4 wt% in the average number of colonies, but this difference is not significant when compared to the area of the colonies. It is also observed that the percentage of reduction was greater the higher the concentration of CuO-NPs in the polymeric film. The fungicidal action of copper oxide nanoparticles against *Aspergillus niger* was also reported by Safaei et al.⁸⁵, in which the compound was present in an alginate polymer matrix, obtaining an inhibition percentage of 83.17% of the fungus using a concentration corresponding to 0.4% of copper oxide nanoparticles.

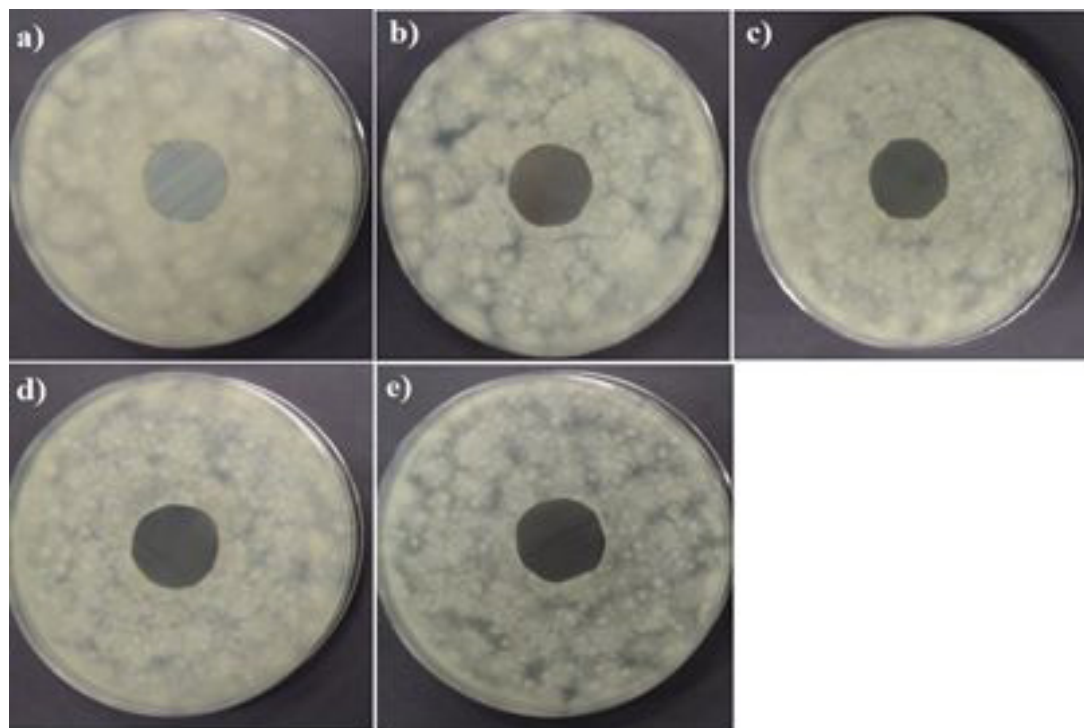


Figure 5. Results of the solid medium diffusion test for polymeric films: (a) without CuO-NPs; (b) with 1 wt% CuO-NPs; (c) with 1.5 wt% CuO-NPs; (d) with 2 wt% CuO-NPs; (e) with 4 wt% CuO-NPs, against 1×10^5 spores·mL⁻¹ of *Aspergillus niger*.

Table 2. Results of the microbiological analysis of the growth curve of *Aspergillus niger* for the polymeric films added with 1 wt%, 1.5 wt%, 2 wt% and 4 wt% CuO-NPs and for the polymeric film without additive, expressed in number of formed colonies, area of formed colonies and percentage of inhibition of the fungus *Aspergillus niger* in the polymeric film compared with the film without CuO-NPs.

Polymer film sample	Average number of colonies	Average colony area (cm ²)	Inhibition Percentage (%)
Pure Ecoflex®	Countless**	59.42±0**	0
Ecoflex® +1.0 wt% CuO-NPs	5.3±0.6 ^{a*}	9.27±6.7 ^b	84.39
Ecoflex® +1.5 wt% CuO-NPs	3.7±1.2 ^{a,b}	5.92±2.7 ^b	90.04
Ecoflex® +2.0 wt% CuO-NPs	2.7±0.6 ^b	2.27±1.2 ^b	96.17
Ecoflex® +4.0 wt% CuO-NPs	1.3±1.2 ^b	1.45±1.9 ^b	97.57

* Equal lowercase letters represent that there are no significant differences in the column ($p < 0.05$) ** Fungus growth across the plate.

Given the exposed data, it appears that the polymeric films added with CuO-NPs, at concentrations of 1 wt% to 4 wt%, are effective in terms of inhibiting the fungus *Aspergillus niger*, considerably decreasing its growth compared to the film without nanoparticles, making the material promising for use in food packaging that are deteriorated by the action of fungi. Some studies present possible mechanisms of action of copper against the fungus *Aspergillus niger*, which may be related to the antifungal activity of polymeric films added with CuO-NPs obtained in this work, among them the work by Tsekova et al.⁸⁶, which suggests that the action of copper on the fungus *Aspergillus niger* is related to the production of acid phosphatases during mycelial growth in the presence of cupric ions, as microorganisms secrete phosphatases that cleave organic phosphorus compounds, which can then be used for its growth, and in the presence of metal ions this process is interrupted⁸⁷. It is also due to defects in the

structure of nucleic acids and proteins, as well as changes in oxidative phosphorylation reactions, which alter the osmotic balance of living cells of microorganisms, may occur due to the capacity of Cu²⁺ to form organic complexes with the functional groups present in microorganisms³⁵.

Other studies suggest that CuO-NPs induce fungal cell death through cell wall destruction or cell wall penetration mechanism, causing an effect on intracellular processes⁸⁸⁻⁹⁰. The cell walls of fungi are composed of polysaccharides such as chitin, glucan, and chitin-glucan, and CuO-NPs can bind to these walls through the adsorption mechanism, damaging the membrane and interrupting the diffusion processes. Furthermore, nanoparticles can create oxidative stress, leading to apoptosis⁸⁸⁻⁹⁰. According to Safaei et al.⁸⁵ nanoparticles smaller than 50 nm can penetrate the nucleus of cells, interrupting processes such as replication and transport, causing apoptosis. These facts may have occurred with

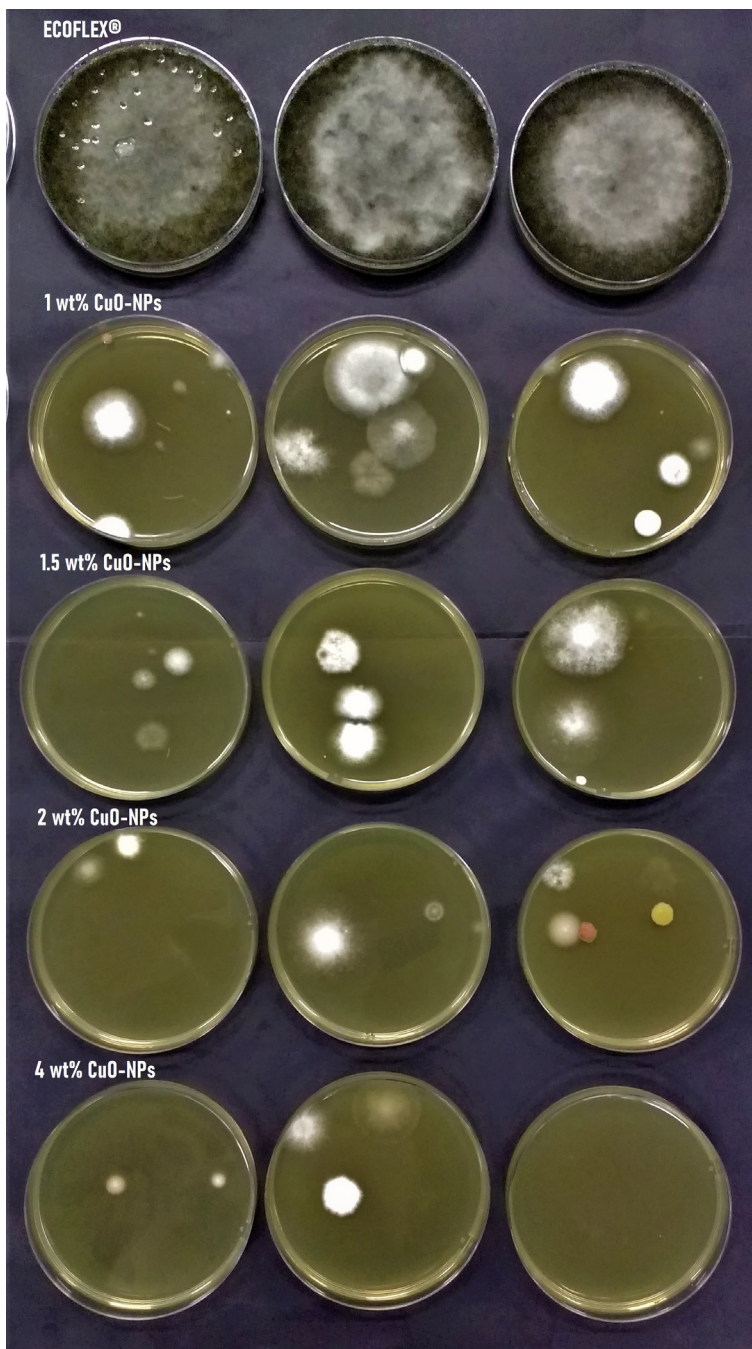


Figure 6. Result of the *Aspergillus niger* fungus growth/death assay for pure Ecoflex® polymeric film and for polymeric films added with 1 wt%, 1.5 wt%, 2 wt% and 4 wt% CuO-NPs.

Aspergillus niger when in contact with the surface of the polymeric film added with CuO-NPs.

3.5. Results of the Differential Scanning Calorimetry (DSC) analysis of the polymeric obtained film

The cooling and heating DSC thermograms obtained for the polymeric films added with 1 wt%; 2 wt% and 4 wt% CuO-

NPs and for the pure Ecoflex® polymeric film are presented in Figure 7. In Figure 7a are shown the DSC thermograms obtained during the cooling process and Figure 7b shows the DSC thermograms obtained during the second heating. Table 3 presents the value of the parameters related to the enthalpy variation (ΔH), crystallization temperature (T_c) in the cooling phase and melting temperature (T_m) in the heating phase. The crystallinity degree of the polymeric

films was determined from the variation of the enthalpy of crystallization values in relation to the value of the enthalpy of crystallization of the Ecoflex® film. Therefore, the values of the degree of crystallinity are relative percentage values (% degree of crystallinity) and represent the percentage variation of the crystallinity of each film in relation to the Ecoflex® film.

It is possible to observe the formation of a single exothermic peak for the pure Ecoflex® material, related to its crystallization, which occurs at an average temperature of 124.02 °C and an enthalpy of -7.7041 J·g⁻¹. The pure Ecoflex® film showed two well-defined endothermic peaks during heating, while for the additive films only one endothermic peak was observed, and this one had a larger area when compared to the pure Ecoflex® film, which became less evident as increases the concentration of CuO-NPs. The polymeric films added with CuO-NPs showed an increase in enthalpy variation, leaving them with more crystalline phases and a greater volume of crystals when compared to the polymeric film of pure Ecoflex®, except in the concentration of 4 wt% of CuO-NPs, a fact evidenced by the greater area value (enthalpy variation) (Figure 7, Table 3). Result like that found by Capelezzo et al.¹³, Valentim et al.⁹¹ also found similar results when adding 1 wt% TiO₂-NPs to Poly (ethylene vinyl acetate) – EVA. The authors found that the degree of crystallinity is reduced due to molecular disorganization and the increase in the amorphous phase of EVA. The

displacement of the melting temperature referring to the first endothermic peak of the pure Ecoflex® film indicates that the higher the concentration of CuO-NPs, the larger the size of the crystals, requiring a higher temperature for the melting of the crystallites. When evaluating the entire formation process, the T_m of the polymers with additives is lower, that is, in a molding process, the temperature for extrusion of the polymeric film with additives will be shorter compared to the time for extrusion of the pure Ecoflex® film. Despite the crystallization temperature of all samples being similar, the degree of crystallinity changed, decreasing with the increase in the concentration of CuO-NPs, making the polymer more crystalline with the addition of 1 wt% and 2 wt% of CuO-NPs, and less crystalline with the addition of 4.0 wt% CuO-NPs. Mohr et al.¹² added NPs-TiO₂ in the biodegradable polymer Ecovio® and obtained a reduction in the degree of crystallinity from 52.96% to 44.72% when the concentration of NPs-TiO₂ increased from 1 wt% to 5 wt%, and the authors suggest that these phenomena are probably due to the TiO₂ nanoparticles could disrupt the regularity of the chain structures in the polymer and increase the spacing between the chains. The increase in crystallinity in the polymer implies a material with greater density, rigidity, dimensional stability, chemical resistance, melting temperature (T_m), glass transition temperature (T_g), and temperature of use, among others⁹².

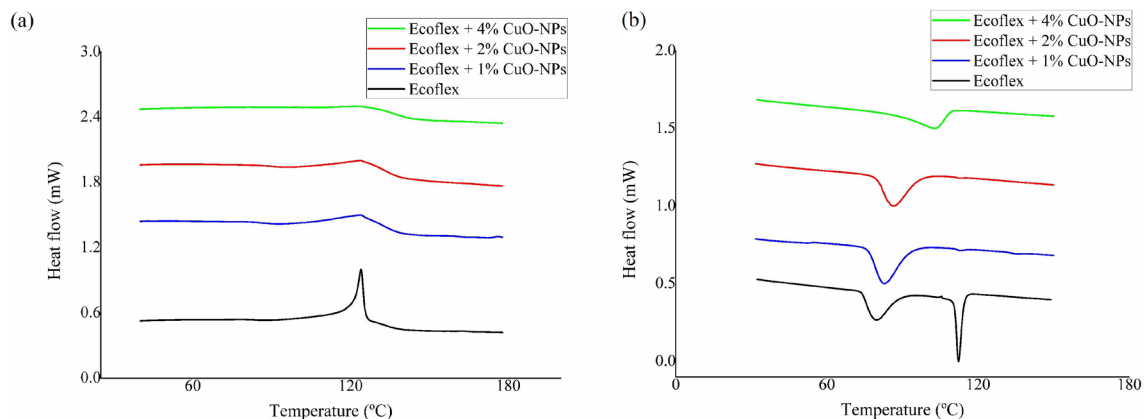


Figure 7. DSC thermograms for polymeric films obtained with 1 wt%; 2 wt% and 4 wt% CuO-NPs and without the additive: (a) cooling; (b) second heating.

Table 3. Variation values of enthalpy of fusion and crystallization (ΔH), crystallization temperature (T_c), melting temperature (T_m) and percentage increase/reduction of relatively degree of crystallinity of films with 1 wt%, 2 wt% and 4 wt% CuO-NPs in relation to Ecoflex® polymeric film.

Polymer film sample	ΔH (J·g ⁻¹) Heating	ΔH (J·g ⁻¹) Cooling	T _m (°C)	T _c (°C)	% degree of crystallinity (Ecoflex® as reference) *
1 wt% CuO-NPs	13.4699	-9.1276	82.83	123.85	18.5
2 wt% CuO-NPs	11.7526	-8.7744	86.36	123.93	13.9
4 wt% CuO-NPs	6.6017	-3.6832	102.85	123.85	-52.2 ³
Ecoflex®	10.0851 ¹	-7.7041	79.49 ¹	124.02	0.0
	7.0499 ²		112.25 ²		0.0

* Used the Ecoflex® as reference. ¹Referring to the first endothermic peak. ²Referring to the second endothermic peak. ³The negative sign indicates the reduction in the degree of crystallinity.

Table 4. Average values and standard deviation of rupture tension, maximum tension, deformation, and modulus of elasticity of polymeric obtained films with 1 wt%, 1.5 wt%, 2 wt% and 4 wt% CuO-NPs and pure Ecoflex® polymeric film.

Polymer film sample	Breaking Stress (MPa)	Rupture tension (MPa)	Strain (%)	Modulus of Elasticity (MPa)
Ecoflex® puro	5.78±0.86 ^{a*}	2.81±0.49 ^{a*}	84.95±32.70 ^{a*}	3.63±1.02 ^{a*}
1 wt% CuO-NPs	11.88±1.95 ^b	5.71±0.52 ^b	161.52±51.46 ^b	3.16±0.21 ^a
1.5 wt% CuO-NPs	12.61±1.08 ^b	6.00±0.20 ^b	176.07±41.20 ^b	3.54±0.69 ^a
2 wt% CuO-NPs	12.68±0.48 ^b	5.55±0.41 ^b	185.24±21.88 ^b	3.03±0.49 ^a
4 wt% CuO-NPs	12.72±0.41 ^b	5.80±0.52 ^b	207.89±11.01 ^b	2.80±0.25 ^a

*Equal lowercase letters represent that there are no significant differences in the column ($p < 0.05$).

3.6. Mechanical characterization of polymeric obtained films

To evaluate the influence of CuO-NPs on the mechanical properties of the polymeric film of pure Ecoflex®, the polymeric obtained films with 1 wt%, 1.5 wt%, 2 wt%, and 4 wt% CuO-NPs were submitted to the mechanical test and the results are presented in Table 4.

It is observed, through Table 4, that the addition of CuO-NPs in Ecoflex® increased the values of the mechanical properties of the polymeric obtained films. From the DSC results it is possible to verify that the CuO-NPs increase the degree of crystallinity when added in low concentrations, which reflects on the mechanical properties of these films⁹². The polymeric films added with CuO-NPs did not differ significantly among themselves, but they differed significantly from the pure Ecoflex® polymeric film about the properties of rupture tension, maximum tension, and deformation. The increase in the rupture tension and maximum tension of the additive films, when compared to the Ecoflex® film, indicate an increase in the material rigidity, making it more resistant and with greater tenacity. Canevarolo⁹² comments that this behavior may be associated with the strong interactions between the copper oxide (CuO) and the polymer used, since the nanoparticles caused an increase in the degree of crystallinity of the additive polymeric obtained films.

According to Zehetmeyer⁹³, these changes in the mechanical properties of polymeric films can be attributed due to the empty space in the polymer matrix that was occupied by the nanoparticles, increasing the strength of the material. Regarding the modulus of elasticity, the pure Ecoflex® film showed no significant difference when compared to the additivated polymeric films, demonstrating that the CuO-NPs do not interfere with this property. Roy et al.⁹⁴, also obtained significant results in changing the mechanical properties of agar films added with copper sulfide nanoparticles, and the tension and flexibility of the films increased after the addition of nanoparticles. According to the authors, the increase in these properties can be attributed to the strong intermolecular interaction between the matrix and the nanoparticles, since the nanoparticles have a high surface area, consequently, a small amount of charge is enough to significantly change the properties of the matrices used.

Other metals in the form of nanoparticles also showed a positive effect on the mechanical properties of polymers, as reported by Díez-Pascual and Díez-Vicente⁹⁵, using NPs-ZnO in PHBV and Yu et al.⁹⁶, using Ag-NPs in cellulose/poly (lactic acid). According to Sarantopoulos et al.⁹⁷, the rupture tension of polymeric films is a key property that

Table 5. Copper concentration in aqueous medium of polymeric obtained films with 1 wt%, 1.5 wt%, 2 wt% and 4 wt% CuO-NPs and of pure Ecoflex® polymeric film.

Polymer film sample	Copper concentration in solution (mg·L ⁻¹)
Pure Ecoflex®	3.80×10 ⁻⁴ ±2×10 ^{-6a*}
Ecoflex® + 1.0 wt% CuO-NPs	1.82×10 ⁻³ ±9.7×10 ^{-5b}
Ecoflex® + 1.5 wt% CuO-NPs	7.0×10 ⁻⁴ ±3.5×10 ^{-5c}
Ecoflex® + 2.0 wt% CuO-NPs	8.6×10 ⁻⁴ ±2.3×10 ^{-5c}
Ecoflex® + 4.0 wt% CuO-NPs	1.08×10 ⁻³ ±5.6×10 ^{-5d}

*Equal lowercase letters represent that there are no significant differences in the column ($p < 0.05$).

directly impacts their mechanical performance during conversion in equipment, packaging in production lines, as well as storage and distribution conditions. In addition, an increase in the rupture tension of these films results in higher productivity, better stackability and transportability, and improved handling conditions for the end consumer.

3.7. Release test of copper present in the polymeric obtained films in an aqueous medium

To verify whether the polymeric films added with nanoparticles release the copper compound and whether the concentration released complies with ANVISA legislation⁶⁴, a release test was carried out in an aqueous medium and the results are presented in Table 5 along with the statistical analysis.

Table 5 shows that a small amount of copper was released into the aqueous medium, but this amount is below the limit established by ANVISA⁶⁴, which is 5 mg·L⁻¹ for copper in plastic packaging and equipment intended to be in contact with food. Therefore, packaging produced with the polymeric obtained films can be an alternative for packaging foods, such as cereals (wheat, barley, corn), fruits, oilseeds, vegetables such as onions and peanuts, since there is no possibility of contamination of the product by copper^{98,99}. Furthermore, it appears that the higher the CuO-NPs concentration in the polymeric obtained film, the greater the amount of copper released in the aqueous solution, except in the sample with 1 wt% CuO-NPs. During the experimental test, the formation of copper precipitates was not observed and there was no change in the color of the solutions containing the polymeric film samples. Capezezzo et al.¹³ evaluated the release of zinc from biodegradable films additivated with Zn-NPs, obtaining maximum release of 0.41 ppm, for concentration

of 1 wt%. Kirschner et al.¹⁰⁰ studied the release of Zn in LDPE sheets, obtaining results compatible with the present study, as there was a release of concentrations below 1 ppm. The release test showed that the amount of copper that diffuses into the aqueous medium was very small, and this characteristic corroborates the microbiological result obtained in the diffusion test in the solid medium of the polymeric obtained films with additives (Figures 5), where there was no formation of the inhibition halo. Regarding the amount of copper released from the obtained films, studies have reported that copper is part of a small group of metallic elements essential to human health and to most living organisms, in which it acts as an electron donor/receptor by alternating between the oxidative states Cu(I) and Cu(II), therefore at low concentrations, such as those obtained in this study, it does not present human toxicity when compared to other biocide materials^{101,102}.

3.8. Biodegradation test of polymeric obtained films

Because Ecoflex® is a biodegradable commercial polymer, this biodegradability test aimed to determine whether the presence of CuO-NPs interferes with the rate of biodegradation of the pure polymer. Through the analysis of the composting soil carried out, which presented a C/N ratio of 4, it can be said that it presents itself in a favorable condition for this type of study, simulating the composting conditions, since residues that present a C/N ratio of N less than 20 has a higher mineralization rate, since nitrogen is the essential element for the growth of microorganisms since it is a basic component of nucleic acids and amino acids^{96,103}. Table 6 presents the accumulated amount of carbon dioxide (CO₂) produced during the biodegradation of polymeric

films added with 1 wt%, 2 wt%, and 4 wt% of CuO-NPs, of the pure Ecoflex® film, as well as the soil in the absence of film polymer (control), in 140 days of biodegradability test.

It can be seen from Table 6 that the polymeric films added with CuO-NPs did not differ significantly among themselves and neither with the pure Ecoflex® polymeric film nor with the control (soil without polymer), that is, the addition of CuO-NPs in Ecoflex® did not affect the biodegradability of pure Ecoflex® polymer at the evaluated concentrations. When comparing the total amount of CO₂ generated during the biodegradability process of pure Ecoflex® with the work by Capelezzo et al.¹³, which was 1,363.96±73 mg, it is noted that the compost soil used in this work provides greater CO₂ production (8,455.90±290 mg), accelerating the biodegradation process, about 6 times more, when compared to the use of virgin soil used by the authors. Accordingly, it is possible to confirm that the biodegradation process is taking place in the polymeric films when Figure 8 is

Table 6. Total amount of CO₂ produced for pure Ecoflex® polymeric films, added with 1 wt%, 2 wt% and 4 wt% of CuO-NPs and soil without polymer, after 140 days of analysis of the biodegradability test.

Polymer film sample	Amount of CO ₂ produced by 140 days of biodegradation test (mg)
Pure Ecoflex®	8,455.90±290.00 ^{a*}
Ecoflex® +1 wt% CuO-NPs	8,218.80±168.50 ^a
Ecoflex® +2 wt% CuO-NPs	8,268.90±99.80 ^a
Ecoflex® +4 wt% CuO-NPs	8,219.40±52.30 ^a
polymer free soil	8,370.50±156.60 ^a

*Equal lowercase letters represent that there are no significant differences in the column (p<0.05).

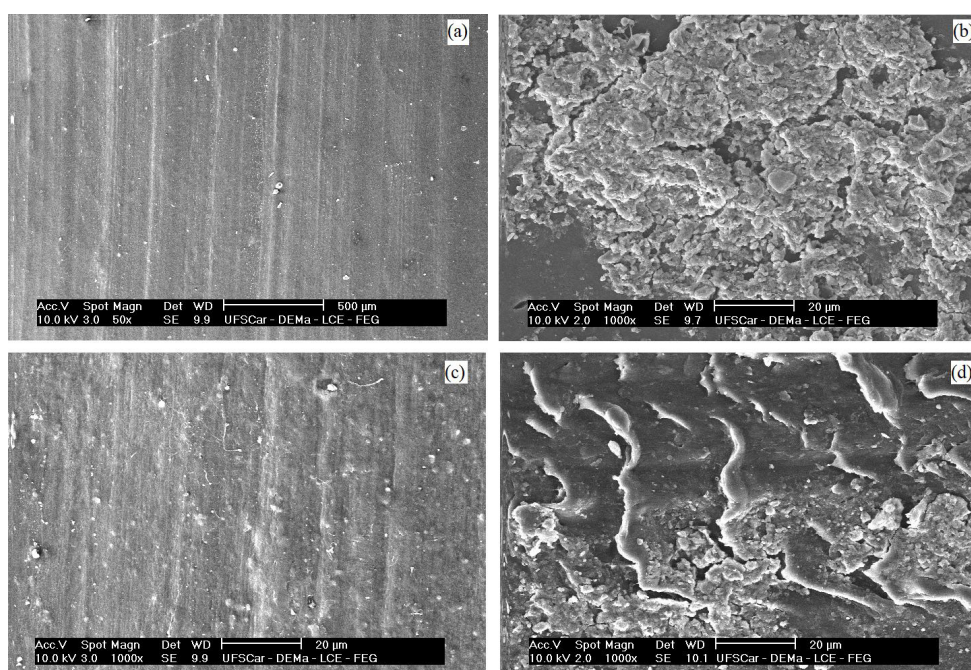


Figure 8. Micrographs of polymeric films: (a) pure Ecoflex® 0 days; (b) pure Ecoflex® after 140 days of biodegradation; (c) Ecoflex® +2 wt% CuO-NPs 0 days; (d) Ecoflex® +2 wt% CuO-NPs after 140 days of biodegradation.

analyzed, which presents the images obtained in the SEM/FEG of the pure Ecoflex® polymeric film and with 2 wt% CuO-NPs at time zero and after 140 days of biodegradation test. A similar result was also obtained by Capezezzo et al.¹³, and by Mohr et al.¹², in which the presence of NPs-ZnO and NPs-TiO₂ did not interfere with the biodegradability of pure Ecoflex® and Ecovio®, respectively.

By analyzing Figure 8, it is possible to confirm the beginning of the biodegradation process, both in the pure polymeric film and in the polymeric film added with 2 wt% CuO-NPs. In all images it was possible to observe the presence of cavities in line shape that were originated by the extrusion process in the manufacture of the films. This evidence is visualized through the existing cracks in Figures 8b and 8d, when compared to Figures 8a and 8c, corroborating the fact that the films are being biodegraded under the studied conditions. It is also possible to observe in Figure 8b and 8d (after 140 days of biodegradation test) that the biodegradation of the polymeric films was not complete, to both pure polymeric film, Figure 8b, as the polymeric film added with 2 wt% CuO-NPs, Figure 8d, requiring a longer period for its conclusion. Šerá et al.¹⁰⁴, who studied the biodegradation of pure Ecoflex® in soil under aerobic conditions at 25 °C, obtained a mineralization level of approximately 6% after 100 days of incubation.

The biodegradability of the polymeric material depends on the conditions in which the material is exposed, for example, exposure to light, heat, humidity, and oxygen concentration, which are fundamental parameters for the bacteria responsible for the process to be effective in breaking the chains of flexible polymeric parts of the material¹⁰⁵. Therefore, the fact that the polymeric obtained films in this work were not completely biodegraded may have been due to the experimental conditions adopted, such as a process without aeration, not being exposed to light, and at room temperature. The company BASF brings in its portfolio that Ecovio®¹⁰⁵ plastic packaging is transformed into fertilizer in 45 days, however, the conditions for polymer degradation were composting, where there is aeration, temperature around 60 °C and humidity approximately 70%. In addition, earthworms and springtails were not added, which, if present, could favor the biodegradation of the material. Casarin et al.¹⁰⁶ evaluated the Ecoflex® polymeric film biodegradation process in soil and after 90 days of experiment, where they verified a low mass loss, approximately 3.27±0.21%. The authors also reported that the greater the amount of natural component used in blends, such as saw dust, the greater the mass loss during the biodegradation process.

4. Conclusion

Through this study, it can be concluded that by adding CuO-NPs to the biodegradable polymer Ecoflex® it was possible to add antifungal activity to the polymeric obtained films without compromising its biodegradability, when compared to the polymeric film Ecoflex® without additive. Among the percentages studied, the concentration of 1.5 wt% could be chosen, since it presented a reduction of approximately 90% of the formation of the fungus *Aspergillus niger*. The addition of CuO-NPs provided an increase in the crystallinity of the added polymeric films

and favoring the formation of crystals in the cooling phase. In terms of mechanical properties, the addition of CuO-NPs promoted an increase in maximum tension and rupture and deformation, desirable characteristics for flexible packaging for possible applications in the food sector. The results of the copper release test revealed that the values are within the limit allowed by ANVISA, which makes the material safe for use in contact with food. The biodegradable films developed from the addition of CuO-NPs in Ecoflex® have potential application for biodegradable packaging with antifungal action against the fungus *Aspergillus niger*.

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6. References

1. Poveda PNS, Molari JA, Brunelli DD, Silva LGAE. Study of different process additives applied to polyethylene. In: Bowen L, editor. Characterization of minerals, metals and materials. Cham: Springer International Publishing; 2018. p. 661-7.
2. Oliveira RR, Oliveira TA, Silva LRC, Barbosa R, Alves TS, Carvalho LH, et al. Effect of reprocessing cycles on the morphology and mechanical properties of a Poly(Propylene)/Poly(Hydroxybutyrate) blend and its nanocomposite. Mater Res. 2021;24(4):e20200372. <http://dx.doi.org/10.1590/1980-5373-MR-2020-0372>.
3. Gonçalves LMG, Rigolin TR, Frenhe BM, Bettini SHP. On the recycling of a biodegradable polymer: multiple extrusion of poly (Lactic Acid). Mater Res. 2020;23(5):e20200274. <http://dx.doi.org/10.1590/1980-5373-MR-2020-0274>.
4. Lamberti FM, Román-Ramírez LA, Wood J. Recycling of bioplastics: routes and benefits. J Polym Environ. 2020;28(10):2551-71. <http://dx.doi.org/10.1007/s10924-020-01795-8>.
5. Brito GF, Agrawal P, Araújo EM, Mélo TJA. Biopolímeros, polímeros biodegradáveis e polímeros verdes. REMAP. 2011;6(2):127-39.
6. Casarin SA, Agnelli JAM, Malmonge SM, Rosário F. Blendas PHB/Copoliésteres biodegradáveis – biodegradação em solo. Polímeros. 2013;23:115-22. <http://dx.doi.org/10.1590/S0104-14282013005000003>.
7. Vaezi K, Asadpour G, Sharif SH. Effect of coating with novel bio nanocomposites of cationic starch/cellulose nanocrystals on the fundamental properties of the packaging paper. Polym Test. 2019;80:106080. <http://dx.doi.org/10.1016/j.polymertesting.2019.106080>.
8. Hopmann C, Schippers S, Höfs C. Influence of poly(lactic acid) recycling on packaging relevant properties. J Appl Polym Sci. 2015;132(9):1-6. <http://dx.doi.org/10.1002/app.41532>.
9. Rabelo LH, Munhoz RA, Marini J, Maestrelli SC. Development and characterization of PLA composites with high contents of a Brazilian refractory clay and improved fire performance. Mater Res. 2022;25:e20210444. <http://dx.doi.org/10.1590/1980-5373-MR-2021-0444>.
10. ASTM: American Society for Testing and Materials. ASTM D 833-12: Standard terminology relating to plastics. West Conshohocken: ASTM; 2012.
11. ASTM: American Society for Testing and Materials. ASTM D 5488: standard terminology of environmental labeling of packaging materials and packages. West Conshohocken: ASTM; 2002.
12. Mohr LC, Capezezzo AP, Baretta CRDM, Martins MAPM, Fiori MA, Mello JMM. Titanium dioxide nanoparticles applied as

- ultraviolet radiation blocker in the polylactic acid biodegradable polymer. *Polym Test*. 2019;77:105867. <http://dx.doi.org/10.1016/j.polymertesting.2019.04.014>.
13. Capelezzo AP, Mohr LC, Dalcanton F, Barreta CDM, Martins MAPM, Fiori MA, Mello JMM. Polímero biodegradável antimicrobiano através da aditivação com compostos à base de zinco. *Quim Nova*. 2018;41(4):367-74. <http://dx.doi.org/10.21577/0100-4042.20170187>.
 14. Chen K, Yu J, Huang J, Tang Q, Li H, Zou Z. Improved mechanical, water vapor barrier and UV-shielding properties of cellulose acetate films with flower-like metal-organic framework nanoparticles. *Int J Biol Macromol*. 2021;167:1-9. <http://dx.doi.org/10.1016/j.ijbiomac.2020.11.164>.
 15. Silva LRC, Silva LO, Carvalho LH, Oliveira AD, Bardie MAG, Mesquita ABS, et al. Physical, morphological, structural, thermal and antimicrobial characterization of films based on poly(Lactic Acid), organophilic montmorillonite and oregano essential oil. *Mater Res*. 2022;25:e20220043. <http://dx.doi.org/10.1590/1980-5373-MR-2022-0043>.
 16. Narancic T, O'Connor KE. Plastic waste as a global challenge: are biodegradable plastics the answer to the plastic waste problem? *Microbiology*. 2019;165:129-37. <http://dx.doi.org/10.1099/mic.0.000749>.
 17. Shankar S, Wang L-F, Rim J-W. Incorporation of zinc oxide nanoparticles improved the mechanical, water vapor barrier, UV-light barrier, and antibacterial properties of PLA-based nanocomposite films. *Mater Sci Eng C*. 2018;93:289-98. <http://dx.doi.org/10.1016/j.msec.2018.08.002>.
 18. Zhong Y, Godwin PY, Jin Xiao H. Biodegradable polymers and green-based antimicrobial packaging materials: a mini-review. *Advanced Industrial and Engineering Polymer Research*. 2020;3(1):27-35.
 19. Piletti R, Zanetti M, Jung G, Mello JMM, Dalcanton F, Soares C, et al. Microencapsulation of garlic oil by β cyclodextrin as a thermal protection method for antibacterial action. *Mater Sci Eng C*. 2019;94:139-49.
 20. FAO: Food and Agriculture Organization of the United Nations. Global food losses and food waste: extent, causes and prevention. Rome: FAO; 2011 [cited 2022 Dec 30]. Available from: <https://www.fao.org/3/i2697e/i2697e.pdf>.
 21. Zhang X, Zhang L, Zhou T, Zhou Y. Fungal flora and mycotoxin contamination in tea: current status, detection methods and dietary risk assessment - A comprehensive review. *Trends Food Sci Technol*. 2022;127:207-20. <http://dx.doi.org/10.1016/j.tifs.2022.05.019>.
 22. Sivaram S, Somanathan H, Kumaresan SM, Muthuraman MS. The beneficial role of plant based thymol in food packaging application: a comprehensive review. *Applied Food Research*. 2022;2(2):100214. <http://dx.doi.org/10.1016/j.afres.2022.100214>.
 23. Gan F, Hang X, Huang Q, Deng Y. Assessing and modifying China bentonites for aflatoxin adsorption. *Appl Clay Sci*. 2019;168:348-54. <http://dx.doi.org/10.1016/j.clay.2018.12.001>.
 24. Brandão RM, Batista LR, Oliveira JE, Barbosa RB, Nelson DL, Cardoso MG. In vitro and in vivo efficacy of poly(lactic acid) nanofiber packaging containing essential oils from *Ocimum basilicum* L. and *Ocimum gratissimum* L. against *Aspergillus carbonarius* and *Aspergillus niger* in table grapes. *Food Chem*. 2023;400:134087. <http://dx.doi.org/10.1016/j.foodchem.2022.134087>.
 25. Tam KH, Djurišić AB, Chan CMN, Xi YY, Tse CW, Leung YH, et al. Antibacterial activity of ZnO nanorods prepared by a hydrothermal method. *Thin Solid Films*. 2008;516:6167-74.
 26. Lima MAS, Oliveira MCF, Pimenta ATA, Uchoa PKS. *Aspergillus niger*: a hundred years of contribution to the natural products chemistry. *J Braz Chem Soc*. 2019;30(10):2029-59.
 27. Li Y-X, Erhunmwunsee F, Liu M, Yang K, Zheng W, Tian J. Antimicrobial mechanisms of spice essential oils and application in food industry. *Food Chem*. 2022;382:132312. <http://dx.doi.org/10.1016/j.foodchem.2022.132312>.
 28. Perumal AB, Huang L, Nambiar RB, He Y, Li X, Sellamuthu PS. Application of essential oils in packaging films for the preservation of fruits and vegetables: A review. *Food Chem*. 2022;375:131810. <http://dx.doi.org/10.1016/j.foodchem.2021.131810>.
 29. Massi FP, Sartori D, De Souza L, Iamanaka BT, Taniwaki MH, Carneiro ML, et al. Prospecting for the incidence of genes involved in ochratoxin and fumonisin biosynthesis in Brazilian strains of *Aspergillus niger* and *Aspergillus welwitschiae*. *Int J Food Microbiol*. 2016;221:19-28.
 30. Castro-Mayorga JL, Fabra MJ, Pourrahimi AM, Olsson RT, Lagaron JM. The impact of zinc oxide particle morphology as an antimicrobial and when incorporated in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging and food contact surfaces applications. *Food Bioprod Process*. 2017;101:32-44.
 31. Lotfi M, Tajik H, Moradi M, Forough M, Divsalar E, Kuswandi B. Nanostructured chitosan/monolaurin film: Preparation, characterization and antimicrobial activity against *Listeria monocytogenes* on ultrafiltered white cheese. *Lebensm Wiss Technol*. 2018;92:576-83.
 32. Bryce EA, Velapattino B, Donnelly-Pierce T, Wong T, Dixon R, Akbari Khorami H, et al. Evaluating the antimicrobial activity of copper surfaces against *Pseudomonas aeruginosa* and *Staphylococcus aureus* 1 year after use in a microbiology laboratory. *J Hosp Infect*. 2022;123:186-8. <http://dx.doi.org/10.1016/j.jhin.2022.02.007>.
 33. Xiang Y, Li J, Liu X, Cui Z, Yang X, Yeung KWK, et al. Construction of poly(lactic-co-glycolic acid)/ZnO nanorods/Ag nanoparticles hybrid coating on Ti implants for enhanced antibacterial activity and biocompatibility. *Mater Sci Eng*. 2017;79:629-37.
 34. Silva R, Baretta AM, Silva LL, Ternus R, Colpani GL, Fiori MA, et al. Avaliação da atividade antimicrobiana de ração para frango aditivada de nanopartículas de óxido de zinco. *Research Society and Development*. 2021;10(7):e44610716830-e44610716830.
 35. Arshad M, Qayyum A, Shar GA, Soomro GA, Nazir A, Munir B, et al. Zn-doped SiO₂ nanoparticles preparation and characterization under the effect of various solvents: antibacterial, antifungal and photocatalytic performance evaluation. *J Photochem Photobiol B*. 2018;185:176-83.
 36. Janegitz BC, Lourenção BC, Lupetti KO, Filho O. Desenvolvimento de um método empregando quitosana para remoção de íons metálicos de águas residuárias. *Quim Nova*. 2007;30(4):879-84.
 37. Palza H, Delgado K, Quijada RJ. Antimicrobial polymer composites with copper micro- and nanoparticles: effect of particle size and polymer matrix. *J Bioact Compat Polym*. 2015;30(4):1-15. <http://dx.doi.org/10.1177/0883911515578870>.
 38. Mani VM, Kalaivani S, Sabarathinam S, Vasuki M, Soundari AJP, Ayyappa Das MP, et al. Copper oxide nanoparticles synthesized from an endophytic fungus *Aspergillus terreus*: bioactivity and anti-cancer evaluations. *Environ Res*. 2021;201:111502. <http://dx.doi.org/10.1016/j.envres.2021.111502>.
 39. Grafia AL, Vázquez MB, Bianchinotti MV, Barbosa SE. Development of an antifungal film by polyethylene surface modification with natamycin. *Food Packag Shelf Life*. 2018;18:91-200.
 40. Soni A, Kaushal D, Kumar M, Sharma A, Maurya IK, Kumar S. Synthesis, characterizations and antifungal activities of copper oxide and differentially doped copper oxide nanostructures. *Mater Today Proc*. 2022;22: (In press). <http://dx.doi.org/10.1016/j.matpr.2022.09.133>.
 41. Shende SS, Gaikwad ND, Bansod SD. Synthesis and evaluation of antimicrobial potential of copper nanoparticle against agriculturally important Phytopathogens. *Int J Biol Res*. 2016;1:41-7.

42. Wang Y, Gong Y, Lin N, Yu L, Du B, Zhang X. Enhanced removal of Cr(VI) from aqueous solution by stabilized nanoscale zero valent iron and copper bimetal intercalated montmorillonite. *J Colloid Interface Sci.* 2022;606(2):941-52. <http://dx.doi.org/10.1016/j.jcis.2021.08.075>.
43. Franz RGR. *Revestimento de tinta acrílica com propriedade antifúngica pela incorporação de nanopartículas de óxido de cobre [dissertation].* Chapecó: Unochapecó; 2019.
44. Ganguli P, Chaudhuri S. Nanomaterials in antimicrobial paints and coatings to prevent biodegradation of man-made surfaces: a review. *Mater Today Proc.* 2021;45:3769-77. <http://dx.doi.org/10.1016/j.matpr.2021.01.275>.
45. Lin C-C, Chen W-Y. Effect of paint composition, nano-metal types and substrate on the improvement of biological resistance on paint finished building material. *Build Environ.* 2017;117:49-59. <http://dx.doi.org/10.1016/j.buildenv.2017.02.013>.
46. Sonia S, Kumari LH, Ruckmani K, Sivakumar M. Antimicrobial and antioxidant potentials of biosynthesized colloidal zinc oxide nanoparticles for a fortified cold cream formulation: a potent nanocosmeceutical application. *Mater Sci Eng.* 2017;79:581-9. <http://dx.doi.org/10.1016/j.msec.2017.05.059>.
47. Smijs TG, Pavel S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. *Nanotechnol Sci Appl.* 2011;4:95-112.
48. Khezerlou A, Sani MA, Lalabadi MA, Ehsani A. Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viroses. *Microb Pathog.* 2018;123:505-26.
49. Youssef AM, EL-SAYED SM. Bionanocomposites materials for food packaging applications: concepts and future outlook. *Carbohydr Polym.* 2018;193:19-27.
50. Ghorbani HR, Alizadeh V, Mehr FP, Jafarpourgolroudbary H, Erfan K, Yeganeh SS. Preparation of polyurethane/CuO coating film and the study of antifungal activity. *Prog Org Coat.* 2018;123:322-5.
51. Grafia AL, Vázquez MB, Bianchinotti MV, Barbosa SE. Development of an antifungal film by polyethylene surface modification with natamycin. *Food Packag Shelf Life.* 2018;18:91-200.
52. Droval G, Aranberri I, Germán L, Ivano E, Dimitrova E, Kotsilkova R, et al. Thermal and rheological characterization of antibacterial nanocomposites: Poly (amide) 6 and low-density poly (ethylene) filled with zinc oxide. *Journal of Thermoplastic Composite Materials.* 2014;27:268-84.
53. Othman SH. Bio-nanocomposite materials for food packaging applications: types of biopolymer and nano-sized filler. *Agriculture and Agricultural Science.* 2014;2:296-303. <http://dx.doi.org/10.1016/j.aaspro.2014.11.042>.
54. Rehim MHA, Alhamidi J. Tio2/Polymer nanocomposites for antibacterial packaging applications. *Journal of Advancements in Food Technology.* 2018;1:1-8. <http://dx.doi.org/10.15744/2639-3328.1.101>.
55. Alghamdi MH, Abutalib MM, Mannaa MA, Nur O, Abdelrazek EM, Rajeh A. Modification and development of high bioactivities and environmentally safe polymer nanocomposites doped by Ni/ZnO nanohybrid for food packaging applications. *J Mater Res Technol.* 2022;19:3421-32. <http://dx.doi.org/10.1016/j.jmrt.2022.06.077>.
56. Shankar S, Teng X, Rhim J. Properties and characterization of agar/CuNP bionanocomposite films prepared with different copper salts and reducing agents. *Carbohydr Polym.* 2014;114:484-92.
57. Avcu E, Bastanc FE, Guney M, Avcu YY, Rehmanf MAU, Boccaccin AR. Biodegradable polymer matrix composites containing graphene-related materials for antibacterial applications: a critical review. *Acta Biomater.* 2022;151(1):1-44. <http://dx.doi.org/10.1016/j.actbio.2022.07.048>.
58. Ejaz M, Arfat YA, Mulla M, Ahmed J. Zinc oxide nanorods/clove essential oil incorporated Type B gelatin composite films and its applicability for shrimp packaging. *Food Packag Shelf Life.* 2018;15:113-21.
59. Rangel WM, Boca Santa RAA, Riella HG. A facile method for synthesis of nanostructured copper (II) oxide by coprecipitation. *J Mater Res Technol.* 2019;9:994-1004.
60. Perez C, Pauli M, Bazerque P. An antibiotic assay by the well agar method. *Acta Biol Med Exp.* 1990;15:113-5.
61. CLSI: Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M07-A8.* Wayne, PA: CLSI; 2009.
62. JIS: Japanese Industrial Standards. *JIS Z 2801:2010. Antibacterial products - Test for antibacterial activity and efficacy.* Tokyo: JIS; 2010.
63. ASTM: American Society for Testing and Materials. *ASTM D-882-10: standard test method for tensile properties of thin plastic sheeting.* West Conshohocken: ASTM; 2010.
64. Brasil. ANVISA: Agência Nacional de Vigilância Sanitária. *Resolução nº 51 de 26 de novembro de 2010. Dispõe sobre migração em materiais, embalagens e equipamentos plásticos a entrar em contato com alimentos. Diário Oficial da República Federativa do Brasil; Brasília; 26 nov 2010.*
65. ASTM: American Society for Testing and Materials. *ASTM D 5988-18: standard test method for determining aerobic biodegradation of plastic materials in soil.* West Conshohocken: ASTM; 2018.
66. Lasfargues M, Sead G, Amjad M, Ding Y, Wen D. In situ production of copper oxide nanoparticles in a binary molten salt for concentrated solar power plant applications. *Materials (Basel).* 2017;10(5):537. <http://dx.doi.org/10.3390/ma10050537>.
67. Usman M, Zowalaty ME, Shamel K, Zainuddin N, Salama M, Ibrahim N. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. *Int J Nanomedicine.* 2013;8:4467-79.
68. Zhang Y, Jiao Z, Hu Y, Lv S, Fan H, Zeng Y, et al. Removal of tetracycline and oxytetracycline from water by magnetic Fe₃O₄ graphene. *Environ Sci Pollut Res Int.* 2017;24:2987-95.
69. Ethiraj AS, Kang DJ. Synthesis and characterization of CuO nanowires by a simple wet chemical method. *Nanoscale Res Lett.* 2012;7:70. <http://dx.doi.org/10.1186/1556-276X-7-70>.
70. Gupta D, Meher SR, Lilyaskutty N, Alex ZC. Facile synthesis of Cu₂O and CuO nanoparticles and study of their structural, optical and electronic properties. *J Alloys Compd.* 2018;743(1):737-45.
71. Jiang T, Bujoli-Doeuff M, Gautron E, Farre Y, Cario L, Pellegrin Y, et al. Cu₂O@CuO core-shell nanoparticles as photocathode for p-type dye sensitized solar cell. *J Alloys Compd.* 2018;769(1):605-10.
72. Hosseini-Koupaei M, Shareghi B, Saboury AA, Davar F, Sirotkin VA, Hosseini-Koupaei MH, et al. Catalytic activity, structure and stability of proteinase K in the presence of biosynthesized CuO nanoparticles. *Int J Biol Macromol.* 2019;122(1):732-44.
73. Yathisha RO, Nayaka YA, Manjunatha P, Purushothama HT, Vinay MM, Basavarajappa KV. Study on the effect of Zn²⁺ doping on optical and electrical properties of CuO nanoparticles. *Physica E.* 2019;108(1):257-68.
74. Nakhate AV, Yadav GD. Cu₂O nanoparticles supported hydrothermal carbon microspheres as catalyst for propargylamine synthesis. *Mol Catal.* 2018;451(1):209-19.
75. Guo Y, Dai M, Zhu Z, Chen Y, He H, Qin T. Chitosan modified Cu₂O nanoparticles with high catalytic activity for p-nitrophenol reduction. *Appl Surf Sci.* 2019;480(1):601-10.
76. Suryawanshi SR, Singh A, Deo M, Late DJ, Sinha S, More MA. 3D Hetero-architecture of GdB6 nanoparticles on lessened cubic Cu₂O nanowires: enhanced field emission behaviour. *CrystEngComm.* 2015;17:3936-44. <http://dx.doi.org/10.1039/C4CE02530J>.
77. Cano AP, Gillado AV, Montecillo AD, Herrera MU. Copper sulfate-embedded and copper oxide-embedded filter paper and

