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GAMMA-IRRADIATION MODIFIED POLYPROPYLENE AND NANOSILVER HYBRID FILMS-ANTIBACTERIAL ACTIVITY

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ABSTRACT

This paper presents a study of films based on blends of polypropylene (PP) with radiation modified PP and insertion of silver nanoparticles aiming bactericide effect. The use of silver (Ag) gives important antibacterial properties since silver is highly toxic for bacteria.
The blend of 50/50 PP and gamma irradiated PP was processed in a twin screw extruder. The polypropylene was processed for five PP-Nanocomposite AgNPs in different concentrations of 0.25%; 0.5%; 1.0%; 2.0% and 4.0% in wt%. The material was characterized by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), transmission electron microscopy (TEM), cytotoxicity assay and reduction colony-forming unit (CFU). The analyzed films showed agglomeration of silver particles and regions with homogeneous distribution of the particles. The interactions of the nanosilver bactericidal effect with *E. coli* and *S. aureus* were assessed.

Keywords: polypropylene, AgNPs, gamma irradiation, bactericide

1. INTRODUCTION

Commodity isotactic PP possesses many desirable physical properties such as good resistance to chemicals, high stiffness, high rigidity, high service temperature, and good temperature stability compared to other polyolefins [1,2] and its relatively low cost [3]. However, the lack of melt elasticity strain hardening behavior has limited its applications in extrusion foaming, extrusion coating, blow molding, and thermoforming where the extensional viscosity dominates [1,2].

Branching effects answer to crosslinking efficiency in two opposing directions. One effect is suppressing the crosslinking due to the presence of tertiary carbon and another is enhancing

the crosslinking due to increased molecular mobility. The hydrogen atom attached to the tertiary carbon atom at the branching might be particularly susceptible to fracture by radiation [4].

Our institute developed the production of branched PP, based on the grafting of long chain branches on to PP backbone using acetylene as a crosslinker under gamma radiation process. The resulting grafting reactions occurred with rearrangement of the radicals formed in the polymer [5,6] and the main characteristic is the property of melt strength, defined as the resistance of a melt to draw-down, of great importance to all processing technologies where elongational flows and stretching of polymer melt are required. This material is applied to optimize foam extrusion, coating extrusion, blow moulding and fibre spinning [7].

In spite to antimicrobial activity, substances can be incorporated directly into polymeric materials coated onto polymer surface or immobilized into the polymers. Thermal processing such as melt blending, extrusion, and injection molding has been applied for incorporate the antimicrobials into polymers, but the thermal stability of active component and chemical compatibility of polymer matrix and antimicrobials should be considered in order to evenly distribute antimicrobial substance [8].

Silver is particularly attractive because it combines the high toxicity for bacteria with a low toxicity for bacteria with a low toxicity for humans [9].

Important research used silver nanoparticles (AgNPs) with different surfactants, polyvinyl pyrrolidone (PVP) and oleic acid (OA) to facilitate dispersion. The polypropylene-AgNPs compounds were prepared by melt mixing, and the effects of the processing conditions on nanoparticles dispersion were investigated, as well as, antimicrobial properties of polypropylene filled with coated AgNPs [10].

The large surface area of silver nanoparticles provides better contact with microorganisms and in consequence antimicrobial properties are very efficient. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria. The bactericidal property of silver is related to the amount of silver and the rate of silver released. Direct contact between metallic silver and moisture on the skin or fluids in a wound leads to its ionization [11].

The objective of this work is to obtain polypropylene nanocomposite with silver nanoparticles by extrusion processing and to evaluate the biocide action of films obtained ahead bacteria *E. coli* and *S. aureus*.

2. EXPERIMENTAL

2.1. Materials

The isotactic polypropylene with MFI = 1.5 dg min^{-1} and M_w = 338,000 g mol^{-1} Braskem - Brazil, was provided in the form of pellets. The iPP pellets were placed in a nylon container and subjected to a pressure of 110 kPa acetylene 99.8%, supplied by White Martins [12]. The irradiation process of beads was performed in a ^{60}Co source at a dose rate of 5 kGy h^{-1}. The radiation dose used was 12.5 kGy monitored by a Harwell Red Perspex Dosimeter 4034. After irradiation, the granules were subjected to heat treatment at 90 °C for 1 hour to promote

recombination and annihilation of residual radicals [13,14]. The silver nanoparticles (AgNPs) were supplied by Sigma Aldrich.

2.2. Preparation of Polypropylene-AgNPs nanocomposite films

The blend of iPP and PP 12.5 kGy (50/50) was prepared in pellets and was mixed with Irganox B 215 ED in a rotary mixer and maintained under these conditions for 24 hours. After this period the mixture was processed with the addition of AgNPs (0.25, 0.5, 1.0, 2.0, 4.0) wt% in a twin screw extruder Haake co-rotating, Model Rheomex PTW 16/25, with the following processing conditions: temperatures of the zones were 180 to 195 °C and screw speed of 50-100 rpm. Immediately after extruding the material was pelletized. The films PP-AgNPs were obtained by compression molding at 190 °C for 10 min without pressure and 5 min at a pressure of 80 bar, after that it were dipped in a water bath at 23 °C.

2.3. Characterization

2.3.1. Scanning electron microscopy and Energy dispersive spectroscopy

Details of the nanocomposite morphology was investigated using scanning electron microscopy, EDAX equipment brand Philips Model XL-30. The samples were fixed on metal support adequate and coated with gold by sputtering technique. The EDS spectrum of polypropylene-AgNPs films were also obtained.

2.3.2. Transmission electron microscopy

The morphology of the silver nanoparticles was examined by TEM JEOL JEM-2100 operating at an accelerating voltage of 80 kV. The ultrathin sections (80nm) were prepared using an ultra-microtome (Leica EM FC6) at room temperature with diamond knife blade. The samples were prepared on a carbon-coated standard copper grid (400 mesh).

2.3.3. Cytotoxicity assay

The test was conducted based on International Standard Organization (ISO 10993) [15] and in the literature [16] by neutral red uptake methodology. It was used the cell line: NCTC Clone 929 from American Type Culture Collection (ATCC). The cells were cultured in Eagle's minimum medium (MEM) supplemented with 10% fetal bovine serum (FBS), 0.1 mM sodium pyruvate and non-essential amine-acids (MEM-use). High density polyethylene (HDPE) was used as negative control and natural rubber latex film as positive control, 96 wells were used: spread 200 µL of cell suspension containing ($5x10^5$ cells mL^{-1}) in each well, incubated at 37 °C in atmosphere with 5% CO_2 for 24h. The samples and controls extracts were prepared by immersion in MEM-use and incubated during 24h at 37ºC ($1cm^2$ mL^{-1}). A serial dilution was made to obtain the following dilutions: 100, 50, 25, 12.5 and 6.25%. The culture medium of the microplate was replaced by diluted extracts of controls and samples, in triplicates. The extracts were replaced by neutral red solution and the plate was incubated for 3 hours, and after was washed twice with PBS and once with washing solution. Each well received 200 µL of extracting solution. The absorbance were read in a ELISA reader, Sunrise-Tecan at 540nm with 620nm reference filter and the cell viability percentages were calculated in relation to cell control [17].

2.3.4. Reduction colony-forming unit

It was used the adapted standard JIS Z 2801: 2010 [18]. The cell suspension for inoculum was $900x10^6$ CFU mL^{-1} for each tested step. The following procedure was performed separately for each microorganism. Samples of the films of PP-AgNPs were placed in a sterile petri dish and inoculated on the surface of 50 µL of suspension of each organism in an area of 40x40 mm^2. The samples were inoculated for 24 hours at 37 °C.

3. RESULTS AND DISCUSSION

3.1. Scanning electron microscopy and Energy dispersive spectroscopy

The Fig.1 shows SEM images of 6 types of polypropylene films with different nanosilver concentrations. Only in the sample at Fig. 1D was used PVP with nanosilver solution in order to avoid agglomerates of silver nanoparticles.

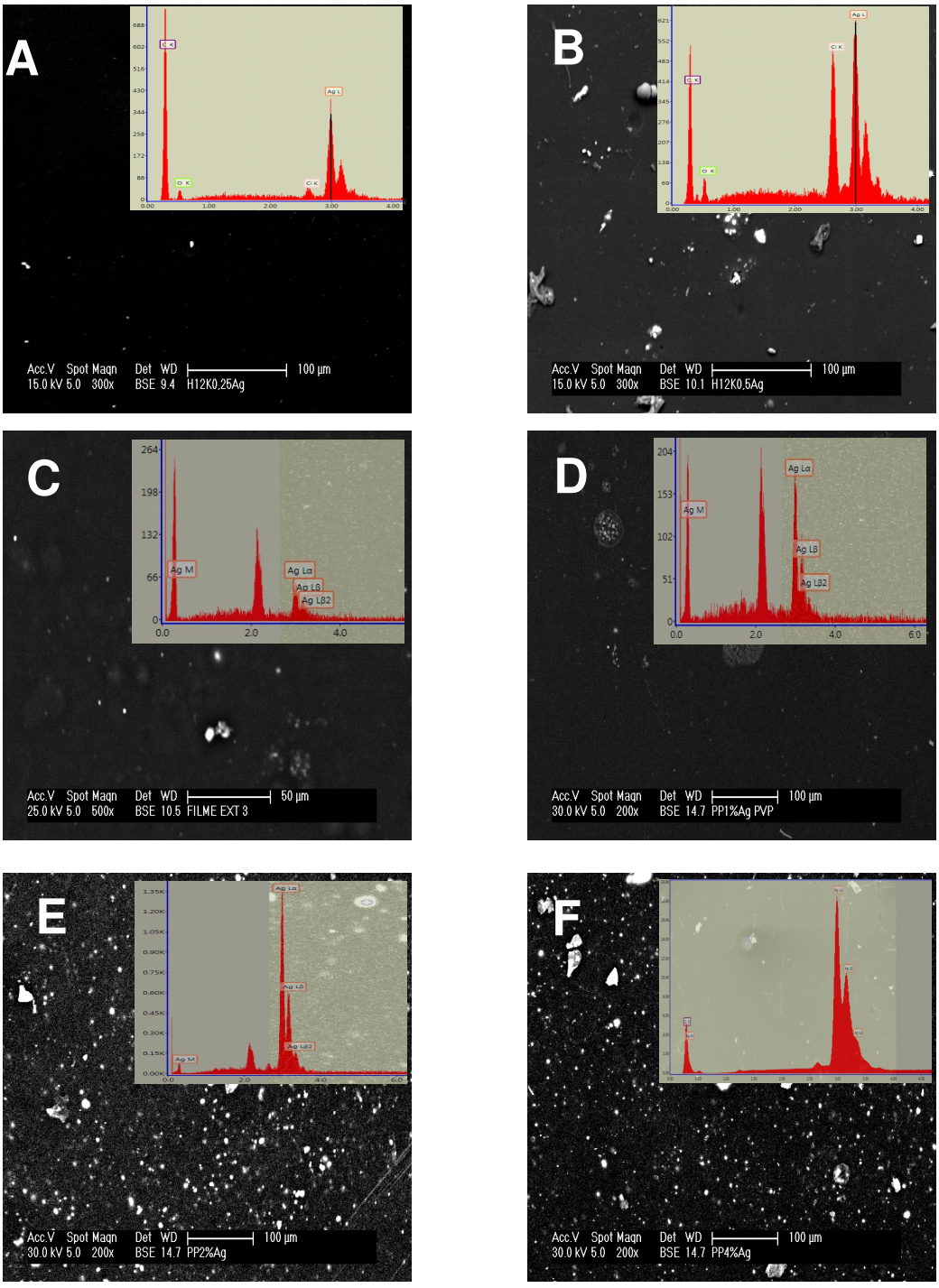

Figure 1: SEM image and EDS mapping of polypropylene films with AgNPs: (A) PP+0.25%AgNPs; (B) PP+0.5%AgNPs; (C) PP+1.0%AgNPs; (D) PP+PVP+1.0%AgNPs; (E) PP+2.0%AgNPs and (F) PP+4.0%AgNPs

In Fig.1, PP films with AgNPs, shows bright spots that corresponds to the presence of silver nanoparticles. The elemental analysis of AgNPs was investigated by EDS and the result, confirmed the presence of silver in films. The peaks around 3.40 keV are energy bands corresponding to AgL [19-21].

3.2. Transmission electron microscopy

TEM images of the PP-AgNPs nanocomposites containing 1 wt% of silver nanoparticles are shown in Fig.2.

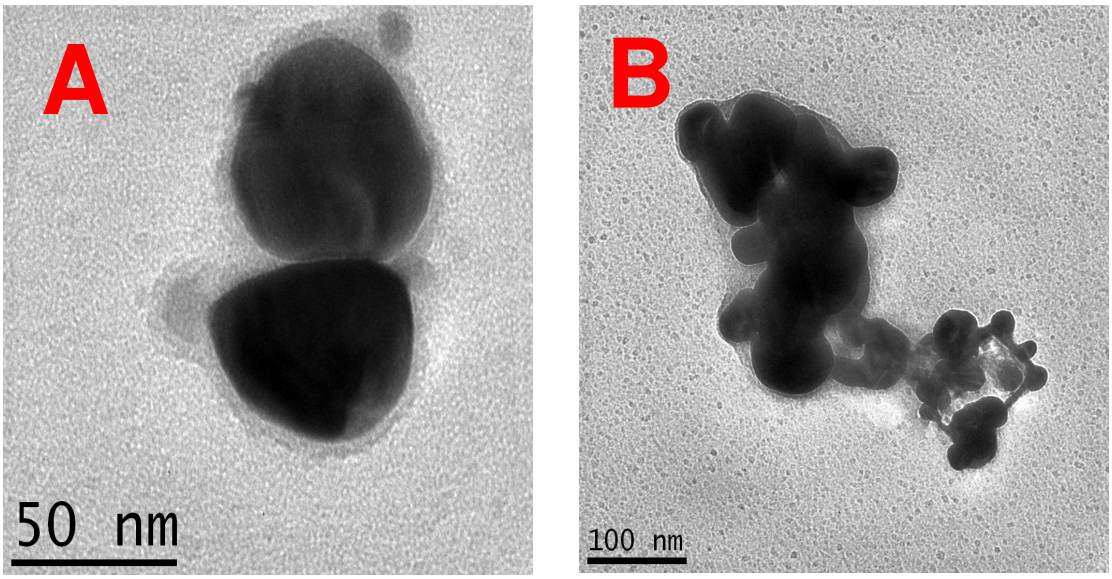

Figure 2: Picture of TEM polypropylene film with AgNPs: (A) PP + 1%AgNPs; (B) PP + 1%AgNPs agglomerates

The TEM image Fig.2A supports the spherical shape of AgNPs as well as the formation of agglomerates, Fig.2B, in polypropylene film. The size of the silver nanoparticles ranges from 50 to 55 nm in diameter.

3.3. Cytotoxicity assay

The sample with cell viability curve above $IC_{50\%}$ line is considered non-cytotoxic and under $IC_{50\%}$ line is considered toxic. The cytotoxicity index was obtained by projecting a line from 50% cellular viability axis to extract concentration, Fig.3.

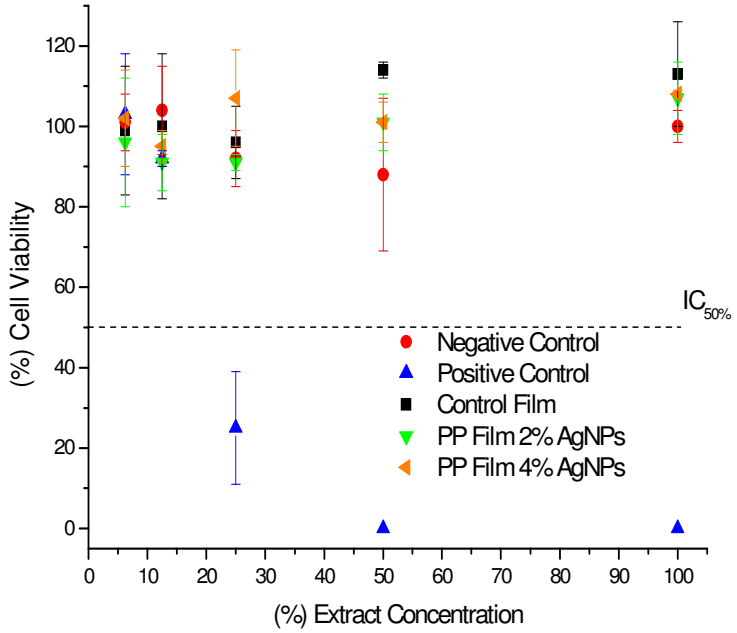

Figure 3:.Cell viability curves of polypropylene film with AgNPs in the cytotoxicity test by the neutral red uptake method

The polypropylene film with AgNPs showed similar behavior to the negative control, i.e., the film is no toxic. The film-PP-AgNPs was characterized as non-cytotoxic in this assay.

3.4. Reduction colony-forming unit

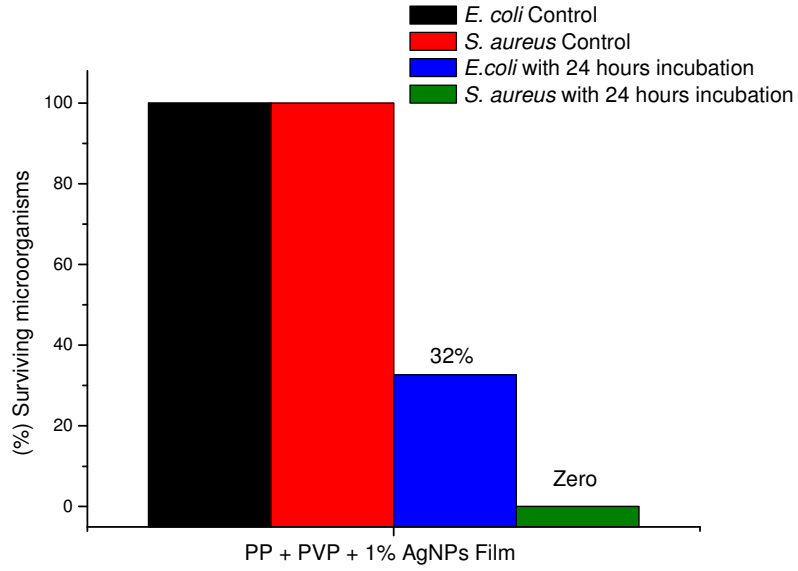

Figure 4: Surviving microorganisms percentage (*S. aureus* and *E. coli*) against polypropylene film with AgNPs

The test of reduction (%) for CFU for the film PP-AgNPs, Fig.4 showed an excellent effect against *S. aureus*, demonstrating the effectiveness of the biocide AgNPs after 24 hours of incubation. The *E. coli* 24 hours incubation left 32% of surviving microorganisms also confirming the efficiency of AgNPs as an antimicrobial agent. For the studied films, only the polypropylene with surfactant PVP and 1% AgNPs presented satisfactory results from the antibacterial interaction.

4. CONCLUSIONS

The addition of coated silver nanoparticles (AgNPs) to a polypropylene matrix by extrusion process represents an interesting solution to increase protection against *S. aureus* and *E. coli*. Some of these AgNPs showed be located inside the PP film which hinders the antibacterial activity but the overall effect was a high antibacterial efficiency. Classic organic biocides have limited their applications due to: low heat resistance, high decomposability, short life and high toxicity. One feasible alternative are inorganic biocides such as polymers composites. Presence of AgNPs also slightly improves thermal stability of PP-AgNPs compounds thus enabling easy processing; this could be done to the interaction between polypropylene chains and (PVP) surfactant-coated silver nanoparticles. Reduction (%) CFU assay showed positive biocide results for *S. aureus* and for *E. coli.* The cytotoxicity test for polypropylene film with AgNPs showed no cytotoxicity effect.

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