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Full Length Article

Effect of natural ageing on surface of silver loaded TPE and its influence in antimicrobial efficacy

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ABSTRACT

The aim of this study is to characterize the modifications in silver loaded TPE surfaces exposed to weathering and their relation to susceptibility to microbial attack. Silver loaded TPE materials were exposed to natural ageing for nine months and modifications in antimicrobial properties and surface characteristics were evaluated. Chemical changes were investigated by using the infrared spectra. The average surface roughness and topography were determined by atomic force microscopy. Contact angle was measured to verify wettability conditions and surface free energy (SFE). After nine months of exposure, a decrease in the antimicrobial properties of loaded TPE compounds was observed. A reduction in surface roughness and improvement in wettability and high values of polar component of SFE were verified. The best antibacterial action was noticed in the sample with high Lewis acid force, lower roughness and lower carbonyl index.

1. Introduction

Styrene-ethylene/butylene-styrene copolymer (SEBS)-based thermoplastic elastomers (TPE's) is widely used to produce devices with soft touch features. Bacterial contamination in SEBS-based TPE surfaces represents a health problem mainly in nosocomial settings. Despite SEBS-based TPE's is being used in a broad range of applications, there is a lack of information about the processes involved in microbial colonization of this type of surface. In addition, the different sorts of substances used in the processing of these polymers, such as the plasticizer (mineral oil) [1], can be a carbon source, with an influence on microbial proliferation by providing nutritional sources for the microorganisms' consumption [2,3].

Biofilm formation begins with the attachment of microorganisms to a surface followed by the permeation of the cells inside extracellular substances originated from bacterial metabolism, ensuing biofilm maturation which is no longer possible to remove with usual cleaning methods [4]. To prevent biofilm formation, one strategy is to develop materials less susceptible to bacterial attachment. Seeking this aim, many studies have been conducted to create antimicrobial materials either by incorporating biocidal additives [5,6] or through surface modification [7–9].

A study performed by this research group proved the antimicrobial efficacy of silver loaded TPE [6]. An ensuing study showed that upon the exposition to natural ageing, even with the amount of silver unchanged, the loaded TPE compounds presented a reduction in their biocide action [10]. These results suggesting that, modifications in the polymer surface can be more significant to microbial colonization, than the leach of antimicrobial additive. This means that some characteristics of polymer surfaces can provide conditions to microbial attachment even in materials loaded with biocide substances. Pursuing this questioning, studies have been done relating the characteristic of surface of implantable materials and prostheses [11,12] and the polymer biodegradation process [3,13]. In all these studies the rating of wettability, carbonyl groups presence, modification in surface free energy and topography were addressed; however, further clarifications are needed.

Reckoning with the above discussions, the aim of this study is to characterize the modifications on silver loaded TPE surfaces exposed to weathering and to relate polymer surface characteristics to its susceptibility to microbial attack. The research hypotheses are that surface properties (wettability, surface free energy and topography) may have an effect on bacterial adherence and development

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in TPE surface and, thereafter, in the loss of antimicrobial efficacy of silver loaded compounds.

2. Materials and methods

2.1. Materials

The additives tested were bentonite organomodified with silver (referred to here as "Ag^+_bentonite"), silver ions supported in phosphate glass (referred to here as "Ag^+_phosphate") and nanosilver adsorbed on fumed silica (referred to here as "AgNp_silica"). The proportions used were 2.0%, 0.3% and 0.05% which were set in line with previous studies. The main characteristics of the additives are shown in Fig. 1 based on a previous characterization reported in Tomacheski et al. [14]. The additives were incorporated into a TPE formulation compounded by styrene-ethylene/butylene-styrene copolymer (SEBS, 32% styrene, ethylene/butylene 32/68, linear, Mw 214.8 Da) polypropylene homopolymer (PP, melt flow index 1.5 g 10 min^{-1} at 230 °C), and white mineral oil (64% paraffinic and 36% naphtenic), at a ratio of 30/20/50, respectively. An antioxidant was added at a proportion of 0.1% to prevent thermal degradation during processing. An additive-free compound (Standard) was also used.

2.2. TPE compound preparation

The samples were prepared using a co-rotating double screw extruder (L/D 40 and 16 mm screw diameter, AX Plásticos) with temperature profile from 170 °C to 190 °C, speed of 300 rpm, feed rate of 1.5 kg h^{-1} and melt discharge temperature of 200 °C. Test specimens in 2 mm thick plate form were prepared using injection molding machine (Haitian, PL860) at 190 °C and an injection pressure of 17 bars.

2.3. Natural ageing experiments

The specimens were exposed to weathering for nine months (from August 2015 to May 2016), under real climatic conditions in accordance with ASTM 1435-13. The specimens were exposed in an outdoor station with 30° inclination from the ground located in Campo Bom city, southern Brazil (29°40′54"S and 51°03′25"W), 20 m above sea level. The samples were collected every three months to study the effects of weathering.

2.4. Analytical methods

2.4.1. Microbiological studies

Japan Industrial Standard (JIS) Z 2801:2010 was applied to evaluate antimicrobial abilities of metal loaded TPE samples against *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and *Escherichia coli* ATCC 8739 (*E. coli*). After weathering exposure, the TPE specimens (50 mm × 50 mm) were placed in a sterile Petri dish, and 400 μL of bacterial suspension of *E. coli* and *S. aureus* were inoculated on the specimen surfaces. All of them were incubated for 24 h at 35 ± 1 °C. The results were expressed as a microbial value calculated from the difference between the number of colony forming units (CFU) per square centimeter at zero hour (initial) and after 24 h of incubation.

The Brazilian Association of Technical Standards (ABNT) NBR 15275 was used to evaluate the additive compounds' antimicrobial properties against the fungus *Aspergillus niger* (*A. niger*) ATCC 6275. TPE specimens (25 mm × 25 mm) were placed in a sterile Petri dish with agar, and 100 μL of fungus suspension were inoculated on the specimen surfaces. All of them were incubated for 7 days at 30 ± 2 °C. The presence of inhibition zone (after 48 h of incubation) and hyphal growth (after 7 days of incubation) were evaluated with a stereoscopic microscope. The results were expressed as a percentage of the specimen area covered by the fungus.

2.4.2. Biofilm formation on SEBS-based TPE samples

Scanning electron microscopy (SEM) was performed to verify *in vitro* susceptibility of non-loaded TPE samples (Standard) exposed to the bacteria *Escherichia coli* ATCC 8739 (*E. coli*) and *Staphylococcus aureus* ATCC 6538 (*S. aureus*). To this end, the Standard sample was incubated for 24 h at 35 ± 1 °C with inoculums of *E. coli* and *S. aureus*. After incubation, the TPE samples were immersed in a glutaraldehyde buffer (1.2 mL glutaraldehyde 25%, 5.0 mL 0.2 M PO_4^{3-}

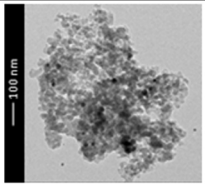
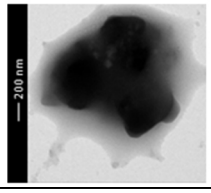
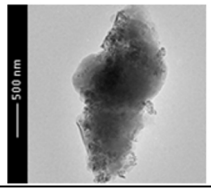
	Micrographs obtained by TEM	Average diameter by TEM, μm	Diameter by laser diffraction, μm		SSA, m^2 g^{-1}	Zeta potential at pH, mV	
A		0.02	D_{10}	4.70	293.90	3	0.12
			D_{50}	9.20		5	-6.48
			D_{90}	28.99		7	-27.70
			Average	12.97		9	-27.53
						11	-35.77
B		1.0	D_{10}	0.86	6.16	3	15.7
			D_{50}	1.50		5	6.82
			D_{90}	2.49		7	-21.80
			Average	1.61		9	-1.06
						11	-3.64
C		-	D_{10}	2.08	36.73	3	-8.31
			D_{50}	6.32		5	3.77
			D_{90}	13.92		7	-33.53
			Average	7.30		9	-32.17
						11	-42.30

Fig. 1. Micrographs and average diameter determined by transmission electron microscopy (TEM), diameter determined by laser diffraction, surface specific area (SSA), and zeta potential at different pH determined for A) AgNp_silica, B) Ag^+_phosphate, and C) Ag^+_bentonite.