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ANTIBACTERIAL EVALUATION OF SOME POLYURETHANE MEMBRANES MODIFIED BY ZINC OXIDE NANOPARTICLES

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In this paper a series of polyurethane (PU) membranes modified by zinc oxide nanoparticles was prepared. The polymer utilized in this study was synthesized from 1,6-hexamethylene diisocyanate (HDI), a mixture of polyesters consisting of poly(1,4-butylene adipate) diol end capped (PBA, M 2000) and polycaprolactone diol (PCL, M 2000), dimethylolpropionic acid (DMPA) as anionic centre and 1,4-butane diol (BD) as chain extender. Different concentrations of nano-ZnO powder were incorporated into the PU matrix solution in dimethylformamide (DMF). The PU/nano-ZnO membranes were prepared by precipitation in worm water, washed with distilled water and then dried at room temperature for several days. These membranes were used for Dynamic Vapours Sorption (DVS), contact angle measurements and mechanical characterization. The structure of samples was confirmed by FT-IR analysis. The antibacterial evaluation was made on *Escherichia coli*, a Gram-negative bacterium. The results suggest that the polyurethane membranes modified by nano ZnO have important antibacterial properties and can be used as biocidal materials.

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Keywords: Polyurethane membranes, Zinc oxide nanoparticles, Escherichia coli

1. Introduction

The manufacture of a biomaterial scaffold is of great importance for successful tissue. Polyurethane based materials are widely used in coatings [1], paints [2], foams [3], elastomers [4-6], adhesives [7] and medical devices [8-11]. The application of polyurethane materials has been the object of several studies aimed at producing advanced materials with high performances [12-16]. Zinc oxide nanoparticles (nano-ZnO) are useful as antibacterial and antifungal agents when incorporated into materials, such as surface coatings (paints), textiles, and plastics [17, 18]. The enhanced surface area of ZnO nanoparticles allows for increased 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 UV radiation when compared to bulk materials [23].

The objective of this study was to synthesize and characterize a series of polyurethane membranes modified by different proportions of nano-ZnO. The membranes morphology was analyzed by SEM images. Were also investigated the antibacterial activity against *Escherichia coli*, a Gram-negative bacterium, by determining the optical density of culture cells in agar solution. Wetting and mechanical properties complete this study.

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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, Reagent Plus, 99% (1,4-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%. Rest of chemicals were used as received without further purifications. For biological test were used Lysogeny broth (LB) media (10 g Bacto Tryptone, 5 g Yeast Extract, 5 g NaCl in 1 L; pH adjusted to 7.0), and cells (DH5α from a T7 Express Sampler, New England, BioLabs).

2.2. Characterizations

FTIR was used to examine changes in the molecular structures of the samples after mixing. The spectra were measured on a Bruker Vertex 70 FT-IR instrument, equipped with a Golden Gate single reflection ATR accessory, spectrum range 600-4000 cm^{-1}, at ambient temperature.

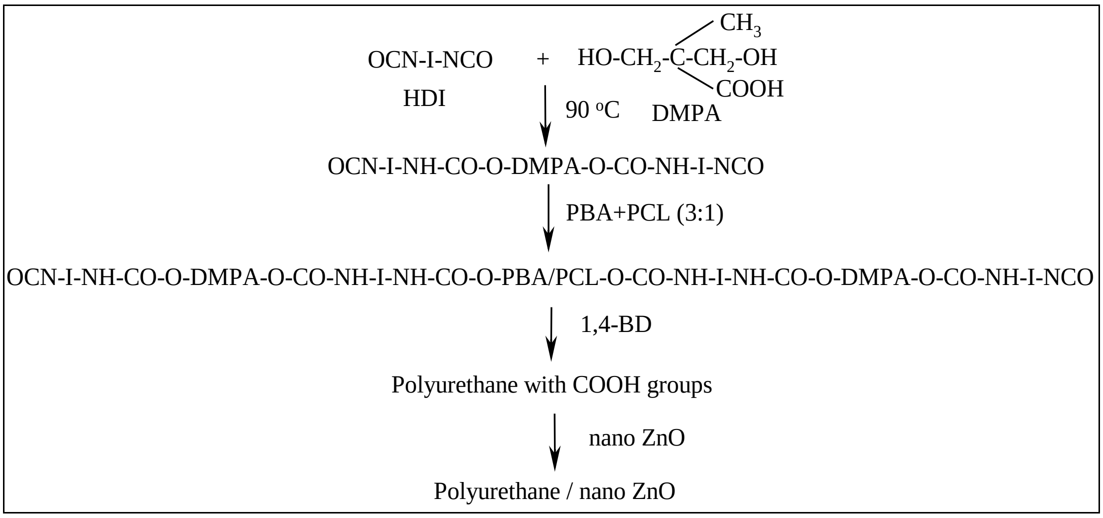
Contact angles were measured by the static drop technique at room temperature, using a KSV CAM 101 goniometer, equipped with a special optical system and a CCD camera connected to a computer to capture and analyze the contact angle (five measurements for each surface). A drop of liquid (~1 μl) was placed, with a Hamilton syringe, on a specially prepared plate of substratum and the image was immediately sent via the CCD camera to the computer for analysis. The angle formed between the liquid/solid interface and the liquid/vapour interface is the contact angle. Temperature and moisture was constant during the experiment (25°C and 65 % respectively).

Dynamic vapours sorption (DVS) capacity of the samples has been measured in dynamic regime by using an IGAsorp apparatus (a fully automated gravimetric analyzer, supplied by Hiden Analytical, Warrington - UK). This apparatus is used to study water sorption at atmospheric pressure by passing a humidified stream of gas over the sample, and can be applied to a wide range of studies from fundamental research to routine quality assurance/control. The IGAsorp is a standard sorption equipment, which has a sensitive microbalance (resolution 1μg and capacity 200 mg), which continuously registers the weight of the sample together with the temperature and relative humidity around the sample.

Stress–strain measurements were performed on dumbbell- shaped cut from thin films on a TIRA test 2161 apparatus, Maschinenbau GmbH Ravenstein, Germany. Measurements were run at an extension rate of 50 mm/min, at room temperature. All samples were measured three times and the averages were obtained.

2.3. Synthesis of polyurethane nano-ZnO membranes

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 until the NCO content reached the desired value. The content of NCO was determined by the standard dibutylamine titration method. 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 the theoretical value. Then the prepolymer was extended with 1,4-BD (0.2 mole) at 35 °C and diluted with dry distilled DMF. 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. Finally, to the 10% solutions of PU were added different proportion of the nano-ZnO (2.5, 5.0, 7.5 and 10.0% w/w) and then by precipitation in worm water were obtained the PU/nano-ZnO membranes, which were used in this study. The polyurethane samples of 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. Synthetic route for obtaining of polyurethane membranes modified by nano-ZnO

3. Results and discussions

3.1. FT-IR characterization

FTIR spectra of the polymers were used to confirm the structure of polyurethanes. The characteristic bands are found as mentioned in the literature. Formation of the polyurethane from this study is confirmed by the disappearance of NCO stretch at 2260 cm^{-1} (Fig. 1).

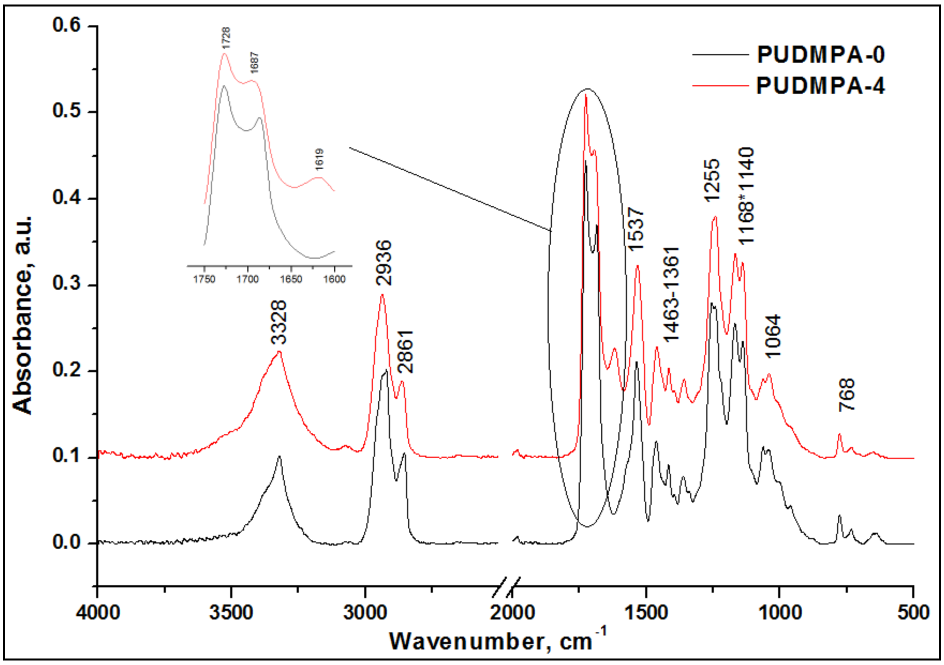


Fig.1. Representative ATR-FTIR spectra of the blank polyurethane sample and respectively polyurethane membrane modified by nano-ZnO

The absorption at 3320 cm^{-1} is assigned to hydrogen-bonded -NH groups. The absorption bands at 2936 and 2861 cm^{-1} are associated with asymmetric and symmetric -CH$_2$ groups. The strong band at 1728 cm^{-1} corresponds to the free and hydrogen-bonded –C=O groups. The absorption of ν(C=O) (amide I) and δ(NH) with ν(CO-N) (amide II) appears at 1687 cm^{-1} and 1537 cm^{-1}, respectively. The bands between 1463 and 1361 cm^{-1} manifest diverse modes of -CH$_3$ and -CH$_2$ vibrations. The band at 1255 cm^{-1} corresponds to ν(C-N) with δ(NH) (amide III) of aliphatic -R-NH-COO- groups. The bands at 1168 and 1140 cm^{-1} are assigned to the stretching vibration of the ester group –CO-O-C-. The bands at 1064 cm^{-1} is assigned to deformation of O-C=O stretching vibration in the hard urethane group and the band at 778 cm^{-1} is assigned to the out-of-plane bending of the ester group. Difference between spectrum of the polyurethane membranes without nano-ZnO and the other samples modified by nano-ZnO consist in attenuation of the peak at 1687 cm^{-1} and appearance of a new peak at 1619 cm^{-1}, the other signals are the same.

3.2. Wetting properties

3.2.1. Contact angle, work of adhesion and surface free energy

Following equations were used for calculation of the surface tension parameters and work of adhesion:

$\boxed{\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cdot \cos\theta}$ Young equation (1); $\boxed{W_a = \gamma_{SV} + \gamma_{LV} - \gamma_{SL}}$ Dupré equation (2);

$\boxed{W_a = \gamma_{LV}(1 + \cos\theta)}$ Young-Dupré equation (3); $\boxed{\gamma_{SV} = \gamma_{SV}^d + \gamma_{SV}^p}$ Fowkes equation (4);

$$\boxed{\frac{1+\cos\theta}{2} \cdot \frac{\gamma_{LV}}{\sqrt{\gamma_{LV}^d}} = \sqrt{\gamma_{SV}^p}\sqrt{\frac{\gamma_{LV}^p}{\gamma_{LV}^d}} + \sqrt{\gamma_{SV}^d}} \quad \text{Owens - Wendt geometric mean equation} \quad (5)$$

where: θ is the contact angle determine for water and ethylene glycol, subscripts '*LV*' and '*SV*' denote the interfacial liquid-vapour and surface-vapour tensions, while superscripts '*p*' and '*d*' denote the polar and dispersive components of total surface tension, γ_{sv}.

To determine the surface free energy of polyetherurethane membranes modified by nano-ZnO with its polar and dispersive portions, the contact angle is measured with a number of test liquids and evaluated according to the geometric mean equation (eq. 5). The method requires the use of at least two test liquids with known surface tension and its polar and dispersive contributions. Each additional liquid will increase the accuracy of the estimation. Table 1 shows the contact angles, work of adhesion and surface free energy of polyetherurethane membranes modified with zinc oxide nanoparticles.

Table 1. Contact angles, work of adhesion and surface free energy of polyetherurethane membranes modified by nano-ZnO

Sample	Contact angle, deg	Work of adhesion, mN/m	Surface free energy γ_{SV}, mN/m	Polar portion γ_{SV}^p, mN/m	Dispersive portion γ_{SV}^d, mN/m	Surface free energy γ_{SL}, mN/m
PUDMPA-0	104.7	54.23	22.68	0.29	22.39	48.38
PUDMPA-1	98.2	62.43	34.18	0.32	33.86	44.55
PUDMPA-2	94.7	68.55	42.57	0.38	42.19	43.34
PUDMPA-3	94.0	67.21	38.12	0.42	37.70	41.45
PUDMPA-4	80.2	85.16	33.81	6.47	27.34	21.45

Hydrophilic behaviour of polyurethane modified by nano-ZnO increase in the order: PUDMPA-0 < PUDMPA-1 < PUDMPA-2 < PUDMPA-3 < PUDMPA-4, proving that the increase of ZnO amount in sample increase hydrophilicity.

The surface free energy of the samples is particularly influenced by the polar portion, which is to be expected from the creation of polar functional groups. A large amount of polar portion, from surface free energy, corresponds to a pronounced hydrophilic character. The polar portions of the surface energy can be quantitatively collected by means of the contact angle measurement.

3.2.2. Humidity absorption study

One purpose of this paper is to determine the water vapour sorption behavior and physical stability of polyurethane samples modified with nano ZnO, using the IGAsorp system. The interaction of materials with water vapour is of interest to a broad spectrum of science and industry. Almost all materials have some interaction with moisture that is present in their surroundings. The effects of water can be both harmful and beneficial depending on the material and how it is used. Isothermal studies can be performed as a function of humidity (0-95%) in the temperature range 5°C to 85°C, with an accuracy of ± 1% for 0 - 90% RH and ± 2% for 90 - 95%RH. The relative humidity (RH) is controlled by wet and dry nitrogen flows around the sample. The RH is held constant until equilibrium or until a given time is exceeded, before changing the RH to the next level.

Water vapours sorption behaviour of this sample was analyzed using moisture sorption isotherms. The role played by water molecules in the sample was interpreted on the basis of two models, Brunauer-Emmet-Teller (BET) [24] and Guggenheim-Anderson-de Boer (GAB) [25-27] which allows a good fit of the water sorption data.

The BET (6) and GAB (7) equations are very often used for modelling of the sorption isotherms:

$$\boxed{W = \frac{W_m \cdot C \cdot p/p_0}{(1 - p/p_0) \cdot (1 - p/p_0 + C \cdot p/p_0)}} \quad (6)$$

$$\boxed{W = \frac{W_m \cdot C \cdot K \cdot p/p_0}{(1 - K \cdot p/p_0) \cdot (1 - K \cdot p/p_0 + C \cdot K \cdot p/p_0)}} \quad (7)$$

where: W- the weight of adsorbed water,
W_m- the weight of water forming a monolayer,
C – the sorption constant,
p/p_o- the relative humidity,
K – the additional constant for the GAB equation.

Both of these methods have been used in many fields where the theory of mono and multilayer adsorption is very applied to the water sorption in order to find useful information about the compounds of interest. Contrarily to the GAB model, which represents the sorption isotherm on a wide range of water activity values (a_w), the BET model enables the representation of the sorption isotherms only in a range of activities from: 0 to 0.35 according to Brunauer and all [24]. For water activities lower than 0.35, the BET model is better fitting the experimental results than the GAB model. The sorption-desorption isotherms of polyetherurethane samples and the main data obtained from these isotherms are presented in Fig. 2, respectively Table 2.

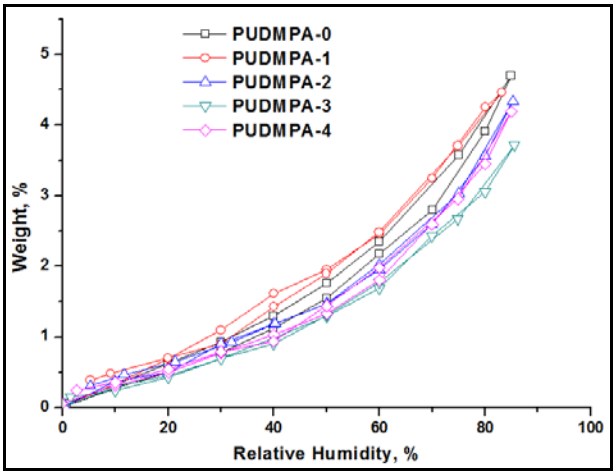


Fig. 2. Sorption/desorption isotherms of polyurethane membranes modified by nano-ZnO

Table 2. The main surface parameters evaluated by sorption isotherms

Sample	Sorption capacity (%)	BET Monolayer (g/g)	BET Area (m^2/g)	GAB Monolayer (g/g)	GAB Area (m^2/g)	Average pore size, BET/GAB (nm)
PUDMPA-0	4.6994	0.011190	39.287	0.010155	35.655	2.39/2.63
PUDMPA-1	4.4602	0.012285	43.133	0.009932	34.872	2.07/2.56
PUDMPA-2	4.3342	0.007966	27.970	0.008559	30.051	3.10/2.88
PUDMPA-3	3.7170	0.008459	29.699	0.006491	22.789	2.50/3.26
PUDMPA-4	4.1890	0.006774	23.782	0.006941	24.369	3.52/3.44

The average pore size was estimated based on desorption branch assuming cylindrical pore geometry by using the following equation:

$$\boxed{r_{pm} = \frac{2\cdot n}{100\cdot\rho_a\cdot A}} \qquad (8)$$

where: r_{pm} is the average pore size (nm), A is the BET or GAB surface area (m^2/g), n is the sorption capacity (%), and ρ_a is the adsorbed phase density (Kg/m^3).

The order of the dynamic water vapour sorption capacity was PUDMPA-0> PUDMPA-1> PUDMPA-2>PUDMPA-4>PUDMPA-3, whereas the order of the BET area values was different: PUDMPA-1> PUDMPA-0> PUDMPA-3> PUDMPA-2> PUDMPA-4, while the order of GAB area values was similar with the order of the dynamic water vapour sorption capacity. The difference between the order by water sorption capacity and area can be generated by the nature of the polar groups on the surface samples. The average pore size also influence the sorption capacity, the order by BET criterion being PUDMPA-4>PUDMPA-2> PUDMPA-3> PUDMPA-0> PUDMPA-1, while by GAB criterion being PUDMPA-4>PUDMPA-3> PUDMPA-2>PUDMPA-0>PUDMPA-1. From the presented study, it is evident that many parameters like amount of nano-ZnO, presence of the polar groups on the polyurethane surface, pore size and surface area, can influence in complex mode the sorption capacity of the samples.

Fig. 3 and Table 3 show drying kinetic curves and respectively the main parameters of this process, for samples of this study (humidity loss and drying speed).

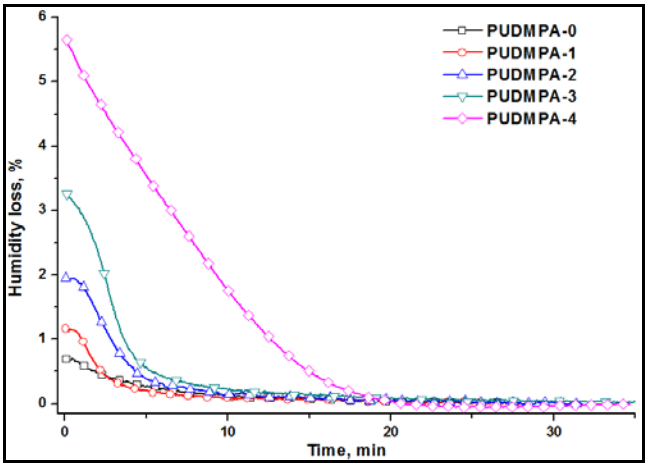


Fig. 3. Drying kinetic curves of polyurethane membranes modified by nano-ZnO

Humidity loss and drying speed of water from the polyurethane membranes are high, for amounts increased of nano-ZnO of the samples. The order of this comportment, in conformity with Fig.3, is: PUDMPA-0< PUDMPA-1< PUDMPA-2< PUDMPA-3< PUDMPA-4 < PUDMPA-5.

Table 3. Main drying parameters of polyurethane membranes modified by nano-ZnO

Sample	m (mg)	t_o (s)	m_{usc} (mg)	t_f (s)	Humidity loss, g % $\Delta W = \frac{m - m_{usc}}{m_{usc}} \cdot 100$	Δt	Drying speed, g%/s $\eta = \frac{\Delta W}{\Delta t} \cdot 10^3$
PUDMPA-0	4.2434	28	4.2151	1499	0.6714	1471	0.456
PUDMPA-1	3.3315	24	3.2946	1500	1.1200	1476	0.758
PUDMPA-2	2.7925	11	2.7396	1496	1.9309	1485	1.30
PUDMPA-3	3.0584	14	2.9663	1530	3.1048	1516	2.048
PUDMPA-4	2.4416	15	2.3137	1493	5.5279	1478	3.740

3.3. Mechanical tests

The stress strain curves measurements of the polyesterurethane samples are shown in Fig. 4.

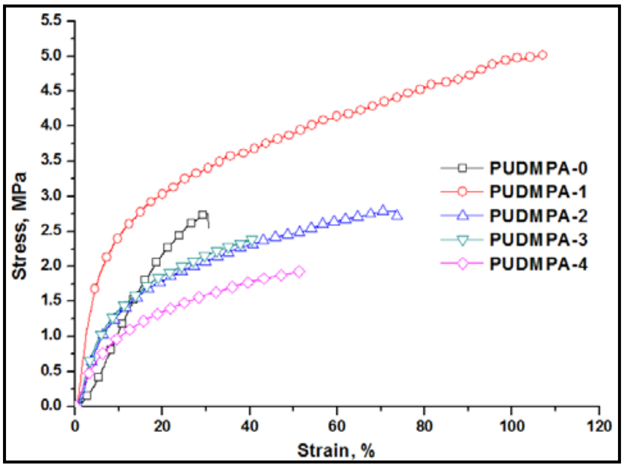


Fig. 4. Stress-strain measurements of polyurethane membranes modified by nano-ZnO

Characteristic values of these curves are presented in Table 4. The tensile strength of the polyurethane samples can be affected by factors such as the content of soft and hard segments in the polyurethane structure, their cohesion energy, packing degree of macromolecules, phase separation, cross-linking degree of polyurethane samples, etc [15]. It is noted that the influence on mechanical characteristics is given by the percentage of zinc oxide nanoparticles in the sample.

Table 4. The main parameters of physico-mechanical tests of polyetherurethane membranes modified by nano-ZnO

Sample	Young's modulus, MPa	Tensile strength, MPa	Elongation at break, %	Hardness, °ShA
PUDMPA-0	11.78±0.15	2.75±0.07	28.74±0.55	80.2±1.1
PUDMPA-1	47.19±0.65	5.02±0.10	105.96±1.10	77.6±1.0
PUDMPA-2	17.14±0.27	2.79±0.07	69.58±0.77	76.5±1.0
PUDMPA-3	15.24±0.25	2.39±0.05	39.88±0.66	70.3±0.9
PUDMPA-4	7.25±0.10	1.92±0.04	52.77±0.70	64.9±0.8

3.5. Antibacterial activity

For the growth measurements in LB media (10 g Bacto Tryptone, 5 g Yeast Extract, 5 g NaCl in 1 L; pH adjusted to 7.0), the cells (DH5α from a T7 Express Sampler, New England, BioLabs) were initially grown for 20 hrs at 28° C with continuous stirring (50 rpm), in a 200 mL flask containing 40 mL media. Sterile 100 mL flasks, containing 20 mL of LB medium were inoculated with 1 mL pre-culture (OD 1.15) and grown at 37 ºC and 100 rpm. The cells density was determined at 5 respectively 20 hrs by reading the absorbance (optical density-OD) at 580 nm using a Libra UV/Vis Spectrophotometer (Biochrom, UK).

Analysis of antimicrobial test was carried out by making samples from nano-ZnO free PU membranes and nano-ZnO containing ones in a solution of agar with Escherichia coli cells, a Gramm negative bacterium. Before using, the samples were sterilized by autoclaving at 120 °C. The polyurethane samples were screened for antibacterial activity using Turbidimetric method and turbidity produced is measured by taking absorbance and compared with turbidity produced by standard probe. Through a qualitative analysis can be seen that the samples become more clear based on increasing the concentration of zinc oxide in samples (Fig. 5). Antibacterial activity was determined by the reduction in OD of the cell culture test relative to controls.

Fig. 5. Cell culture test on polyurethane membranes modified by nano-ZnO after 20 hrs at 37 °C

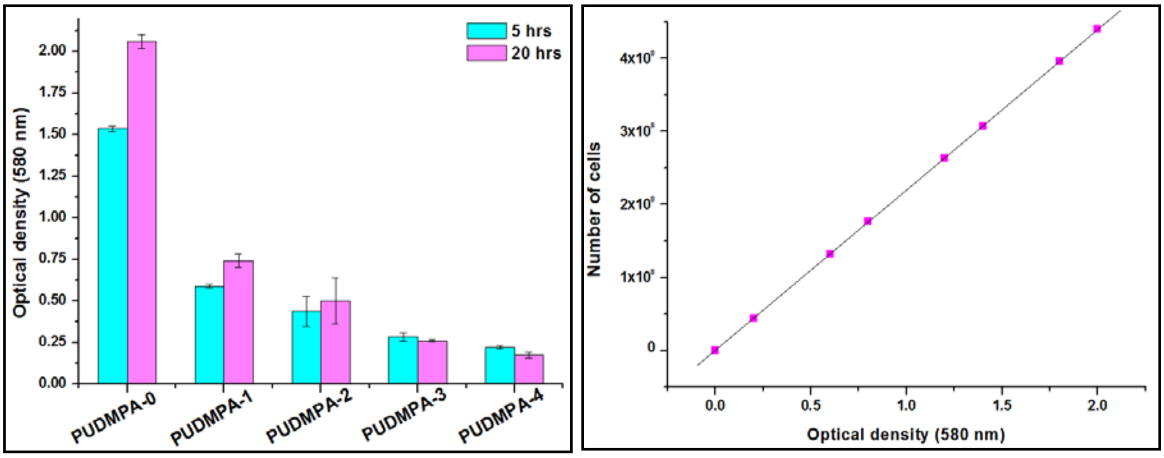


Fig. 6. Optical density values and correlation with number of cells growth for polyurethane membranes modified by nano-ZnO

Fig. 6 presents the OD values and correlation with number of cells growth for polyurethane membranes modified by nano-ZnO. The reduction in optical density of the cell culture test relative to controls proved a high antibacterial activity when the amount of nano-ZnO from material is increased.

4. Conclusions

The study was performed on a series of polyurethane membranes in whose matrix have been introduced different percentages of zinc oxide nanoparticles. Wetting characteristics are influenced by nano-ZnO content of the material studied. Hydrophilicity of these polyurethane membranes has increased as a percentage increase of nano-ZnO in the sample. Based on the sorption/desorption isotherms registered by DSV, BET and GAB surface area as well as average pore size were estimated, and placed these polymers, set by IUPAC, between microporous and mesoporous materials. Humidity loss and drying speed of water from these polyurethane samples depend of the amount of nano-ZnO. Antibacterial activity was evaluated by determining the degree of turbidity by measuring the optical density of the solutions analyzed. The antibacterial activity of these membranes is raised as the nano-ZnO content is higher. The results suggest that the polyurethane membranes modified by nano ZnO have important antibacterial properties and can be used as biocidal materials.

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References

[1] H. Ni, J. L. Daum, M. D. Soucek, W. J. Simonsick, J. Coating. Technol., **74** (928), 49 (2002).
[2] S. Vessot, J. Andrieu, P. Laurent, J. Galy and J. F. Gérard, J. Coating. Technol., **70** (882), 67 (1998).
[3] S.-R. Yang, O.-J. Kwon, D.-H. Kim, J.-S. Park, Fiber. Polym., **8** (3), 257 (2007).
[4] P. Z. Zhao, Y. S. Wang, J. H. Zhu, X. Y. Hua, Q. Z. Wen, Sci. China Ser. B-Chem. **51** (1), 58 (2008).
[5] K. Stokes, R. McVenes, J. M. Andersion, J. Biomater. Appl., **9**, 321 (1995).
[6] D. Klempner, K. Frisch, Advances in Urethane Science and Technology, Rapra Publisher, UK, 2001.
[7] S. D. Desai, A. L. Emanuel, V. K. Sinha, J. Polym. Res., 10 (4), 275 (2003).

[8] S. Vlad, M. Butnaru, D. Filip, D. Macocinski, A. Nistor, L. M. Gradinaru, C. Ciobanu, Dig. J. Nanomater. Bios. **5** (4) 1089 (2010).

[9] S. Vlad, C. Ciobanu, M. Butnaru, D. Macocinschi, D. Filip, L. M. Gradinaru, M. Mandru, Dig. J. Nanomater. Bios. **6** (2) 643 (2011).

[10] D. Macocinschi, D. Filip, S. Vlad, M. Cristea, M. Butnaru, J. Mater. Sci. Mater. Med., **20**, 1659 (2009).

[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] L. M. Gradinaru, M. Mandru, C. Ciobanu, D. Filip, D. Macocinschi, S. Vlad, J. Optoelectron. Adv. M.,**13** (3) 286 (2011).

[17] D. Zvekic, V. V. Srdic, M. A. Karaman, M. N. Matavulj, Processing and Application of Ceramics **5** (1) 41 (2011).

[18] C. Guo, Z. Zheng, Q. Zhu, X. Wang, Polym. Plast. Technol., **46** (12) 1161 (2007).

[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] S. Brunauer, L.S. Deming, W.E. Deming, E. Teller, J. Am. Chem. Soc. **62**, 1723 (1940).

[25] E. A. Guggenheim, Application of Statistical Mechanics, Clarendon Press, Oxford, pp. 186-206 (1966).

[26] R. B. Anderson, J. Am. Chem. Soc. **68**, 686 (1946).

[27] J. H. De Boer, The Dynamical Character of Adsorption, Clarendon Press, Oxford, pp. 200-219 (1968).