Book Chapter

Evaluate The Relationship Between Particle Size, Anti-Microbial Activity, and Leachability of Copper Particles in Liquid Suspension and Compounded in Polypropylene

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Abstract

Testing the anti-microbial efficiency of plastics with good precision and repeatability remain a challenge in the plastic industry, as commonly used standards can give unreliable data. We show in this paper that the "Bacterial Liquid Suspension Test" is a very robust method that allows measuring from poor to very potent biocides. We used this technique to discriminate the performance of two Cu-based biocides, either in nanoparticle (NP) or macroparticle (MP) size, at three different loadings (0.02, 0.2 and 2 wt.%) in PP. With this technique, we could also test the anti-bacterial performance of PP as powders, pellets, and injection molded disks. As anticipated, the technique shows that both the increased loading and the smaller particle size show higher anti-microbial activity than the bigger particles size due to the increased surface area. Also, PP powders gave a higher bacterial reduction compared to pellets and disks. While the PP with 2 wt.% Cu NPs showed the best anti-microbial performance, the detection of Cu at the surface (using SEM-EDX) and in the water leachate (using ICP-MS) were below the LODs, indicating how potent they are to kill bacteria.

Keywords

Anti-Microbial Activity, *E. coli* K-12 MG1655, Copper Particles, Leachability, Compounding

Introduction

The global demand for materials possessing anti-microbial properties recently took a different perspective following the COVID-19 pandemic. The hygiene and safety of plastics, concerning the presence or growth of microbes (fungi, bacteria,

and viruses) their surface have become major consumer's concern. Warmth, humidity and presence of nutrients are a source of microbial growth and plastics typically lack defense against this [1]. Microorganisms adhering to artificial surfaces in damp conditions have the ability to survive and multiply. When the number of microbial cell number increases, it leads to a biofilm formation, a matrix of polysaccharides containing embedded cells. Biofilms enable microbial survival in challenging environments and exhibit significantly reduced susceptibility, approximately 1000-fold, to most biocidal agents [2]. Toxins excreted from biofilms spread pathogenic and resilient infections and can lead to multiresistant bacterial strain [3]. This led to the development of anti-microbial materials, meaning materials that "are capable of inhibiting or killing the microbes on their surface or within their surroundings" [4]. The field of anti-microbial materials has been extensively investigated, and several approaches were developed as shown by the plethora of articles in literature and patents [5]. Regarding the production of anti-microbial polyolefins in particular, there are different ways to confer such property to the material with either (i) direct incorporation of anti-microbial agents into polyolefin through a compounding step, (ii) applying an anti-microbial coating, and (iii) chemical incorporation using copolymerization or grafting (e.g. through reactive extrusion) to immobilize anti-microbial agents or to impart anti-microbial functionalities onto polyolefin by either ionic or covalent linkages [6,7].

Heavy metal biocides such as silver and copper nanoparticles (NPs), traditionally used as anti-microbial agents, it can be readily integrated into polyolefin through extrusion techniques, potentially imparting effective anti-microbial properties to the materials with a short contact duration of several hours [8]. When exposed to moisture, an electrochemical process occurs, leading to the release of silver ions. These ions infiltrate microbes, incapacitating their ability to function, proliferate, or reproduce [9]. Therefore, their mode of action is through a leaching phenomenon, from the plastic to the microorganism. Some studies have shown that prolonged exposure to silver ions has adverse effects on human health (skin discoloration known

as argyria or eye discoloration termed as argyrosis can occur, and exposure to soluble silver compounds may result in adverse effects such as liver and kidney damage, as well as irritations of the eyes, skin, respiratory system, and intestinal tract) [10]. Additionally, waste management is a concern when looking at the amount of silver disposed in nature, especially in marine environments where the release of silver was shown to be harmful to aquatic life [11]. Through immobilization of silver onto carriers (ZnO, glass, zeolite) a limited release of silver into nature can be achieved, nevertheless, it was advised by European Chemicals Agency (ECHA) to no longer approve these technologies for food contact applications. Therefore, there is the need for more environmentally friendly and less toxic anti-microbial additives in plastics where not only the type of inorganic biocide is important, but also its size and shape. Copper exhibits anti-microbial properties, and like silver, it has been utilized to sterilize liquids or treat human wounds for centuries [12,13]. The mechanism of the biocidal action of Cu ions is complex, since some copper ionic species can be formed (e.g. Cu^{2+} and Cu^{+}). Additionally, Cu NPs can be oxidized to CuO or Cu_2O and all these different moieties can interact in various ways with the cell's vital functions [8]. In general, Cu^{+} is connected with the production of highly reactive species like hydroxyl radicals which disrupt the membrane's integrity and cause oxidative stress to the cell, while Cu^{2+} forms complexes with bacteria's functional groups (proteins, DNA etc.), resulting in ion substitution and enzyme deactivation [14-16]. For most anti-microbial additives compounded into plastics, their migration properties and leachability propensity are crucial since the biocidal mechanism involves ions migrating from the plastic matrix, to the surface and then to the microbe. Therefore, there is a key distinction to make when dealing with dry and humid surfaces as illustrated in (**Figure 1**).

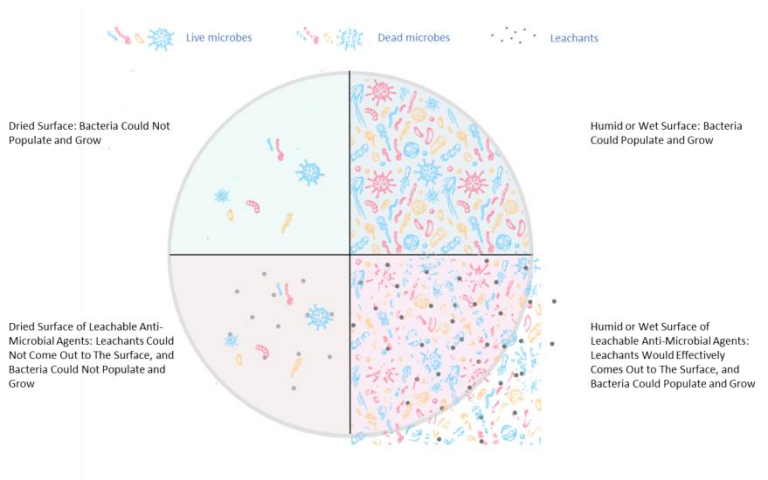

Figure 1: The living mechanism of bacteria on dry and wet surface of PP composites.

Different factors affect the bacterial survival on inanimate surfaces and depending on the type of bacteria, [17] their survival rate on dry surfaces can range from days to months [18]. This article particularly focuses on studying the influence of particle size of copper (macro vs. nano with spherical particle shape) onto anti-microbial properties using a specific anti-microbial testing procedure that allows constant renewal of bacterial cultures on the surface of neat PP disks, and PP composites (powders, pellets, and disks) to avoid any miss-leading data due to the intrinsic wetting issues (hydrophobicity). The PP composites with spherical shaped Cu-nanoparticles and spherical shaped Cu-microparticles were processed at three different mixing ratios (0.02 wt.%, 0.2 wt.%, and 2 wt.%). The morphology of PP composites was studied using SEM and EDX. The thermal analysis was analyzed using DSC and TGA, and the leachability was characterized using ICP-MS for the composites of PP disks.

Experimental

Materials

Polypropylene homopolymer (PP 500 P grade) was obtained from SABIC (Riyadh, Saudi Arabia) as a powder with the following properties: melting point: 230 °C, density: 0.905 g/cm^3, and melt flow MFR = 3 g/10 min at 230°C / 2.16 kg; Mw ~ 300 kg/mol, isotacticity ~ 96%. Copper nanoparticles (purity: 99.99 %) were obtained from Nanomaterial Powder (Istanbul, Turkey) as a dry powder with an average particle size of 570 nm. Copper microparticles (purity: 99.99 %) were obtained from Sigma Aldrich (Missouri, USA) as a dry powder with an average particle size of 425 μm.

Melt-Compounding and Injection Molding of PP with Anti-Microbial Agents

Physical mixing of the additive package with the PP resin was carried out for each formulation, ensuring enough homogeneity of the mixtures applied prior to the melt mixing. Extrusion was performed using a twin-screw extruder from Thermofisher (Process 11: D 11 mm, L/D 40). The operating conditions were kept the same for all experiments. The extruder temperature profile was set from 60 to 230 ºC. The extruder screw rotation speed was kept at 223 rpm. The mixing ratio for both Cu-nanoparticles and Cu-microparticles were (i) 0.02 wt.%, (ii) 0.2 wt.%, and (iii) 2 wt.%. Extrusion compounding led to PP polymer pellets with the desired level of additives. The composite pellets were furtherly processed to make a PP powder of both Cu-nanoparticles and Cu-microparticles composites at (i) 0.02 wt.%, (ii) 0.2 wt.%, and (iii) 2 wt.% using a lab scale pellets grinder model EBERBACH E3300.00, Mini Cutting Mill, MI, USA (**Figure 2**). Injection molding of the pellets was performed using a Babyplast 6/12 machine. The processing temperature was 230 °C, and the mold temperature was 60 °C using 5x5x0.32 cm molds. The neat PP and six compounded PPs were injection molded into disks (the disk measures 25 millimeters in diameter and has a thickness of 1.55 millimeters). The disks were of a size that could be used in anti-microbial testing equipment. The untreated PP did not display anti-microbial activity; therefore, it was selected as a negative control sample.

Figure 2: Lab scale grinder.

Anti-Microbial Testing Method

The anti-microbial activities of the disks were assessed using *E. coli* K-12 MG1655 obtained from the American Type Culture Collection in Manassas, VA. The stock culture was stored at -80 °C and streaked onto TSA plates from BBL/Difco in Sparks, MD, USA. After incubation at 37 °C for 24 hrs, a single colony was selected and transferred to 5 mL of TSB also from BBL/Difco and incubated at 37 °C for 18 hours. Following incubation, 1 mL of culture was centrifuged at 13,000 x g for 5 minutes using a Fisher Scientific accuSpin micro 17 R centrifuge, and the supernatant was discarded. The cells were then suspended in 1 mL of Phosphate Buffered Saline (PBS) from Crystalgen in Commack, NY, USA, by vortexing. This cell suspension was transferred to a 15 mL tube, and 11.5 mL of PBS was added. Aliquots of this suspension were then exposed to the different types of (i) anti-microbial particles (powders), (ii) neat PPs (powders, pellets, and disks), and (iii) composite PPs (powders, pellets, and disks) that contain anti-microbial additives.

Each single material (20 g of powders, 20 g of pellets, and 1 disk which is weighs ~ 20 g) was individually positioned in a pod,

consisting of contact lens cases manufactured by Bosch + Lomb. For each pod containing a single type of material such as (i) anti-microbial particles (powders), (ii) neat PPs (powders, pellets, and disks), and (iii) composite PPs (powders, pellets, and disks), 1 mL of bacterial suspension was added to submerge the material into the culture broth and the pods were closed. The pods were affixed onto a mini rotator (Benchmark Scientific, Roto Mini Plus R 2024, Sayreville, NJ, USA) and set to rotate at 20 rpm around the machine's horizontal axis, ensuring continuous agitation of the broth and facilitating liquid renewal on the tested materials. At intervals of 0, 4, and 24 hrs, a 100 µL sample of the bacterial suspension was extracted for appropriate 1:10 dilutions, plated in TSA, and subsequently incubated overnight at 37 °C. The number of colony-forming units (CFUs) was enumerated to assess cell viability

Experimental Design

A 2x3x3 factorial experiment was used to investigate the effect of the anti-microbial agents on the melt compounded PPs against *E. coli* K-12 MG1655. The factorial experiments were performed as follows: anti-microbial particle size (570 nm and 425 µm), anti-microbial agent concentration (0.02 wt.%, 0.2 wt.%, and 2 wt.%), and melt compounded composite shape (powders, pellets, and disks).

Statistical Analysis

Each experiment was conducted with three separate biological replicates. Variations in the means of *E. coli* K-12 MG1655 cell density resulting from direct exposure to the following were analyzed: (1) pristine PP disk (serving as the negative control), and (2) PP composites containing anti-microbial agents were assessed at 0, 4, and 24 hrs using Tukey's honest significant difference test and Student's *t*-test at a confidence level of 96% ($p \leq 0.05$). Interactions between the particle size of anti-microbial agents (nano and micro-size), mixing ratio of the anti-microbial agents with PP (0.02 wt.%, 0.2 wt.%, and 2 wt.%), composite sample shape (powders, pellets, and disks), and the anti-microbial activity was evaluated using four-way analysis of variance (ANOVA) with Origin 2022b software (version 9.9.5.167; OriginLab Corporation, Massachusetts, USA).

Sample Characterization

Bacteria in Aqueous Suspension Test

The anti-microbial activity of the agents (pure particles without polymer) was tested in aqueous bacterial suspension, and the reduction in *E. coli* K-12 MG1655 cell density was observed at two-time intervals (4 and 24 hrs). 18 mg of the anti-microbial particles (powders) was added to a 1 mL of *E. coli* K-12 MG1655 in bacterial aqueous solution (liquid) using a microcentrifuge tube and rotated at 20 rpm using a sample mini rotator. 100 µL of the solution was extracted for plating in TSA at 4 and 24 hrs.

Anti-microbial Test for Neat and Composite PPs

The anti-microbial activity of neat PP and composite PPs were tested in three different shapes (powders, pellets, and disks), Three levels of concentration in PPs (0.02 wt.%, 0.2 wt.%, and 2 wt.%), and two sizes of Cu anti-microbial agents with spherical shapes (nanoparticles and microparticles).

Scanning Electronic Microscopy (SEM) and Energy Dispersive X-ray (EDX) Preparation Methods

The anti-microbial particles (pure powders of Cu-nanoparticles or Cu-microparticles), the compounded and injection molded PP disks with (0.02 wt.%, 0.2 wt.%, and 2 wt.% of either Cu-nanoparticles or Cu-microparticles) were characterized by SEM using a JEOL 7500F field emission emitter (JEOL Ltd. Tokyo, Japan), and by energy dispersive X-ray EDX analysis using an Oxford Instruments Aztec system (Oxford Instruments, High Wycomb, Bucks, England). Samples for these analysis were prepared by mounting on aluminum stubs using the Epoxy glue, System Three Quick Cure 5 (System Three Resins, Inc. Aubur, WA). All the mounted samples were coated with (~ 2.7 nm thickness) iridium in a Quorum Technologies/Electron Microscopy Sciences Q150T turbo pumped sputter coater (Quorum Technologies, Laughton, East Sussex, England BN8 6BN) and purged with argon gas.

Differential Scanning Calorimetry (DSC)

The melting point and crystallinity temperature of PP samples

were determined using a TA Instrument Model Q100 system with nitrogen flow set at 70 mL min-1. Samples were analyzed within a temperature range of -20 to 250 °C at a rate of 10 °C.min^{-1}, with a one-minute hold to eliminate prior thermal history. Additionally, samples were cooled to -20 °C at 10 °C.min^{-1} and reheated to 250 °C at the same rate to record thermal responses. Each sample underwent at least three replications. The degree of crystallinity was calculated from heat of fusion values obtained during the second heating runs and assessed using Equation (1)"

$$X_c(\%) = \left[\frac{\Delta H_c}{\Delta H_0 . W}\right] x100 \quad (1)$$

Where X_c represents the crystallinity of PP samples, ΔH_c denotes the heat of fusion, ΔH_0 stands for the enthalpy of fusion for 100 % crystalline PP [209 J/g] [19], and W indicates the fraction (weight) of PP in the composite.

Thermal Gravimetric Analysis (TGA)

Thermal characterization of the PP samples was conducted with a TA Instrument (Model Q50, thermogravimetric analysis (TGA) technique). A sample weighing (8 ± 2 mg) was placed into an aluminum pan, and the temperature was gradually increased from (20 to 600 °C) at a heating rate of (10 °C/min) within a nitrogen atmosphere flowing at a rate of (40 mL/min).

Cu Leachability Measurements of PP Composites

The leachability of the compounded polypropylene (disks) and with Cu-nanoparticles and Cu-macroparticles at the highest level of dosing (2 wt.%) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The tests were performed according to the procedure illustrated in (**Figure 3**). A razor was used to cut the disks into small pieces. A ratio of 200 mg of sample per 0.5 mL of water was used for all the samples and the amount of copper leached was measured at two intervals (4 and 24 hrs) at room temperature (20 ºC). Water samples were 100 - 5000 times diluted in 5 % nitric acid (trace metal grade). The elements in the water samples were quantified by ICP-MS using a multi-element calibration set from Inorganic Ventures and an Agilent 8900 ICP-MS system.

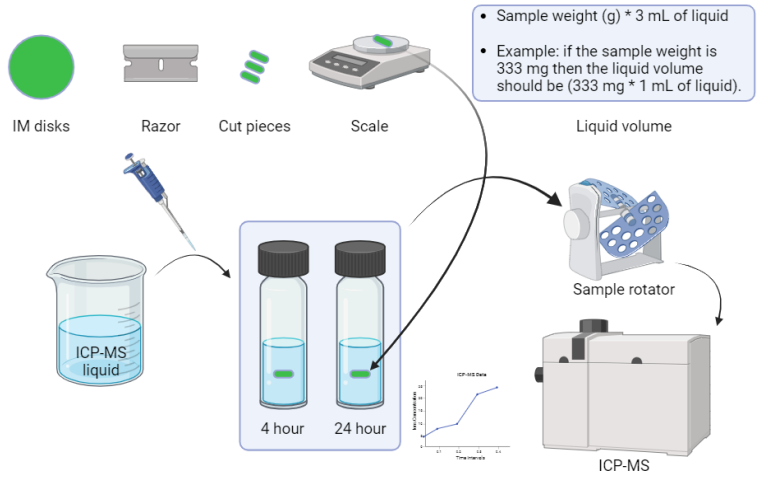

Figure 3: Illustration of ICP-MS experimental procedure.

Results and Discussion

Bacterial Liquid Suspension Test

We first assessed the anti-microbial efficacy of the two copper additives themselves, prior to compounding them in PP using the liquid suspension test. The amount of additive used in the test corresponds to the total amount dosed in the PP materials when using 2 wt.% loading. We looked at the *E. coli* bacterial reduction at two different time intervals (4 and 24 hrs). Both nano-Cu and macro-Cu gave outstanding bacterial reductions. While after 4 hrs, there is no strong distinction between the two forms, after 24 hrs, the nano-Cu gave a slightly better result with a log 8 reduction vs a log 7 for the macro-Cu (**Figure 4**). This first confirms the slight superiority of the anti-microbial ability of nanoparticles compared to macroparticles.

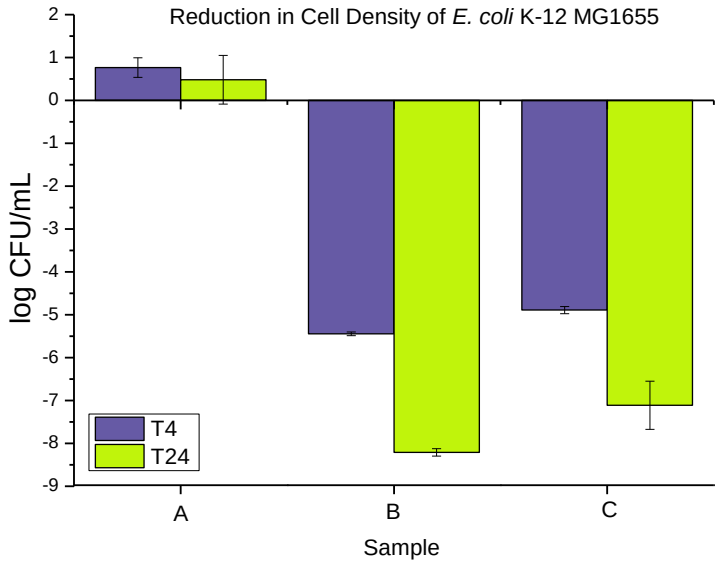

Figure 4: Represent the anti-microbial activity of (A) neat E. coli bacterial cultures, (B) Cu-nanoparticles, and (C) Cu-microparticles.

Anti-microbial Test for Neat and Composite PPs Samples

In the first set of experiments, we looked at the effect of the two additives compounded at (2 wt.%) in PP and tested in three different shapes (powders, pellets, and disks). First, as a control, we confirmed that the untreated PP disk showed no anti-microbial activity, with even a slight growth of *E. coli* observed over 24 hrs (**Figure 5 A**). As expected, the anti-bacterial effect observed is slightly lower in compounded PP vs the neat additives as only a small amount of Cu leached into the liquid suspension. Again, the best results were obtained when using nano-Cu with a bacterial reduction up to log 5.5 in PP powder after 24 hrs. The sample form also had an influence, and better results were obtained for the PP powders, which has an enormous surface area than the pellets and disks. These two parameters (nano vs. macro and powders vs. pellets vs disks) are, however, only visible after 24 hrs, as after 4 hrs of experiments,

all results were fairly comparable. This could be explained by the very high loading of the copper additive used here, which is potentially present in enough quantities on the surface of the PP at the start of the experiment to already have a very good biocidal effect. After 24 hrs, a migration of the Cu through the PP matrix can potentially occur where the shape of the PP and the size of the Cu would have an influence on such a phenomenon.

*Anti-microbial activity of neat PP, and **0.02 wt.%** nanoparticles and microparticles composites is presented in (**Figure 5**) as (i) powders, (ii) pellets, and (iii) disks at T4 and T24*

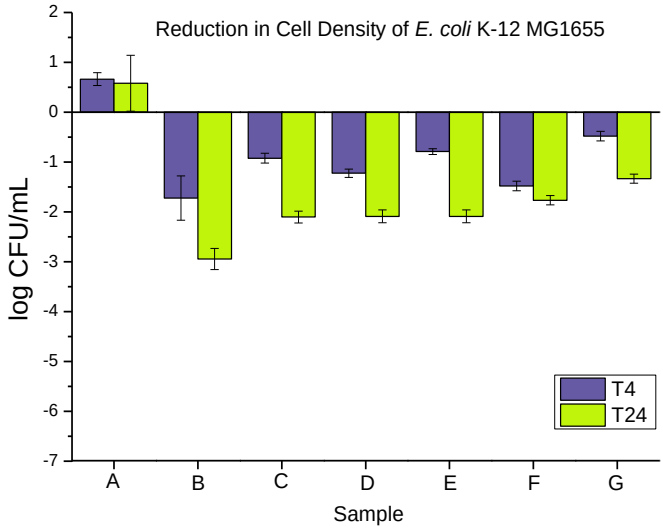

Figure 4: Represent the anti-microbial activity of (A) neat PP, (B) PP powders compounded with 0.02 wt.% NPs, (C) PP powders compounded with 0.02 wt.% MPs, (D) PP pellets compounded with 0.02 wt.% NPs, (E) PP pellets compounded with 0.02 wt.% MPs, (F) PP disks compounded with 0.02 wt.% NPs, and (G) PP disks compounded with 0.02 wt.% MPs.

*Anti-microbial activity of neat PP, and **0.2 wt.%** nanoparticles and microparticles composites is presented in (**Figure 6**) as (i) powders, (ii) pellets, and (iii) disks at T4 and T24*

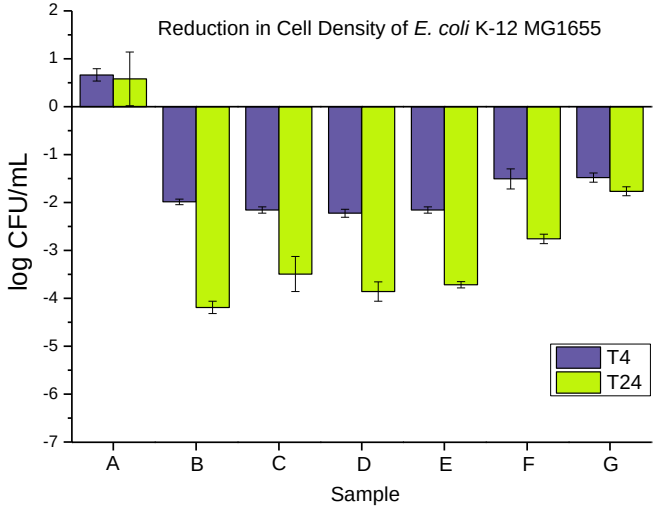

Figure 5: Represent the anti-microbial activity of (A) neat PP, (B) PP powders compounded with 0.2 wt.% NPs, (C) PP powders compounded with 0.2 wt.% MPs, (D) PP pellets compounded with 0.2 wt.% NPs, (E) PP pellets compounded with 0.2 wt.% MPs, (F) PP pellets compounded with 0.2 wt.% MPs, (F) PP disks compounded with 0.2 wt.% NPs, and (G) PP disks compounded with 0.2 wt.% MPs.

When reducing the loading in PP to 0.2 wt.%, bacterial reduction was obviously reduced with only up to log 4 in the best case (PP powders with nano-Cu). Similar observations can be made compared to the 2 wt.% experiments in terms of the effect of nano vs macro and PP form. However, in that set of experiments, the PP pellets proved to be far superior to the disks for both T4 and T24. A reduced loading means less additive on the surface, which can exacerbate the effect of surface area to an even higher degree than at higher Cu loading. This hypothesis is further validated when using a very low loading of Cu (0.02 wt.%). In this case, we can expect that very little amount of Cu is present initially on the surface which leads to very low bacterial reduction at T4, barely reaching log 2 in the best cases.

*Anti-microbial activity of neat PP, and **2 wt.%** nanoparticles and microparticles composites is presented in (**Figure 7**) as (i) powders, (ii) pellets, and (iii) disks at T4 and T24*

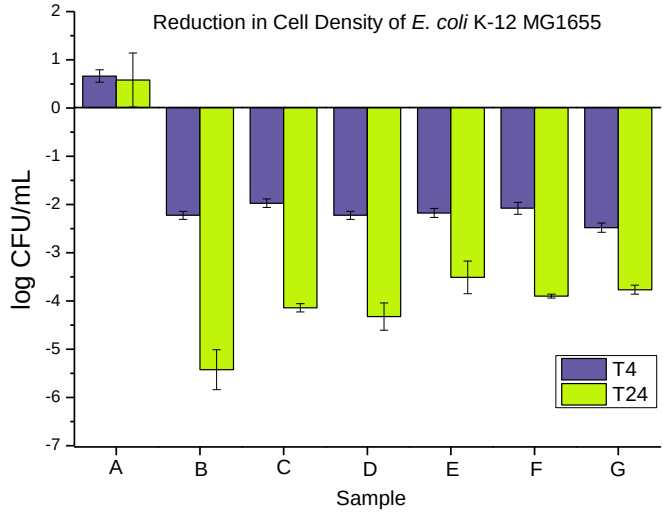

Figure 6: Represent the anti-microbial activity of (A) neat PP, (B) PP powders compounded with 2 wt.% NPs, (C) PP powders compounded with 2 wt.% MPs, (D) PP pellets compounded with 2 wt.% NPs, (E) PP pellets compounded with 2 wt.% MPs, (F) PP pellets compounded with 2 wt.% MPs, (F) PP disks compounded with 2 wt.% NPs, and (G) PP disks compounded with 2 wt.% MPs.

Overall, we showed that the liquid suspension test proved to be a very good tool for assessing the anti-microbial efficiency of either additives alone or compounded articles. It can clearly discriminate the effect of the morphology of additives (nano vs macro) and the surface area of the articles. The method is very robust with typically small standard variation and a high log CFU scale, which allows measuring from poor to very potent biocides. This is not always the case in other methods such as the ISO 22196:2011 (and similar methods such as JIS Z 2801), which is the most commonly used standard for the measurement of anti-bacterial activity on plastic surfaces. While this standard has the advantage of relative ease of use and low cost, this technique is quite notorious for having a high variation in the results from laboratory to laboratory [20].

Scanning Electronic Microscopy (SEM)

SEM of Dry Powder of Cu Nanoparticles is Presented in (Figure 8) and Cu Microparticles is Presented in (Figure 9)

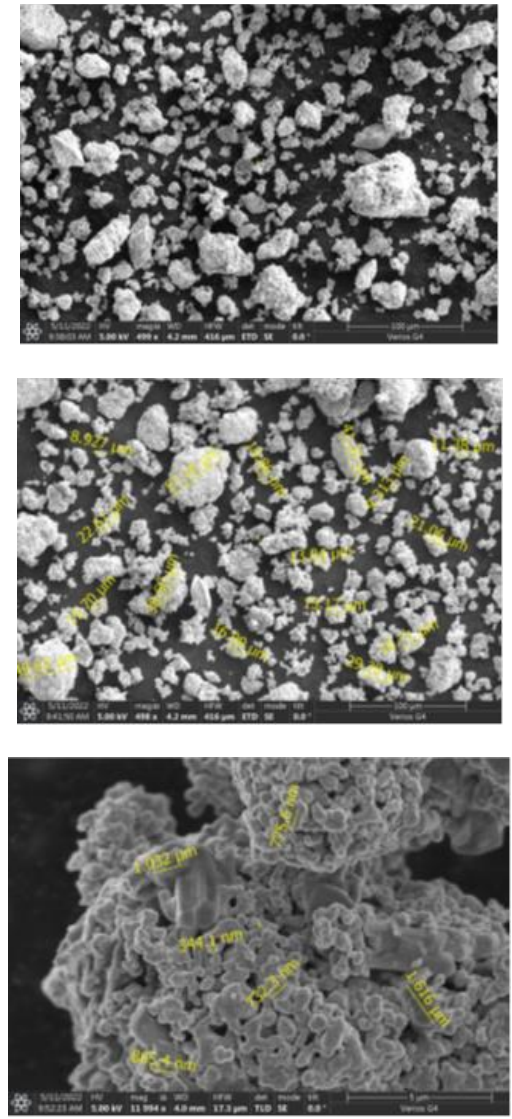

Figure 7: Shows the SEM images of pure Cu Nanoparticles.

In both cases, the Cu NPs and Cu MPs crystals have a reddish brown color. The SEM images of the Cu in (**Figure 8**) show the particles were spherical. The particles were about 344-865 nm, and the average particle size was about 570 nm. The spherical particles seem to have clustered together, and in certain instances, there appear to be crystal formations growing over them. Similarly, the Cu MPs particles are also spherical (**Figure 9**) and sometimes present as aggolmerates. In this case, the size of the particles ranged from 260 to 540 µm and the average particle size was about 420 µm.

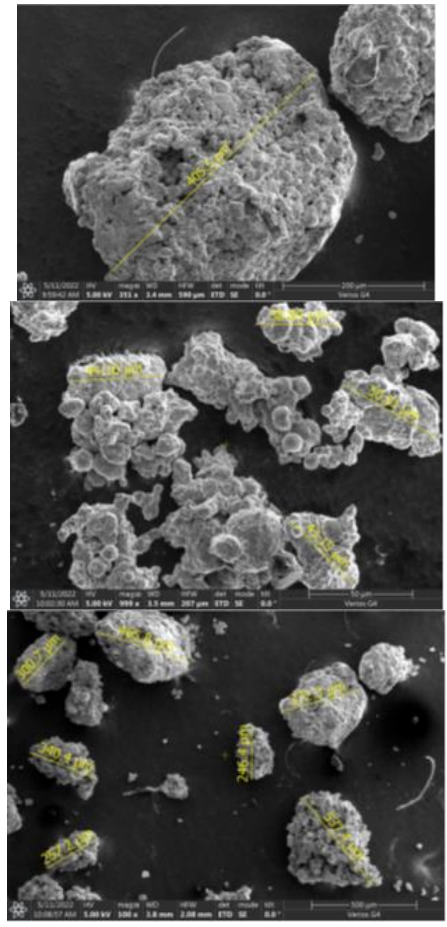

Figure 8: Shows the SEM images of pure Cu Microparticles.

SEM and EDX of Outer Surface View of PP Composites with 2 wt.% Cu NPs is presented in (Figure 10)

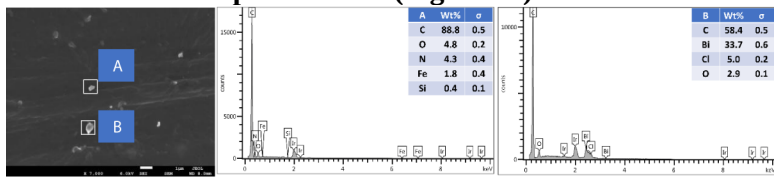

Figure 9: Shows the SEM and EDX of outer surface view of PP composites with 2 wt.% Cu NPs.

SEM and EDX of Cross-Sectional View of PP Composites with 2 wt.% Cu NPs is presented in (Figure 11)

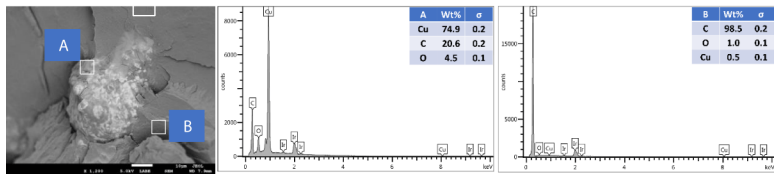

Figure 10: Shows the SEM and EDX of cross-sectional view of PP composites with 2 wt.% Cu NPs.

SEM and EDX of Outer Surface View of PP Composites with 2 wt.% Cu MPs is presented in (Figure 12)

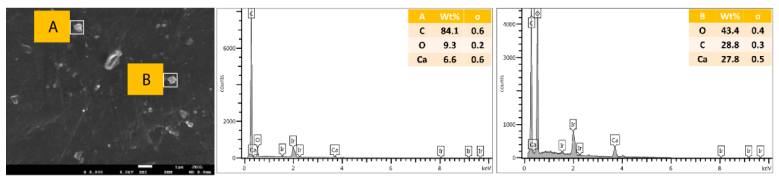

Figure 11: Shows the SEM and EDX of outer surface view of PP composites with 2 wt.% Cu MPs.

SEM and EDX of Cross-Sectional View of PP composites with 2 wt.% Cu MPs is presented in (Figure 13)

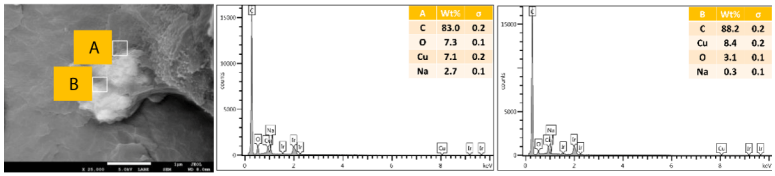

Figure 12: Shows the SEM and EDX of cross-sectional view of PP composites with 2 wt.% Cu MPs.

We further performed SEM-EDX on PP disk samples to better understand the distribution of Cu particles in the composites. We only measured samples containing 2 wt.% of Cu as the sensitivity of the EDX technique is rather poor, with a detection limit of elements at about a 1000 ppm level [21]. The penetration depth is at the micron level, which means, there are overlapping signals from the micron level thick from the surface. We performed analyzes of both the disk surfaces as well as the cross-section to compare the distribution of the Cu within the bulk and the surface of the samples. From the SEM images, we can clearly see the presence of a few particles and agglomerates of Cu, especially for the MPs. For both Cu NPs and MPs, the EDX could not catch the signal of the Cu, meaning that the Cu concentration is below the detection limit of EDX (**Figure 10** and **Figure12**). In contrast, the EDX detected Cu within the bulk of the PP composites, yet at a very low level and irrespective of where the signal was recorded (**A and B zones**, **Figure 11** and **Figure13**), probably due to the detection depth of the technique. Overall, this showed that only a limited amount of Cu is present at the surface and its vicinity which accounts for the anti-microbial performance, and that most of the Cu is located within the bulk of the PP matrix, acting potentially as a reservoir of anti-microbial agents.

Differential Scanning Calorimetry (DSC)

The influence of the anti-microbial additives (loading and size) on the thermal properties of the PP composites was measured by DSC (**Figure 14**). Overall, minor differences between the PP

samples were observed in terms of crystallization temperature (T_c) and melting temperature (T_m) as listed in (**Table 1**). In all cases, T_m was barely affected while minor changes were observed on T_c at 2 wt.% loading of Cu NPs and Cu MPs (up to 2 ºC difference compared to the neat PP sample). Only samples with 2 wt.% of Cu NPs had a noticeable increase in the crystallization degree (from ~40 to ~43%), which overall indicates a slight nucleating aid behavior.

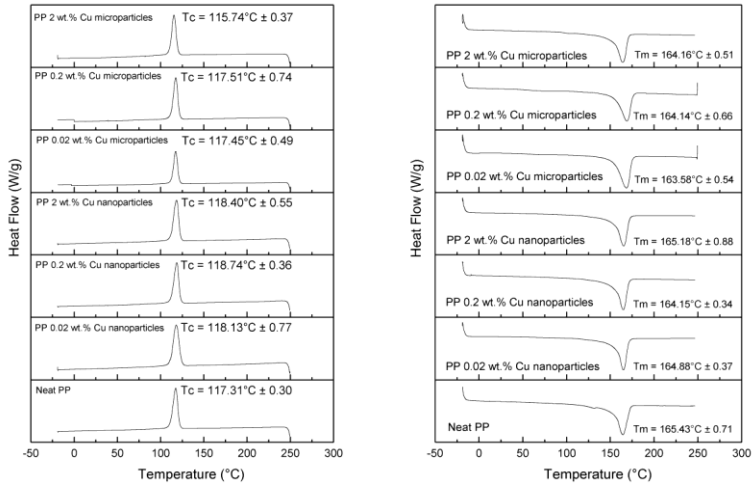

Figure 13: T_c (left) and T_m (right) values were obtained from the DSC data for various samples.

The percentage of crystallinity in various melt-compounded and injection molded PP samples is displayed in (**Table 1**).

Table 1: The degree of crystallinity in PP samples as determined through DSC measurements.

Samples	ΔH_m (J/g)	T_m (°C)	T_c (°C)	Crystallinity (%)
Neat PP	83.59 ± 1.27	165.43 ± 0.71	117.31 ± 0.30	39.99 ± 0.61
PP 0.02 wt% Cu nanoparticles	76.11 ± 1.64	164.88 ± 0.37	118.13 ± 0.77	36.42 ± 0.73
PP 0.20 wt% Cu nanoparticles	84.15 ± 1.43	164.15 ± 0.34	118.74 ± 0.36	40.26 ± 5.73
PP 2.00 wt% Cu nanoparticles	90.23 ± 2.00	165.18 ± 0.88	118.40 ± 0.55	43.17 ± 2.17
PP 0.02 wt% Cu microparticles	74.43 ± 5.47	163.58 ± 0.54	117.45 ± 0.49	35.61 ± 2.74
PP 0.20 wt% Cu microparticles	81.25 ± 7.54	164.14 ± 0.66	117.51 ± 0.74	38.88 ± 1.64
PP 2.00 wt% Cu microparticles	84.48 ± 3.45	164.16 ± 051	115.47 ± 0.37	40.42 ± 3.64

Thermal Gravimetric Analysis (TGA)

The TGA analysis of neat PP and PP composites with the anti-microbial agents were performed to determine their thermos-stability as shown in (**Figure 15**).

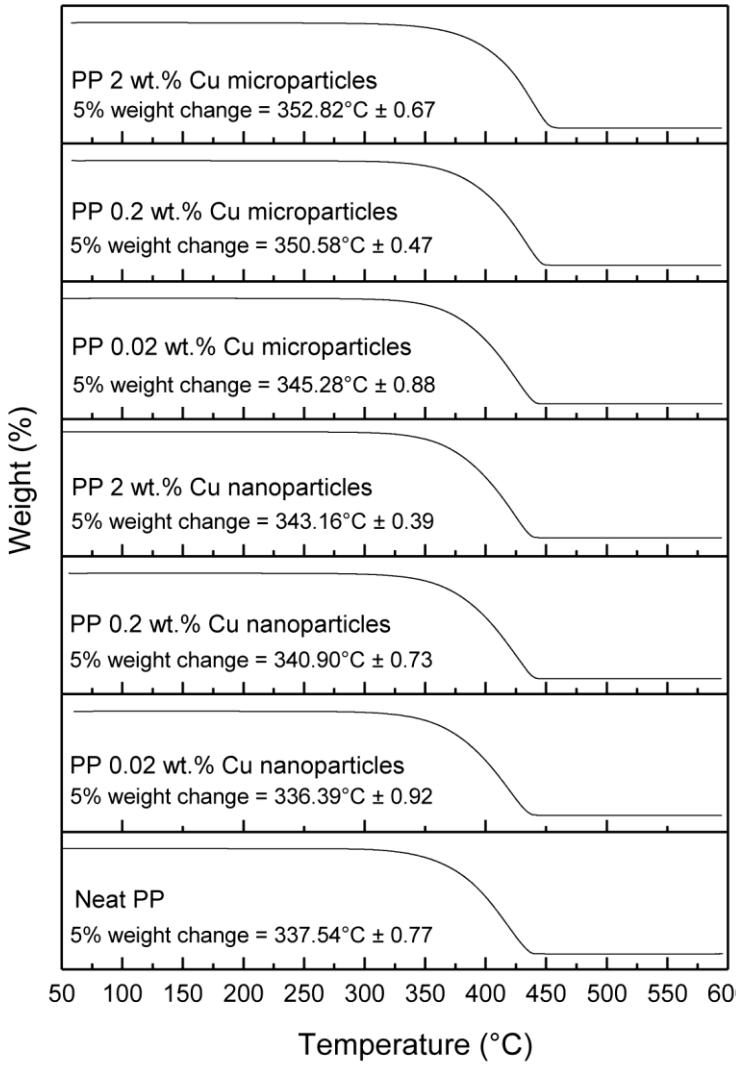

Figure 14: Shows the TGA characterization of neat PP, and various PP composites.

The 5 % weight loss for melt-compounded and injection molded PP disks loaded with Cu NPs at (0.02, 0.2, and 2 wt.%) had occurred at (336.39 ± 0.92 °C, 340.90 ± 0.73 °C, and 343.16 ± 0.39 °C, respectively), and for Cu MPs had occurred at (345.28 ± 0.88 °C, 350.68 ± 0.47 °C, and 352.82 ± 0.67 °C, respectively) as presented in (**Table 2**). Overall, both the Cu NPs and Cu MPs had enhanced the thermal stability of the PP samples at higher loading rate except for Cu NPs at 0.02 wt.% loading.

Table 2: 5 % weight change of PP samples obtained from TGA measurements.

Sample	5 % weight change occurred at temperature (°C)
Neat PP	337.54 ± 0.77
PP 0.02 wt.% Cu nanoparticles	336.39 ± 0.92
PP 0.20 wt.% Cu nanoparticles	340.90 ± 0.73
PP 2.00 wt.% Cu nanoparticles	343.16 ± 0.39
PP 0.02 wt.% Cu microparticles	345.28 ± 0.88
PP 0.20 wt.% Cu microparticles	350.68 ± 0.47
PP 2.00 wt.% Cu microparticles	352.82 ± 0.67

Copper Leachability of The Disks

The leachability of copper into water from the PP disks was measured using the highest Cu loading, as we expected a rather slow phenomenon. The Cu content was measured in the leachate by ICP-MS after 4 and 24 hrs contact time of the PP disks composites (cut into small pieces) with water. We calculated the amount of Cu lost from the initial amount of Cu in the PP (2 wt.%). In this technique, based on the limit of detection of Cu by ICP-MS (0.1 mg/kg), we calculated a LOD of Cu leached vs Cu initially present in PP at ~ 12 ppb level. As can be seen in (**Table 3**), no Cu leaching occurred (below LOD) in PP containing Cu NPs. A slight Cu loss was, however, noticed with Cu MPs, barely reaching 1 ppm after 24 hrs, indicating that, the leaching of Cu is a rather slow phenomenon at room temperature. The anti-microbial results obtained with both samples, it indicates that the Cu NPs do not necessarily work by leaching copper into water and that other surface mechanisms should occur.

Table 3: Amount of Cu leaching out in water vs amount of Cu initialy in PP disks (2 wt.%).

Sample	4 hours	24 hours
PP Cu NPs (2 wt.%)	< 12 ppb	< 12 ppb
PP Cu MPs (2 wt.%)	187 ppb	963 ppb

Conclusion

The "Bacterial Liquid Suspension Test" was successfully used to discriminate the biocidal performance of Cu-based additives in PP. Using this technique, the log reduction of the bacterial count of *E. Coli* was measured after 4 and 24 hrs for PP composites where the type of Cu (NPs or MPs), its loading level in PP (0.02, 0.2 and 2 wt.%) and the shape of PP (powders, pellets, or molded disks) was varied. As anticipated, both the increased loading and the smaller particle size showed a higher anti-microbial activity compared to the bigger particles size as a result of the increased surface area. As anticipated, the technique shows that both the increased loading and the smaller particles size show higher anti-microbial activity compared to the bigger particle size as a result of the increased surface area. Also, PP powder gave a higher bacterial reduction compared to pellets and disks. While the PP with 2 wt.% Cu NPs showed the best anti-microbial performance, the detection of Cu at the surface (using SEM-EDX) and in the water leachate (using ICP-MS) were below the LODs, indicating how potent they are to kill bacteria.

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