## **TITLE:** ANTIMICROBIAL AND ANTIBIOFILM PROPERTIES OF FOUR OZONAZED POLYUREA FORMULATIONS

**AUTHORS:** REINALDI, J.S.; HIGA, B.; CINTRA, B.S.; ANDRADE, G.P.; PIRES, R.H.; MOLINA, E.F.; FERREIRA, J.C.

**INSTITUTION:** University of Franca, Franca, SP (201 Dr. Armando de Sales Oliveira, 14404-600, Franca – SP, Bazil)

## ABSTRACT:

Hydrogels, three-dimensionally organized polymeric structures, have been be used as drug delivery systems. Ozonized vegetable oils have a high concentration of reactive species that offer them germicidal and antibiofilm abilities. The aim of the present study was to describe the antimicrobial and antibiofilm actions, degree of swelling and categories of absorption bands of four ozonized polyurea formulations. Four increasing volumes of ozonized sunflower oil were added to the liquid form of a polyurea-based hydrogel (G1, G2, G3 and G4). Pure polyurea was used as a control group (CG). Standardized samples of the hydrogels (n=3 samples/group) were submitted to infrared spectroscopy and weighing after submerging in distilled water to characterize the absorption bands and the degree of swelling, respectively. The antimicrobial and antibiofilm activity were evaluated against Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 14948, Candida albicans ATCC 90028 and Candida tropicalis ATCC 13803. The hydrogels were submerged in microbial suspensions in a 24-well plate and the suspensions were cultured to enumerate the colony forming units (CFU mL<sup>-1</sup>). Formulations showing germicidal action were tested for antibiofilm ability and were stored for 12 months, when the antimicrobial activity was re-evaluated. Biofilms were grown in 96-well plates. Hydrogel samples were placed on the biofilms for 24h and culture of the suspension was performed to enumerate the CFU mL<sup>-1</sup>. In addition to the absence of the band at 1746 cm<sup>-1</sup> only in the GC, the absorption band related to Amide I (1643-1557 cm<sup>-1</sup>) was detected in all groups. The degree of swelling increased (P<0.001) in all submerged samples, but it was inversely proportional (P<0.001) to the volume of oil added. Similar to the CG, groups G1, G2 and G3 were shown to be inefficient in the antimicrobial test. However, the G4 hydrogel showed germicidal effect against fungi and bacteria and partial antimicrobial action even after 12 months of storage. Finally, the G4 hydrogel reduced the number of CFU mL<sup>-1</sup> from the biofilms of S. aureus, Escherichia coli, C. albicans and C. tropicalis. Considering its antimicrobial and antibiofilm potentials, the hydrogel formulation containing ozonized sunflower oil is promising to the medical community.

Keywords: bacteria, biofilm, fungi, hydrogel, ozone.

**Development Agency:** FAPESP (2020/06265-0), CAPES (001), OZONE&LIFE<sup>®</sup>