ANTIBACTERIAL COMPOUND BASED ON SILICONE RUBBER AND ZnO AND TiO_2 NANOPARTICLES FOR THE FOOD AND PHARMACEUTIC INDUSTRIES. PART II - BIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION

Mihaela NIŢUICĂ (VÎLSAN)[1*], Maria SÖNMEZ[1], Mihai GEORGESCU[1], Maria Daniela STELESCU[1], Laurenţia ALEXANDRESCU[1], Dana GURĂU[1], Carmen CURUŢIU[2,3], Lia Maria DIŢU[2,3]

[1]INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu St., sector 3, Bucharest,

mihaela.nituica@icpi.ro, mihaelavilsan@yahoo.com

[2]University of Bucharest, Faculty of Biology, 1-3 Portocalelor St., 6th district, 060101, Bucharest, Romania

[3]Research Institute of University of Bucharest, 36-46 M. Kogalniceanu St., 5th district, 050107, Bucharest, Romania

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ABSTRACT. The aim of this paper is the biological and morphological characterization of an antibacterial compound based on silicone rubber (Elastosil R701/70-OH), reinforced with ZnO and TiO_2 nanopowders (with antibacterial, antifungal and antimicrobial properties), charged (chalk), crosslinked with peroxide (dicumyl peroxide - powder 40% with calcium carbonate and silica, specific to the food, pharmaceutical, but also medical field). ZnO and TiO_2 nanopowders, by their homogeneous dispersion in the mass of the compound, have a determined role on its antibacterial and antimicrobial sterilization properties. The mixture of antibacterial compound based on silicone rubber was made on a laboratory roller, provided with cooling, in the form of a sheet (maximum thickness 5 mm), in strict compliance with the technological recipe and the established mode of operation. Subsequently, the mixture is processed into forming molds at predetermined parameters by determining the vulcanization time using the Monsanto rheometer (to determine the optimum temperature and vulcanization times), but also the biological and morphological characterization, according to current standards in specific food and pharmaceutical environments.
KEY WORDS: silicone elastomer, biological characterization, nanopowders, antibacterial

COMPOUND ANTIBACTERIAN PE BAZĂ DE ELASTOMER SILICONIC ȘI NANOPARTICULE DE ZnO ȘI TiO_2 PENTRU DOMENIUL ALIMENTAR ȘI FARMACEUTIC. PARTEA II – CARACTERIZARE BIOLOGICĂ ȘI MORFOLOGICĂ

REZUMAT. Scopul acestei lucrări este caracterizarea din punct de vedere biologic și morfologic a unui compound antibacterian pe bază de cauciuc siliconic (Elastosil R701/70-OH), ranforsat cu nanopulberi de ZnO și TiO_2 (cu proprietăți antibacteriene, antifungice și antimicrobiene), șarjat (cretă), reticulat cu peroxid (peroxid de dicumil - pulbere 40% cu carbonat de calciu și silice, specific domeniului alimentar, farmaceutic, dar și medical). Nanopulberile de ZnO și TiO_2 prin dispersia lor omogenă în masa compoundului au rol determinant asupra proprietăților de sterilizare antibacteriană și antimicrobiană ale acestuia. Amestecul de compound antibacterian pe bază de cauciuc siliconic s-a realizat pe un valț de laborator, prevăzut cu răcire, sub forma unei foi (grosime de maxim 5 mm), cu respectarea strictă a rețetei tehnologice și a modului de operare stabilit. Ulterior amestecul este prelucrat in matrițe de formare la parametrii prestabiliți prin determinarea timpului de vulcanizare cu ajutorul reometrului Monsanto (pentru stabilirea temperaturii și timpilor de vulcanizare optimi), dar și a caracterizării biologice și morfologice, conform standardelor în vigoare în medii specifice domeniului alimentar, dar și farmaceutic.
CUVINTE CHEIE: elastomer siliconic, caracterizare biologică, nanopulberi, antibacterian

COMPOSÉ ANTIBACTÉRIEN À BASE DE CAOUTCHOUC DE SILICONE ET DE NANOPARTICULES DE ZnO ET TiO_2 POUR LES INDUSTRIES ALIMENTAIRE ET PHARMACEUTIQUE. PARTIE II - CARACTÉRISATION BIOLOGIQUE ET MORPHOLOGIQUE

RÉSUMÉ. L'objectif de cet article est la caractérisation biologique et morphologique d'un composé antibactérien à base de caoutchouc silicone (Elastosil R701/70-OH), renforcé de nanopoudres de ZnO et TiO_2 (aux propriétés antibactériennes, antifongiques et antimicrobiennes), chargé (craie), réticulé au peroxyde (peroxyde de dicumyle - poudre à 40% avec carbonate de calcium et silice, spécifique aux domaines alimentaire, pharmaceutique et médical). Les nanopoudres de ZnO et TiO_2, par leur dispersion homogène dans la masse du composé, ont un rôle déterminant sur les propriétés de stérilisation antibactériennes et antimicrobiennes. Le mélange de composé antibactérien à base de caoutchouc silicone a été réalisé sur un rouleau de laboratoire, avec refroidissement, sous forme de feuille (épaisseur de maximum 5 mm), en respectant strictement la recette technologique et le mode de fonctionnement établi. Par la suite, le mélange est transformé en moules de formage à des paramètres prédéterminés en déterminant le temps de vulcanisation à l'aide du rhéomètre Monsanto (pour déterminer la température optimale et les temps de vulcanisation), mais aussi la caractérisation biologique et morphologique, selon les normes actuelles dans des environnements alimentaires et pharmaceutiques.
MOTS-CLÉS : élastomère de silicone, caractérisation biologique, nanopoudres, antibactérien

[*] *Correspondence to: Mihaela NIȚUICĂ (VÎLSAN), INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu St., sector 3, Bucharest, mihaela.nituica@icpi.ro, mihaelavilsan@yahoo.com*

INTRODUCTION

Silicone elastomers – silicone rubber is generally processed at high temperatures up to a maximum of +320°C (the processing temperature range is between -100°C to above +320°C), is indoor, non-toxic, has resistance to ozone, resistance to low temperatures and environments (-100°C) while maintaining its properties, also has excellent electrical insulation performance. Silicone rubber is preferred in food, pharmaceutical and medical fields due to these properties and its ease of processing and modeling, but also due to its sterilization properties, as a result of processing at high temperatures up to +320°C [1-7].

The development of new materials coated with various nanoparticles in order to inhibit bacterial adhesion to the substrate, thus eliminating their chance to trigger an infectious process has been intensively studied recently [8-10]. The nanoparticles normally have a high surface/volume ratio, ensuring a much more effective antibacterial and antifungal activity. So, their dispersion in elastomeric compounds have a decisive role in influencing the antimicrobial and antibacterial sterilization properties [11]. Due to their antimicrobial, antibacterial and antifungal activity, ZnO and TiO_2 nanoparticles are used for countless applications in everyday life, such as medical devices, dental implants, the textile industry, plastics, antibacterial coatings, building materials, etc. [12-16].

The aim of the paper was to characterize an antibacterial compound biologically, in specific environments (for the pharmaceutical and food industries: *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25992 and *Candida albicans* ATCC 10231) [17] and morphologically. The tested antibacterial compound is based on silicone elastomer (rubber Elastosil R701/70-OH – the majoritarian material), charged with chalk, stearin as plasticizer, crosslinked with dicumyl peroxide on silica support and reinforced with zinc oxide and titanium dioxide nanopowders in different percentages, obtained by the technique of mixing on an electric roller. The test specimens and the related characterizations were obtained in the electric press at vulcanization times and temperatures established from the rheological analysis [18-22].

EXPERIMENTAL

Materials

Materials used to obtained the antibacterial compound based on siliconic elastomer, reinforced with nanopowders are: Elastosil R701/70-OH (silicon rubber: polydimethylsiloxane with vinyl groups, dynamic viscosity over 9.000.000 mPa*s, in the form of paste, density – 1.32 g/cm^2, colour – opaque); stearin (white flakes, moisture - 0.5% max, ash – 0.025 % max); ZnO (microparticles: white powder, precipitate 93-95%, density – 5.5 g/cm, specific surface – 45-55 m^2/g); ZnO (zinc oxide nanoparticles- white powder, size particles 20 nm, molecular mass – 98.87 g/mol, specific surface area – 23 m^2/g, density – 4.26 g/ml, and concentration – 99,5%); TiO_2 (titanium dioxide nanoparticles: white nanopowder, particle size 21 nm, molecular mass – 79.87 g/mol, specific surface area – 23 m^2/g, density – 4.26 g/ml, assay ≥ 99.5 % trace metals basis); chalk ($CaCO_3$ precipitate – white powder, molecular weight 100.09); PD (dicumyl peroxide, powder 40% with calcium carbonate and silica - Perkadox 14-40B (1.65 g/cm^3 density, 3.8% active oxygen content, pH 7, assay: 39.0-41.0%)).

For the biological characterization were used the following materials: *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25992; *Candida albicans* ATCC 10231. The above-mentioned strains come from American Type Culture Collection (ATCC, US).

Methods

Preparation of Antibacterial Compound

The antibacterial compound mixtures were made by the mixing on a laboratory roller provided with cooling, with strict observance of the technological recipe, Table 1, and the established mixing times (Figure 1) [21]. The silicone rubber - Elastosil R701/70-OH (elastomer) was plasticized between the rolls for approximately 2.5-3 minutes, then the stearin is added and the mixing is continued for 2 minutes. The ZnO microparticles were blended for a maximum of 2 minutes until the mixture became homogeneous, and the ZnO and TiO_2 nanopowders were added to the mass of the compound continuing the mixing for 3 minutes. Then mixing was continued for 3 minutes, then chalk was added, and after that the last ingredient was added - PD (dicumyl peroxide), continuing mixing for 3 minutes. The mixture is homogenized on the roll mill for maximum 2 minutes and taken off in the form of a 3-4 mm thick sheet. After the optimal vulcanization time is established, Figure 1 [21], the specimens are processed in the electric press, then subjected to physical-mechanical, chemical, biological and morpho-structural characterization, according to the standard in force, after their stabilization for 24 h at room temperature [21].

Table 1: Formulation of antibacterial compounds based on silicone rubber reinforced with TiO_2 and ZnO nanoparticles [21]

Symbol	MU [g]	CS_1 (control)	Sample 2	Sample 3	Sample 4
Silicone rubber (Elastosil R701/70-OH)	g	150	150	150	150
Stearin (flakes)	g	7.5	7.5	7.5	7.5
Zinc oxide (active powder)	g	6	4.5	3	1.5
Zinc oxide (nanopowder)	g	-	1.5	3	4.5
Titanium dioxide (nanopowder)	g	-	1.5	3	4.5
Chalk ($CaCO_3$ - powder)	g	15	15	15	15
Dicumyl peroxide (PD)	g	11.25	11.25	11.25	11.25

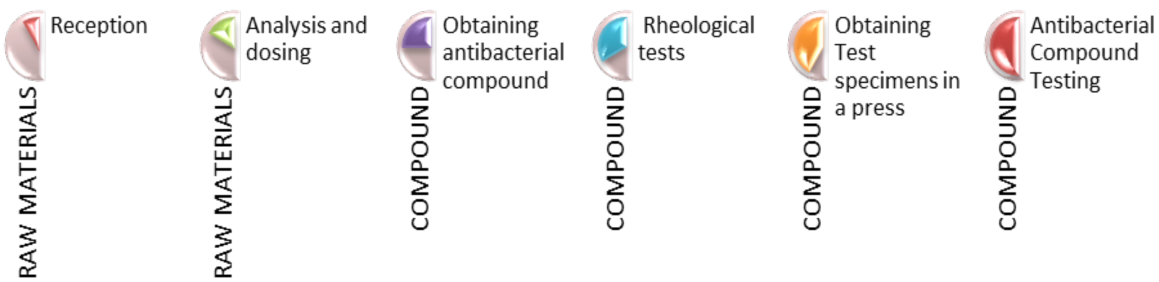

Figure 1. Technological process of obtaining the antibacterial compound reinforced with ZnO and TiO_2 nanopowders [21]

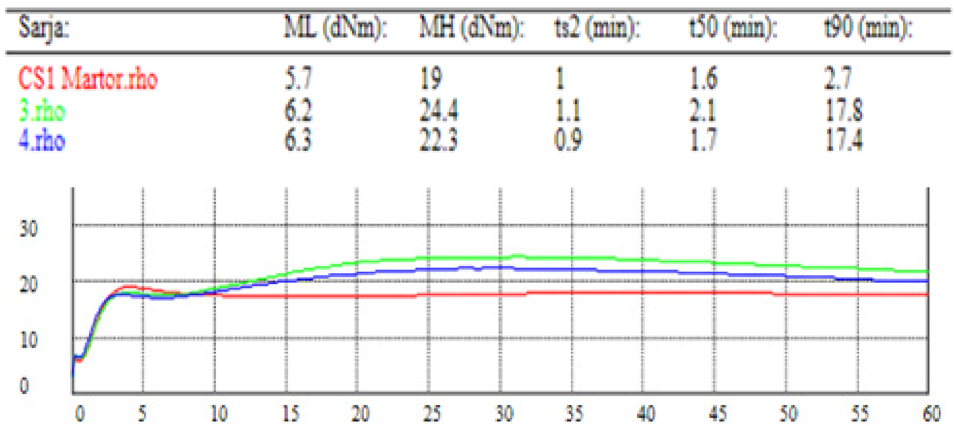

Figure 2. Rheological analysis of sample CS_1 (control), sample 3, sample 4, to establish the curing time in the electrical press [21]

Biological Setup

To analyze the antimicrobial activity (biological setup), the antibacterial compound specimens based on silicone elastomer, reinforced with nanopowders with antifungal, antibacterial and antimicrobial properties, ZnO and TiO_2, were tested and characterized according to ASTM standard: E 2149-10. They were tested on the following strains: *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25992 and *Candida albicans* ATCC 10231 and preserved on glycerol medium, were seeded on nutrient gelatin agar medium and Sabouraud dextrose agar with chloramphenicol, to obtain 24 h cultures. All samples were sterilized and placed in six-well plates - Nunc with 200 µl microbial suspension (density 0.5 McFarland = 1.5 x 108 CFU/mL) and 2 ml broth for bacteria. For fungi, the microbial suspension had a density of 1 McFarland, i.e. 3 x 108 CFU/ml. Incubation took place at 37°C for 24 hours. After the incubation process the colonized materials were washed with sterile distilled water to remove non-adherent microorganisms, then they were introduced into Eppendorf tubes with 1 ml of physiological serum, also sterile (AFS). These were sonicated at maximum power for 15 seconds and then vortexed for 15 seconds at 300 rpm. Then, decimal dilutions were made, which were seeded in triplicate of 10 µl per nutrient agar medium, namely Sabouraud with chloramphenicol, to calculate the number of colony-forming units/ml - CFU.

Morphological Characterisation

The antibacterial compounds based on silicone rubber, reinforced with nanopowders with antifungal, antibacterial and antimicrobial properties and crosslinked with dicumyl peroxide, obtained by mixing technique, were morphologically tested by scanning electron microscopy - SEM. Microscopic analyses were performed using an electron microscope at 15\20 keV, in the primary electron beam, on specimens covered with a gold film, to highlight the degree of dispersion of nanopowders, but also changes in the surface of the elastomer. The microscope generates a flow of electrons that bombard the sample, allowing a clear view of the structure of the sample under analysis. The lower surface, the upper surface, but also the transverse one can be visualized.

RESULTS AND DISCUSSIONS

Biological Characterization of Antibacterial Compounds

There are different types of nanoparticles (nanopowders) that can be incorporated into the elastomer matrix, depending on their properties and their application. Thus, various studies have been performed for the development and use of new materials mixed with different types of nanopowders to limit bacterial adhesion to the surface, thus eliminating the possibility of triggering an infectious process.

The antibacterial compound based on silicone elastomer, reinforced with ZnO and TiO_2 powders (with antifungal, antibacterial and antimicrobial properties) was tested for 24 h at 37°C on:

- *Staphylococcus aureus* as a model for Gram positive bacteria,
- *Escherichia coli (E. coli)* as prototype for Gram negative bacteria,
- *Candida albicans* (*C. albicans)* as a representative of fungi.

This type of species is among the most frequently isolated species from infections, with an increased incidence in nosocomial ones. Thus, in this study, the antimicrobial, antibacterial and antifungal activity of the antibacterial compound surfaces mixed with ZnO and TiO_2 nanopowders was tested.

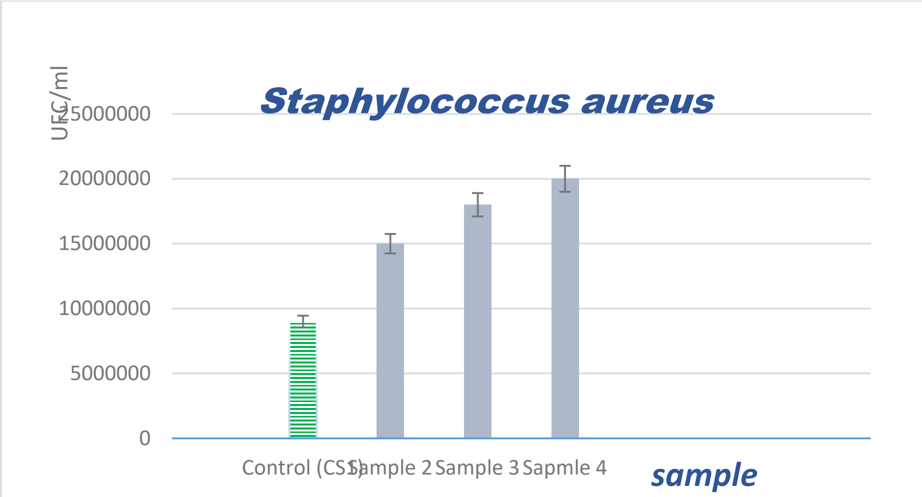

Figure 3. Biological characterization of antibacterial compound samples with ZnO and TiO_2 nanopowders on *Staphylococcus aureus* ATCC 25923 strains

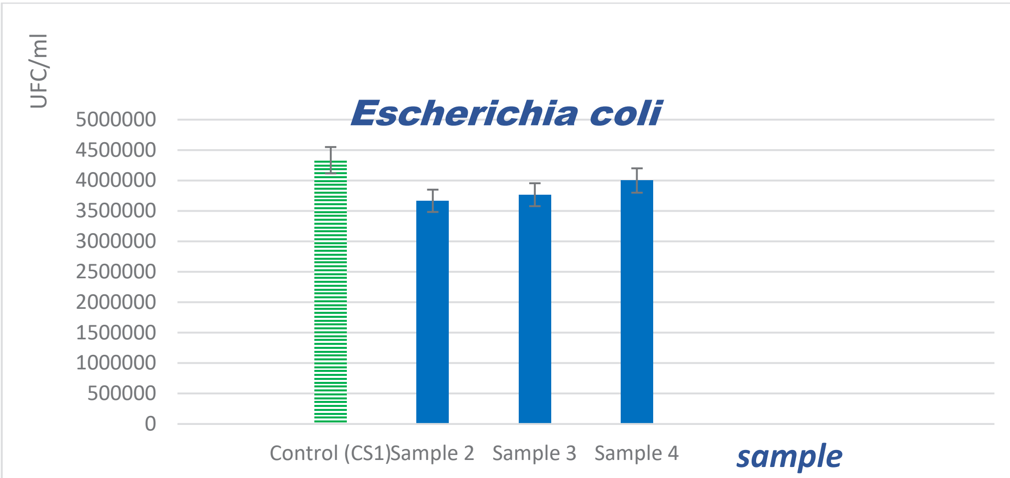

Figure 4. Biological characterization of antibacterial compound samples with ZnO and TiO_2 nanopowders on *Escherichia Coli* ATCC 25992 strains

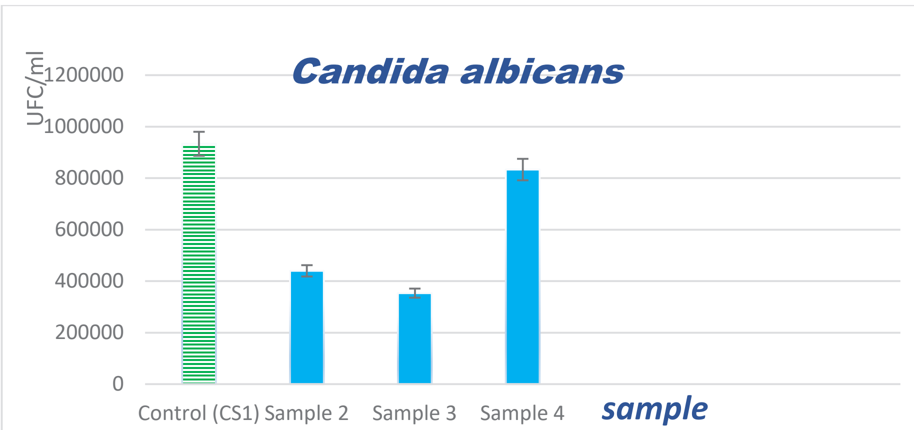

Figure 5. Biological characterization of antibacterial compound samples with ZnO and TiO_2 nanopowders on *Candida albicans* ATCC 10231 strains

Tests performed on these types of strains showed that the specimens with zinc oxide and titanium dioxide nanopowders show adhesion capacity compared to the CS_1 sample (control sample) depending on the concentration of nanopowders introduced in the antibacterial compound mixture.

The tests performed on fungi, *Candida albicans*, proved to be very effective in all the tested samples (Sample 2, Sample 3 and Sample 4), sample 3 showing the most effective values against the mentioned fungal species. Also in the case of Gram-negative bacterial strains - *Escherichia coli*, we can see that all samples, especially sample 2, show effective antimicrobial activity.

The antimicrobial activity tested against Gram-positive bacteria - *Staphylococcus aureus*, in this case favors bacterial adhesion on the tested samples, the CFU/ml values being high compared to the values recorded for the control sample - CS_1.

Morphological Characterization of Antibacterial Compounds

Elastosil R701/70 silicone rubber antibacterial compound specimens (which is the main component) reinforced with ZnO and TiO_2 nanoparticles were analyzed using the Hitachi S2600N electrocopic microscope at 15-20 keV in the primary electron beam, in cross-section, on samples covered with gold film. The SEM images are shown in Figures 6 and 7, where the dispersion of nanoparticles with antimicrobial, antifungal and antibacterial role in the obtained antibacterial compound can be observed.

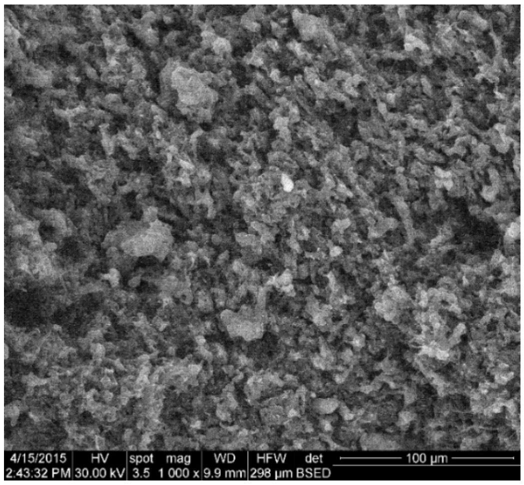

Figure 6. SEM image recorded for sample 2

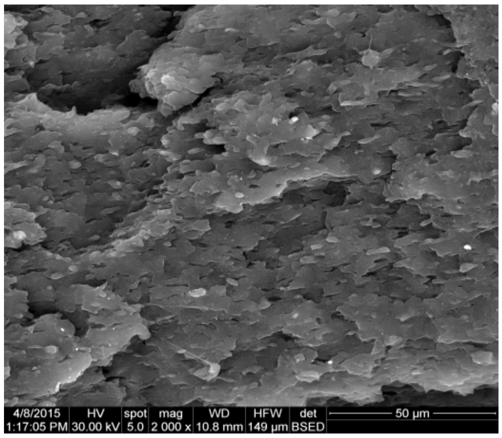

Figure 7. SEM image recorded for sample 3

The images of the samples recorded in cross section show a biphasic morphology, with ZnO and TiO_2 nanopowder particles evenly distributed in the elastomeric matrix (silicone rubber being the major component in which all other components are dispersed), thus leading to an improvement of characteristics of the compound (resistance, elasticity, increased microbial activity, etc.).

There is also a homogeneous dispersion of all components in the mass of the antibacterial compound, thus demonstrating that it was performed at optimal parameters according to the plan.

CONCLUSION

The paper presents the biological and morphological characterization of antibacterial compounds based on silicone rubber, reinforced with zinc oxide and titanium dioxide nanopowders and crosslinked with dicumyl peroxide. The biological characterization was performed on strains specific to the food and pharmaceutical field according to the standards in force, for 24 hours at 37°C. The three strains used are *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25992 and *Candida albicans* ATCC 10231. The standard used for the biological test of the antibacterial compound based on silicone rubber and ZnO and TiO_2 nanopowders was ASTM: E 2149-10 standard.

According to the biological characterization performed, samples 2 and 3 show effective antimicrobial activity against both fungi - *Candida albicans*, and Gram-negative bacteria - *Escherichia coli*.

The morphological characterization showed that the tested specimens were performed according to the vulcanization times and temperatures determined from the rheological analysis (using the Monsanto rheometer) and a homogeneous dispersion of both ZnO and TiO_2 nanopowders and other components used in the mass of the compound.

As a result of the biological and morphological characterization of antibacterial compounds based on sliconic elastomer we can say that they have potential applications in both the pharmaceutical and food fields, especially samples 2 and 3.

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