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Antimicrobial Properties of SEBS Compounds with Zinc Oxide and Zinc Ions

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***Abstract*—**The increasing demand of thermoplastic elastomers is related to the wide range of applications, such as automotive, footwear, wire and cable industries, adhesives and medical devices, cell phones, sporting goods, toys and others. These materials are susceptible to microbial attack. Moisture and organic matter present in some areas (such as shower area and sink), provide favorable conditions for microbial proliferation, which contributes to the spread of diseases and reduces the product life cycle. Compounds based on SEBS copolymers, poly(styrene-b-(ethylene-co-butylene)-b-styrene, are a class of thermoplastic elastomers (TPE), fully recyclable and largely used in domestic appliances like bath mats and tooth brushes (soft touch). Zinc oxide and zinc ions loaded in personal and home care products have become common in the last years due to its biocidal effect. In that sense, the aim of this study was to evaluate the effect of zinc as antimicrobial agent in compounds based on SEBS/polypropylene/oil/ calcite for use as refrigerator seals (gaskets), bath mats and sink squeegee. Two zinc oxides from different suppliers (ZnO-Pe and ZnO-WR) and one masterbatch of zinc ions (M-Zn-ion) were used in proportions of 0%, 1%, 3% and 5%. The compounds were prepared using a co-rotating double screw extruder (L/D ratio of 40/1 and 16 mm screw diameter). The extrusion parameters were kept constant for all materials. Tests specimens were prepared using the injection molding machine. A compound with no antimicrobial additive (standard) was also tested. Compounds were characterized by physical (density), mechanical (hardness and tensile properties) and rheological properties (melt flow rate - MFR). The Japan Industrial Standard (JIS) Z 2801:2010 was applied to evaluate antibacterial properties against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli (E. coli*). The Brazilian Association of Technical Standards (ABNT) NBR 15275:2014 were used to evaluate antifungal properties against *Aspergillus niger* (*A. niger*), *Aureobasidium pullulans* (*A. pullulans*), *Candida albicans* (*C. albicans*), and *Penicillium chrysogenum* (*P. chrysogenum*). The microbiological assay showed a reduction over 42% in *E. coli* and over 49% in *S. aureus* population. The tests with fungi showed inconclusive results because the sample without zinc also demonstrated an inhibition of fungal development when tested against *A. pullulans, C. albicans* and *P. chrysogenum*. In addition, the zinc loaded samples showed worse results than the standard sample when tested against *A. niger*. The zinc addition did not show significant variation in mechanical properties. However, the density values increased with the rise in ZnO additives concentration, and had a little decrease in M-Zn-ion samples. Also, there were differences in the MFR results in all compounds compared to the standard.

***Keywords*—**Antimicrobial, home device, SEBS, zinc.

I. Introduction

EVERYDAY, new materials classified as TPE (thermoplastic elastomers) are developed to be applied in home use devices, like cell phones, toys and other products [1],[2]. TPE compounds based on block copolymer styrene-ethylene/butylene-styrene (SEBS) are widely used in blends with polypropylene (PP) and oil to form materials with soft touch features [1]. These materials are useful to be applied in skin contact products such as toothbrush cable, bath mats, which are exposed to high humidity levels and the action of a range of microorganisms. Due to difficulty of processing and high cost, the addition of PP, plasticizers and fillers to SEBS is necessary [3]-[6]. The addition of PP in the SEBS forms a co-continuous phase. Moreover, the blend of these polymers gives SEBS better processability, improves the mechanical properties and increases the hardness values besides reducing the production costs [5], [6]. Once TPE compounds are a source of carbon and hydrogen, they can be susceptible to microorganism attack [7]. In that sense, there is an interest in materials that have the ability to inhibit the growth of harmful microorganisms. Zinc oxide (ZnO) and zinc ions (Zn-ions) [8] can be loaded in polymer matrix to provide antimicrobial properties to home care products.

This study aims to evaluate the mechanical performance and antimicrobial efficiency of SEBS based TPE zinc compounds to be used in home device.

II. Experimental

A. Compound Preparation

The compounds (SEBS/PP/oil/calcite) were prepared using a co-rotating double screw extruder with L/D ratio of 40/1 and screw diameter of 16 mm, model AX 16DR (AX Plásticos). The processing parameters were 226 rpm of screw rotation rate, with temperature profile from 150 to 190 ºC. After mixing, the materials were compression-molded using the Haitan (PL 860/260 - B) at 190 ºC.

B. Additives

The additives used in this study were two zinc oxides; one supplied by WR Cerâmica (ZnO-WR) and one supplied by Perrin S.A (ZnO-Pe) and, a zinc ion masterbatch (M-Zn-ion)

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supplied by Cristal Master. The concentrations of the investigated additives are listed in Table I.

TABLE I
IDENTIFICATION OF ZINC LOADED COMPOUNDS

Compound	Additive (% weight)	Chemical composition-identification name
Standard	0	-
C-Pe1	1	ZnO-Pe
C-Pe3	3	ZnO-Pe
C-Pe5	5	ZnO-Pe
C-WR1	1	ZnO-WR
C-WR3	3	ZnO-WR
C-WR5	5	ZnO-WR
C-CM1	1	M-Zn-ion
C-CM3	3	M-Zn-ion
C-CM5	5	M-Zn-ion

C. Compounds Characterization

Density was determined by hydrostatic method based on ASTM D 792.

Tensile properties of the compounds were obtained according to ASTM D 412C, in the EMIC DL 2000 machine. The cross-head speed and gauge length of the apparatus were 500 mm/min and 25 mm, respectively. The hardness Shore A was performed according to ASTM D 2240 in durometer Bareiss, model HPE A.

Melt flow rate (MFR) was performed according to ASTM D 1238 in plastometer Dinisco (LMI 4000). The test temperature was set at 200 °C and nominal load was 5 kg.

Japanese industrial standard (JIS) Z 2801:2010 was applied to evaluate antibacterial efficiency of samples against *S. aureus* and *E. coli* bacterial strains. The result was expressed as a percentage value calculated from the difference between the number of colony forming units (CFU) per square centimeter after the incubation period.

To evaluate the antifungal activity of zinc loaded samples, the Brazilian Association of Technical Standards (ABNT) NBR 15275:2014 was used. The microbiological assay was performed using the following fungal species: *A. niger*, *A. pullulans*, *C. albicans*, and *P. chrysogenum*. The presence of inhibition zone (after 48h incubation) and hyphal growth (after 7 days incubation) were evaluated with a stereoscopic microscope. The results were expressed as a percentage of the specimen area covered by the fungus.

D. Data Analysis

Analysis of variance (ANOVA) was applied in all test results using MYSTAT, student version 12 (Systat Software, Inc., CA, USA). The level of significance was set at 0.05.

III. RESULTS AND DISCUSSION

Fig. 1 shows the variance in density of compounds according to their additive concentration. There were significant differences in the density values between the loaded compounds. The density values from ZnO-Pe (C-Pe) and ZnO-WR (C-WR) loaded compounds increased with the increment of ZnO concentration. Zinc oxide has the density of 5.6 g/cm^3 [9]; thus, its addition leads to the rise of compounds density. However, the Zn ion masterbatch loaded compounds (C-CM) presented, according data analysis, no significant difference in density values compared to the standard. This result was expected; due to the density of the additive used (0.90-1.10 g/cm^3- supplier datasheet).

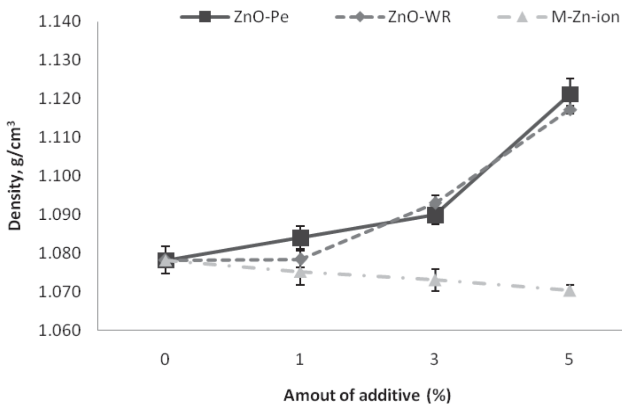

Fig. 1 Variance in density values in zinc loaded compounds

The mechanical properties (hardness and tension) of the compounds with different amount of zinc microparticles are shown in Table II.

TABLE II
MECHANICAL PROPERTIES

Compound	Hardness (Shore A)	Tensile Strength (MPa)	Elongation at Break (%)
Standard	80±0.6	10.8±1.1	662±23
C-Pe1	78±0.4	11.7±0.8	697±21
C-Pe3	79±0.6	12.1±0.6	726±13
C-Pe5	80±0.6	12.4±0.7	711±18
C-WR1	78±0.7	11.6±0.7	739±11
C-WR3	79±0.7	12.0±0.6	726±10
C-WR5	79±0.4	11.9±0.6	720±13
C-CM1	79±0.7	12.2±0.5	734±9
C-CM3	79±0.3	12.1±0.6	736±5
C-CM5	78±0.3	12.5±0.4	741±7

Taking into account each additive, there were observed no significant difference in tensile strength and elongation at break values between the concentrations used. However, an increase in tensile properties values was observed for all zinc loaded compounds when compared to the standard. The results found in tensile strength and elongation at break probably indicates that the addition of zinc, even in a small amount, may have caused a reinforcing effect on the matrix of SEBS/PP. The difference in hardness values was not meaningful, considering the repeatability of the test method.

Fig. 2 shows the variance in MFR of compounds according to their additive concentration. In all zinc loaded samples were observed an increase of MFR values. It was noted that in low amount of additive, there was an increase in MFR, but with high amount, this value decreases. In low amounts, the particles are able to slide in the melt polymer, but in higher amounts of filler there is a strong particle-particle interaction which hinders the flow [10].

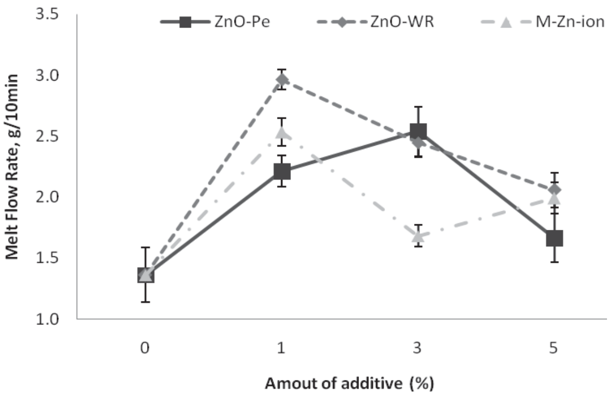

Fig. 2 Variance in MFR values in zinc loaded compounds

Antibacterial properties of zinc loaded compounds were evaluated against *S. aureus* and *E. coli* and compared to the standard samples. Fig. 3 shows the variance in bacterial populations in the standard and loaded samples.

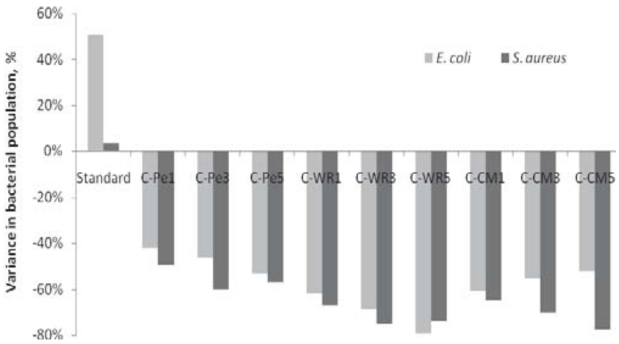

Fig. 3 Variance in bacterial population in zinc containing TPE compounds according to the additive content.

It was observed that standard sample was susceptible to the growth of both bacterial species, showing an increase of 50.7% and 3.5% of *E. coli* and *S. aureus*, respectively. The bacterial proliferation in standard samples was probably due to the absence of antibacterial additive.

In a variable degree, all zinc loaded samples presented an antibacterial effect. The microbiological tests showed a reduction over 42% in *E. coli* and over 49% in *S. aureus* population. It could be noted that Gram-negative bacteria (*E. coli*) were less sensitive to the action of zinc additives. Previous studies reported that lower concentrations of ZnO did not cause any toxic effect to *E. coli* bacteria [11]. On the other hand, Zn loaded samples showed great biocidal effect toward the Gram-positive bacteria (*S. aureus*). These variances may be explained by the nature of the bacterial cell layer, and also due the oxidation of cellular structure with the disorder of the bacterial antioxidant (glutathione) as reported by [12].

Among the Zn additives, the results indicated that ZnO-WR (C-WR) was the most effective against Gram-positive and Gram-negative strains, when compared to the ZnO-Pe (C-Pe) and M-Zn-ion (C-CM) compounds. The small particle size (1.05 µm - results not shown) in ZnO-WR can, in part, explain the results since small particles have better antibacterial effect [13]. Moreover, it is worth to mention that in ZnO-Pe (C-Pe) and ZnO-WR (C-WR) loaded compounds, the concentration of 3% presented improvements in antimicrobial effect. Altan et al. [14] performed an antimicrobial study using nano-ZnO filled composites and reported that ZnO loadings of 1% and 3% presented better antimicrobial results. These findings support the idea that, in high ZnO amounts, like 5%, antibacterial property became worse. This fact can be related to the decrease of photocatalytic activity in agglomerate samples.

In this study, loaded and nonloaded TPE compounds demonstrated resistance to attack by the most of fungus species tested, except *A. niger* (see Table III and Fig. 4). According to [15], the fungicide action of ZnO is related to the harmful effect against fungal hyphae formation. However, the zinc loaded samples showed worse antifungal results than the standard sample when tested against *A. niger*. Muñoz-Bonilla et al. [16] reported that zinc oxide nanoparticles did not produce any activity against *A. niger* in polymers, like polyamide 6 and low density polyethylene. Assumptions can be made considering the morphology and oxidative stress of zinc particles as a factor of toxicity [17].

TABLE III
VARIATION IN *A. NIGER* POPULATION

Compound	Growth
Standard	10%
C-Pe1	30%
C-Pe3	30%
C-Pe5	60%
C-WR1	40%
C-WR3	60%
C-WR5	40%
C-CM1	50%
C-CM3	30%
C-CM5	20%

IV. CONCLUSION

The zinc oxide based additive ZnO-WR (C-WR) was the most effective against Gram positive and Gram negative bacteria and fungus attack. Further studies are required to understand why susceptibility against *A. niger* increase with the incorporation of Zinc in the compounds. The variations in mechanical characteristics are not an exclusion factor to the industrial application of the compound. Finally, the use of commercial ZnO to produce compounds with antimicrobial properties is promising.

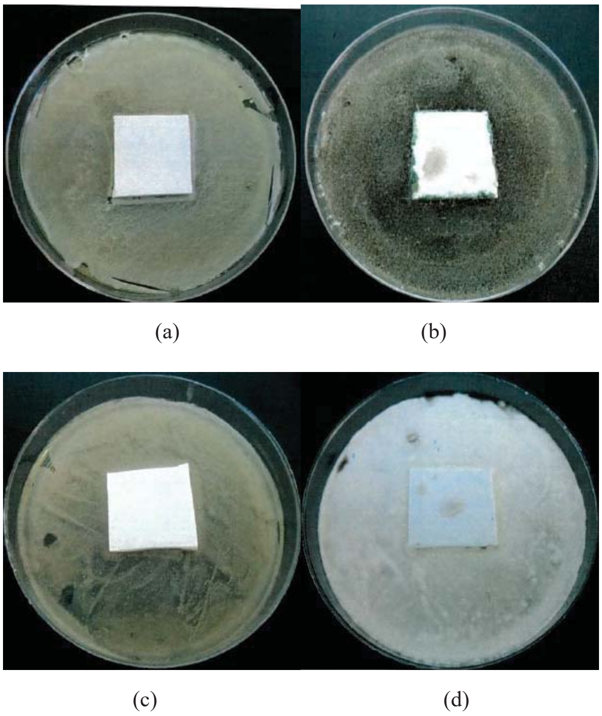

Fig. 4 Antifungal activity of zinc loaded samples: (a) *C. albicans;* (b) *A. niger*; (c) *A. pullulans* and (d) *P. chrisogenum*

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