Digest Journal of Nanomaterials and Biostructures Vol. 7, No. 1, January – March 2012, p. 51 - 58

ANTIFUNGAL BEHAVIOUR OF POLYURETHANE MEMBRANES WITH ZINC OXIDE NANOPARTICLES

S. VLAD*, C. TANASE[a], D. MACOCINSCHI, C. CIOBANU, T. BALAES[a],
D. FILIP, I. N. GOSTIN[a], L. M. GRADINARU
"P. Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Voda Alley, Iasi, 700487, Romania
[a]*"Al. I. Cuza" University, Faculty of Biology, 11 Carol I Street, Iasi, 700505, Romania*

In this paper a series of polyurethane (PU) membranes modified by zinc oxide nanoparticles was prepared. The samples were synthesized from 1,6-hexamethylene diisocyanate (HDI), a mixture of polyesters consisting of poly(1,4-butylene adipate) diol (PBA, M 2000) and polycaprolactone diol (PCL, M 2000), dimethylolpropionic acid (DMPA) and 1,4-butane diol (BD). Different concentrations of nano-ZnO powder were incorporated into PU matrix solution in dimethylformamide (DMF). The PU/nano--ZnO membranes were prepared by precipitation in warm water, washed and then dried at room temperature for several days. A very wide spread aggressive fungal species represented by *Aspergillus brasiliensis* has been used as a biological material. Evaluation of biological activity of these membranes against the attack of these fungi has been done through inoculation onto Sabouraud–agar nutrient medium. Fungal growth was monitored by optical observations at 7, 14, 21, 28 and 60 days after inoculation and by SEM images. The results suggest that the polyurethane membranes modified by nano-ZnO have important antifungal properties and can be used in biomedical applications.

(Received Novembre 17, 2011; Accepted January 10, 2012)

Keywords: Polyurethane membranes, Zinc oxide nanoparticles, *Aspergillus brasiliensis*

1. Introduction

During the 20th century, the polymers have gained a very important role in the human life and all the human activities. Thanks to their properties, among different types of polymers, polyurethane based polymers have played a special part in industrial activities, agriculture, medicine, etc [1-11]. The application of polyurethane materials has been subject of several studies aimed to design high performances advanced materials [12-16]. Because of their widespread usage and the large amount of the production, important quantities of such compounds have been spread in the environment, causing ecological issues.

In recent years, many studies have been focused on the biodegradation or biodeterioration of the synthetic polymers [17-22]. The biodegradation is considered a positive process, and it means the degradation of a chemical compound/material through the biological processes, turning it into small molecular weight compounds or basic elements, participating to the recycling processes. Although the mechanisms implied are similar, the biodeterioration is considered a negative process in which are being caused damages to the materials that lead to loosing of some properties of the material [23].

Because of the above mentioned issues, there are two directions in the production of the polyurethane based polymers: the production of biodegradable polyurethanes, making them more eco-friendly to the environment and the production of biodeterioration resistant polyurethanes [24, 25].

---

* Corresponding author: vladus@icmpp.ro

Many studies have been focused on the biodegradation of the synthetic polymers. Some of them have questioned the degradability of the polyurethanes [26, 27]. Among the tested organisms, some groups of organism proved to be efficient in the biodegradation of the polyurethane based polymers: bacteria [28], white rot fungi [29] and the anamorphic fungi [30]. From all these groups, the fungi proved to be more efficient. The implied mechanisms are different. One of them is the formation of bio-films, which are consortia of microorganisms' adherent to the surface of the material through the secretion of mucilages, like polysaccharides and proteins, these mucilages covering the pores of the material and sometimes penetrating the structure of the material [31]. Another mechanism consists in the secretion of different organic acids that affect the properties and the structure of the polymer, but the most efficient mechanism implies the secretion of exoenzymes like lipases, esterases, ureases, proteases, peroxidases etc., that are breaking down the chain of the polymer [31]. It has been reported that poly (ester urethane)s are more susceptible than poly (ether urethanes) to the attack of esterases (enzymes secreted by different groups of organisms) having specificity for the ester bonds [32]. However, it has been reported that the esterases may cleave the urethane bonds too [32].

The manufacture of a biomaterial scaffold is of great importance for successful tissue engineering. For these reasons, when a new particular polyurethane material is designed for the medical purpose it is vitally important to be tested concerning the resistance to the microbial attack.

The species from the genus *Aspergillus* are widespread, developing in all the media of life. Many authors reported the degradation of different polymeric compounds by these organisms: polyesters [33], paints [34], poly (butylene succinate) [35], and even composite materials based on polyurethanes [36]. *Aspergillus brasiliensis* is one of the most spread and known species of the genus, being possible to be isolated from soil, agricultural products, foods, clothing etc. It has been proved the capacity of this species to colonize plastic made contact lenses [37] and to degrade co-polyesters [38] or poly(vinyl alcohol) based composite materials [39].

Zinc oxide nanoparticles (nano-ZnO) are useful as antibacterial and antifungal agents when incorporated into materials, such as surface coatings (paints), textiles, and plastics [40, 41]. The enhanced surface area of ZnO nanoparticles allows a much stronger interaction with bacteria [19-22]. This permits using a smaller amount of zinc oxide for the same or improved biostatic behaviour. Furthermore, nanoparticles have a large surface area to volume ratio that results in a significant increasing of the effectiveness in blocking the UV radiation when compared to bulk materials [23].

The aim of this study was to synthesize and characterize a series of polyurethane membranes modified by different proportions of nano-ZnO. Were also investigated in vitro the resistance of these composite materials to the attack of the *Aspergillus brasiliensis* isolates. This fact it is very important for the assessment of their potential application as biomedical devices. The membranes morphology was analyzed by sequential observations at 7, 14, 21, 28 and 60 days after inoculation, and SEM images.

2. Experimental

2.1. Materials

Hexamethylene diisocyanate (HDI-Fluka); poly(1,4-butylene adipate) diol end-capped (PBA-Aldrich), Mn 2000; polycaprolactone diol (PCL-Aldrich), Mn 2000; dimethylolpropionic acid (DMPA-Aldrich); 1,4-butane diol, ReagentPlus, 99% (BD-Sigma); nano-ZnO powder, < 100 nm – Aldrich;); dibutyltin dilaurat (DBTL-Fluka); dimethylformamide (DMF-Fluka). Commercial DMF was dried over anhydrous K_2C0_3, and then was distilled from calcium hydride (CaH_2) and kept over 4 Å molecular sieves. Polyols and chain extender were checked for moisture and if was necessary, it was lowered at 0.3%. The rest of the chemicals were used as received without further purifications. For antifungal tests, an *Aspergillus brasiliensis* strain isolated from soil samples was used.

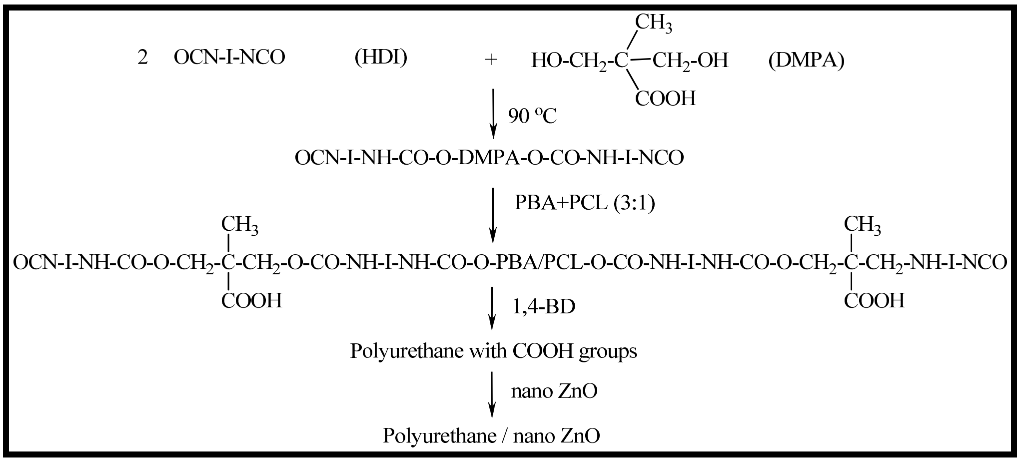
2.2. Characterizations

The polyurethane samples, containing between 0 and 10 wt. % ZnO, were cutted in small pieces of 10 x 15 mm and placed in the middle of sterile 9 cm plattes containing Sabouraud-Agar medium. The medium was inoculated in four points with spores of *Aspergillus brasiliensis*. Other polymer samples having the same size, were inoculated with fungal spores and placed in sterile Petri dishes without medium and used as control. All the samples were incubated at 23 °C for 60 days, in the dark. The dishes were visualized after 7, 14, 21, 28 and 60 days after inoculation.

The surface morphology was performed studying the membrane samples by scanning electron microscopy (SEM). The measurements were taken using a Quanta 200 instrument equipped with an energy dispersive X-ray device, EDX.

2.3. Syntesis of polyurethane nano-ZnO membranes

In the same manner as in a previously paper [18], HDI (0.6 mole) and DMPA (0.3 mole) were added into a dry vessel equipped with a reflux condenser, a mechanical stirrer and a thermometer. The pre-polymerization of polyurethane was carried out at 90 °C under N_2 atmosphere. Then mixture of PBA and PCL (3:1), Mn 2000 (0.1 mole), DBTL (0.5 wt. % of total solid content) were added into the system and reacted at 80 °C until the NCO content reached a theoretical value. Then the prepolymer was extended with BD (0.2 mole) at 35 °C and diluted with dry freshly distilled DMF. Finally, to the 10% solutions of PU different proportion of the nano-ZnO (2.5, 5.0, 7.5 and 10.0% w/w) were added and than the PU/nano-ZnO membranes were obtained by precipitation in warm distilled water. The polyurethane samples used in this study was denoted as follow: sample without nano-ZnO (PUDMPA-0), sample with 2.5 % nano-ZnO (PUDMPA-1), sample with 5 % nano-ZnO (PUDMPA-2), sample with 7.5 % nano-ZnO (PUDMPA-3) and sample with 10 % nano-ZnO (PUDMPA-4). Stages of synthesis of polyurethanes modified with zinc oxide are shown in Scheme 1.



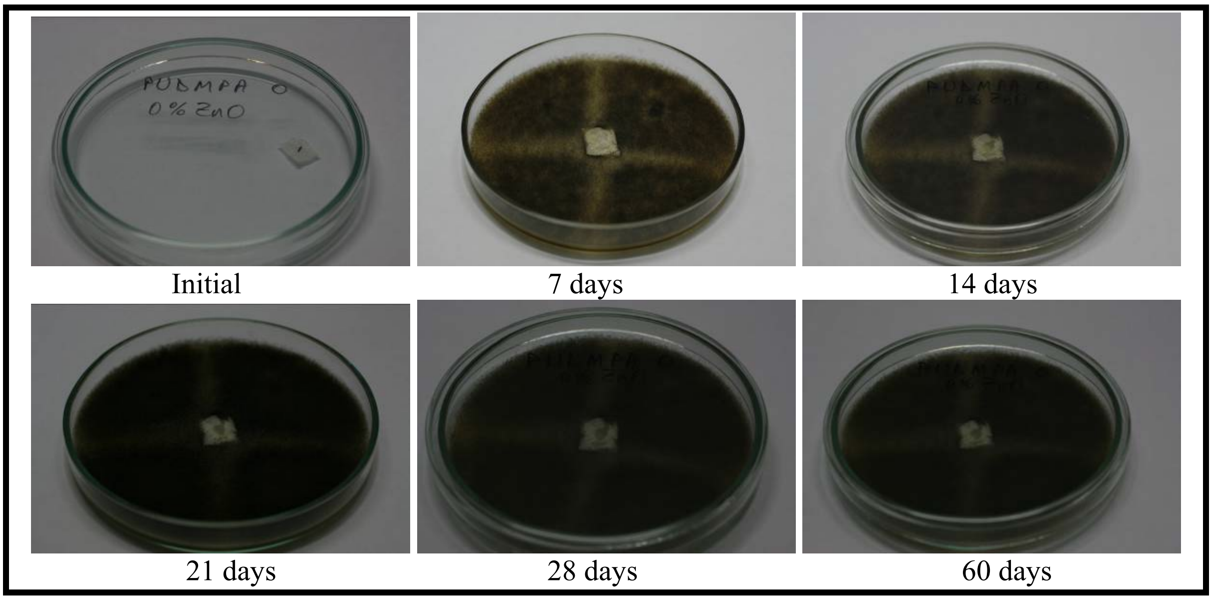
Scheme 1. The synthetic route for obtaining of polyurethane nano-ZnO

3. Results and discussions

These membranes were analyzed by Dynamic Vapours Sorption (DVS), contact angle measurements and mechanical characterization. Also, the structure of these polymers was confirmed by FTIR spectroscopy. These results were reported earlier [18].

3.1. Antifungal behaviour

Following the visual observations it has been found that polymer fragments without nano-ZnO showed no inhibitory effect on the development of fungus. Thus, after 7 inoculation days, the fungal colonies were grown up only to extremity of the polymer fragment. Similar behaviour was observed at higher incubation times (14, 21, 28, and respectively 60 days).

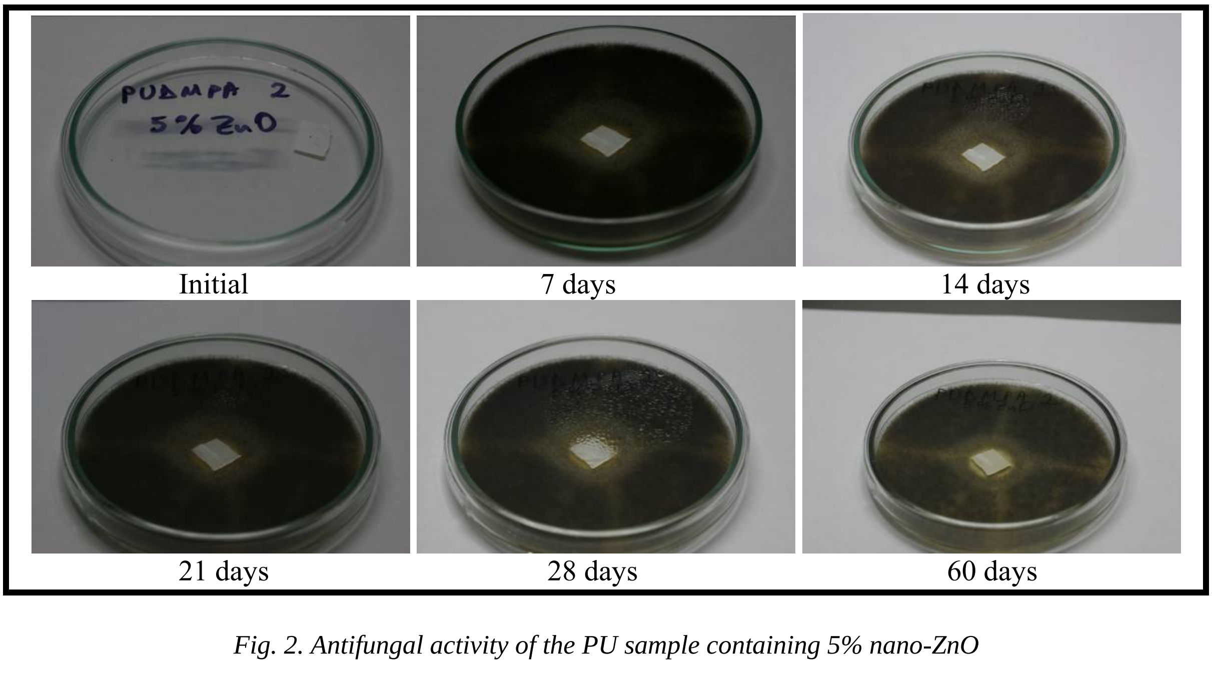


Initial 7 days 14 days

21 days 28 days 60 days

Fig. 1. The fungal growing profile of the sample without nano-ZnO

In comparison, the samples containing 5, respectively 10% nano-ZnO showed obvious inhibitory effects on fungal growth were seen a total inhibition halo of the fungus around of fragment of polymer, increasing the surface area of inhibition depending on the percentage of nano-ZnO in polymer (Fig.2).

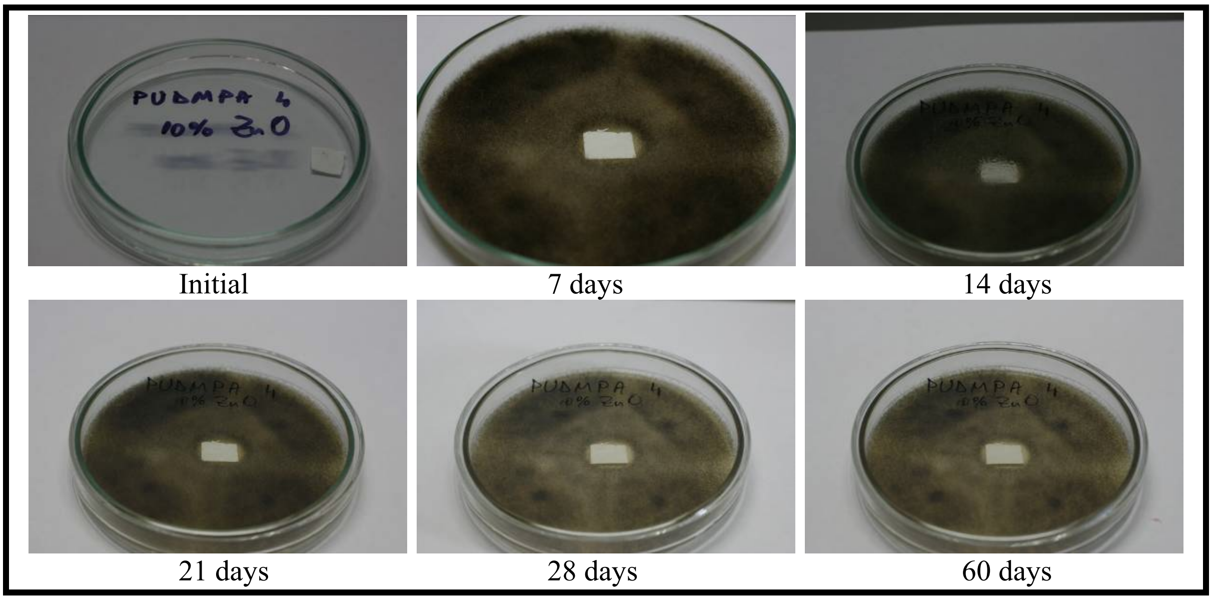


Initial 7 days 14 days

21 days 28 days 60 days

Fig. 2. Antifungal activity of the PU sample containing 5% nano-ZnO

Also, the polymer membrane having 10% nano-ZnO display a partial inhibition area (slow development of fungi and proliferation less abundant) following the total inhibition zone, suggesting the mycotoxic effect of the zinc oxide (Fig.3). These aspects are in agreement with the previous observations carried out by Sawai and Yoshikawa [42].

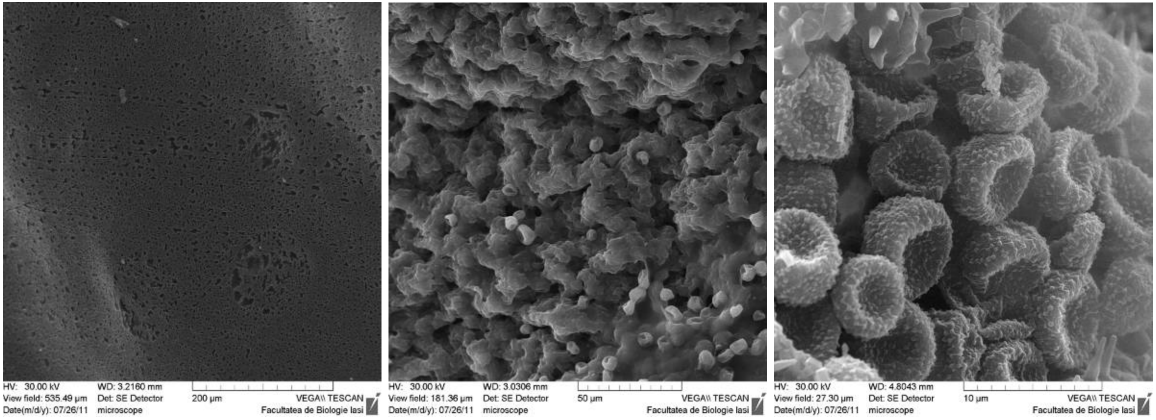


Initial 7 days 14 days

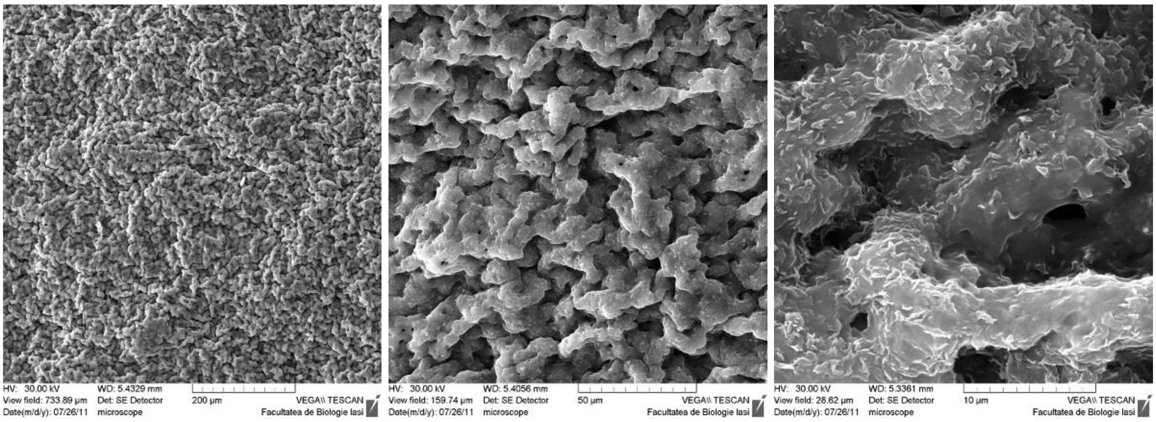
21 days 28 days 60 days

Fig. 3. Fungal evolution of the sample with 10% nano-ZnO

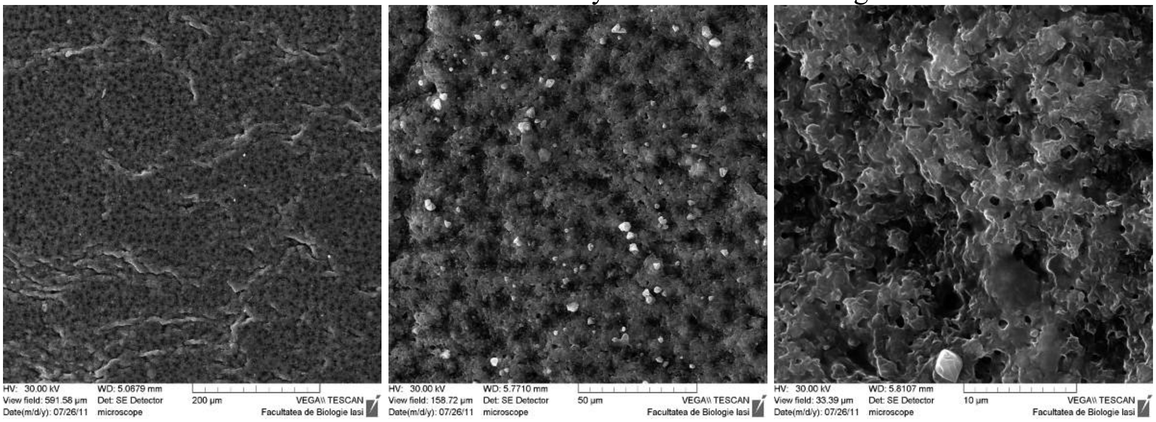
The SEM analysis reveals a lack of polymer surface fungal growth in all three cases. However, in the case of polymer samples without nano-ZnO, the adsorption of fungal conidia on the polymer surface was evidentiated. The SEM observations were made starting from the overall analysis on a wide field (low resolution) to focus on narrow field for observation details (high resolution).



PUDMPA0-initial 60 days fungal conidia-present



PUDMPA2-initial 60 days fungal conidia-absent



PUDMPA4-initial 60 days fungal conidia-absent

Fig. 4. SEM images: fungal growing profile of the samples PUDMPA-0, PUDMPA-2 and PUDMPA-4

4. Conclusion

In this study, the antifungal behaviour for a set of zinc oxide nanoparticles based polyurethanes was envisaged. For these study was used *Aspergillus brasiliensis*, a very wide spread aggressive fungal species from the environment. Evaluation of biological activity of these membranes against attack of these fungi has been done through inoculation onto Sabouraud–agar nutrient medium. The fungal growth was monitored visually by zone diameter inhibition method at 7, 14, 21, 28 and 60 days after inoculation and by SEM images. The presence of fungi conidia was observed for the sample lacking the zinc oxide nanoparticles. Notable, at relatively low concentrations of nano-ZnO, the polymers studied show antifungal behaviour. The results suggest that the polyurethane membranes modified by nano-ZnO have important antifungal properties and can be successfully used in biomedical applications.

Acknowledgements

This work was supported by CNCSIS –UEFISCDI, project number PNII – IDEI code 988/2008, contract nr. 751/2009.

References

[1] D. Klempner, K. Frisch, Advances in Urethane Science and Technology, Rapra Technology Limited, ISBN: 1-85957-275-8 (2001)
[2] P. Vermette, H. J. Griesser, G. Laroche, R. Guidoin, Biomedical Applications of Polyurethanes, Landes Bioscience Georgetown, Texas, USA, ISBN: 1-58706-023-X (2001)
[3] T. Thomson, Polyurethanes as Specialty Chemicals - Principles & Applications, CRC Press, ISBN 0-8493-1857-2 (2005)
[4] J. P. Fisher, A. G. Mikos, J. D. Bronzino, Tissue Engineering, CRC Press, Taylor & Francis Group, ISBN-10:0-8493-9026-5 (2007)
[5] T. Thomson, Design and Applications of Hydrophilic Polyurethanes. Medical, Agricultural and Other Applications, CRC Press, Technomic Publishing Company, Inc., ISBN-1-56676-895-0 (2000)
[6] S. Vlad, Mater. Plast., **42** (1) 63 (2005)
[7] A. Stanciu, V. Bulacovschi, M. Lungu, S. Vlad, S. Balint, S. Oprea, Eur. Polym. J., **35** (11**)** 2039 (1999).
[8] S. Vlad, M. Butnaru, D. Filip, D. Macocinschi, A. Nistor, L. M. Gradinaru, C. Ciobanu, Dig. J. Nanomater. Bios. **5** (4) 1089 (2010)
[9] S. Oprea, S. Vlad, Mater. Plast., **43** (4) 335 (2006)
[10] A. Stanciu, V. Bulacovschi, S. Oprea, S. Vlad, Polym. Degrad. Stab., **72** (3) 551 **(**2001)
[11] D. Macocinschi, D. Filip, S. Vlad, M. Cristea, V. Musteata, S. Ibanescu, J. Biomater. Appl., DOI: 10.1177/0885328210394468 (2011)
[12] M. Butnaru, D. Macocinschi, C. D. Dimitriu, S. Vlad, D. Filip, V. Harabagiu, Optoelectron. Adv. Mat., **5** (2) 172 (2011)
[13] D. Macocinschi, D. Filip, S. Vlad, Polym. Compos., **31**, 1956 (2010)
[14] S. Vlad, D. Filip, D. Macocinschi, I. Spiridon, A. Nistor, L. M. Gradinaru, V. E. Musteata, Optoelectron. Adv. Mat., **4** (3) 407 (2010)
[15] S. Vlad, M. Cristea, C. Ciobanu, D. Macocinschi, D. Filip, A. Nistor, L. M. Gradinaru, J. Optoelectron. Adv. M.,**12** (11) 2278 (2010)
[16] S. Vlad, S. Oprea, J. Optoelectron. Adv. M., **9** (4) 994 **(**2007)
[17] D. Macocinschi, D. Filip, S. Vlad, M. Cristea, M. Butnaru, J. Mater. Sci. Mater. Med. **20**, 1659 (2009)
[18] S. Vlad, C. Ciobanu, R. V. Gradinaru, L. M. Gradinaru, A. Nistor, Dig. J. Nanomater. Bios. **6**(3) 921 (2011)
[19] R. H. Wang, J.H. Xin, X.M. Tao, W.A. Daoud, Chem. Phys. Lett., **398**, 250 (2004)
[20] R. H. Wang, J.H. Xin, X.M. Tao, Inorg. Chem. **44**, 3926 (2005)
[21] R. K. Dutta, P. K. Sharma, A. C. Pandey, Dig. J. Nanomater. Bios. **4** (1) 83 (2009)
[22] N. Vigneshwaran, S. Kumar, A.A. Kathe, P.V. Varadarajan, V. Prasad, Nanotechnology **17**, 5087 (2006)
[23] A. Yadav, V. Prasad, A. A. Kathe, S. Raj, D. Yadav, C. Sundaramoorthy, N. Vigneshwaran Bull. Mater. Sci. **29**, 641 (2006)

[24] F. Cappitelli, C. Sorlini, Appl. Environ. Microbiol., **74** (3) 564 (2008)
[25] M. Gavrilescu, Dyn. Biochem. Process Biotech. Mol. Biol., **4** (1) 1 (2010)
[26] G. R. Silva, A. Silva-Cunha Jr., F. Behar-Cohen, E. Ayres, R. L. Orefice, Polym. Degrad. Stab., **95**, 491 (2010)
[27] Y. V. Zachinyaev, I. I. Miroshnichenko, A. V. Zachinyaeva, Russ. J. Appl. Chem., **82** (7) 1321 (2009)
[28] Y. Akutsu-Shigeno, Y. Adachi, C. Yamada, K. Toyoshima, N. Nomura, H. Uchiyama, T. Nakajima-Kambe, Appl. Microbiol. Biotechnol., **70**, 422 (2006)
[29] V. Šašek, J. Vitásek, D. Chromoková, I. Prokopová, J. Brožek, J. Náhlik, Folia Microbiol., **51** (5) 425 (2006)
[30] J. P. Eubeler, M. Bernhard, T. P. Knepper, TrAC Trends in Analytical Chemistry, **29** (1), 84 (2010)
[31] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, J. E. Nava-Saucedo, Chemosphere **73** (4) 429 (2008)
[32] R. Gautam, A. S. Bassi, E. K. Yanful, Appl. Biochem. Biotech., **141** (1) 85 (2007)
[33] Y. Tokiwa, B. P. Calabia, Polym Environ, **15**, 259 (2007)
[34] G. S. Markov, I. V. Smirnov, V. N. Romanovskii, V. E. Tret'yakov, N. N. Khramov, N. D. Nugaeva and E. V. Lebedeva, Radiochemistry, **44** (1) 96 (2002)
[35] H. Maeda, Y. Yamagata, K. Abe, F. Hasegawa, M. Machida, R. Ishioka, K. Gomi, T. Nakajima, Appl. Microbiol. Biot., **67**, 778 (2005)
[36] O. A. Legonkova, O. V. Selitskaya, Eurasian Soil Sci+, **42** (1) 62 (2009)
[37] M. S. Marqués-Calvo, J. Ind. Microbiol. Biot., **29**, 6 (2002)
[38] F. T. Tan, D. G. Cooper, M. Marić, J. A. Nicell, Polym. Degrad. Stab., **93** (8) 1479 (2008)
[39] A. Stoica-Guzun, L. Jecu, A. Gheorghe, I. Raut, M. Stroescu, M. Ghiurea, M. Danila, I. Jipa, V. Fruth, J. Polym. Environ., **19** (1) 69 (2011)
[40] D. Zvekic, V. V. Srdic, M. A. Karaman, M. N. Matavulj, Processing and Application of Ceramics **5** (1) 41 (2011)
[41] C. Guo, Z. Zheng, Q. Zhu, X. Wang, Polym. Plast. Technol., **46** (12) 1161 (2007)
[42] J. Sawai, T. Yoshikawa, J. Appl. Microbiol., **96**, 803 (2004)