CHARACTERIZATION OF MATERIALS WITH NANOPARTICLES AND THEIR BIOLOGICAL AND ENVIRONMENTAL IMPACT

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Abstract - Materials with nanoparticles have huge environmental impact due to their antimicrobial effects. Their usage is widely spread on many everyday items (including clothing, car materials, food packaging and ambalage, geotextiles and many others), and after usage, large quantities of nanoparticles are released into the environment. In order to assess the possible negative impact of nanoparticles arising from medical textiles and sports materials on the microorganisms in the environment, this work aimed to detect the antimicrobial effects of commercially available nanoparticles (Ag, ZnO and TiO_2) on different model microorganisms (Staphylococcus aureus- MSSA & MRSA). The results have shown that nanoparticles influence environment through interaction with microorganisms in dependance on their size, concentration and chemical composition.

Index Terms - Antimicrobial Testing, Environment, Surface Modification, Nanoparticles;

I. INTRODUCTION

Materials with nanoparticles have strong antimicrobial properties, and among others Ag, ZnO and TiO_2 nanoparticles are the most important and the most widely used non-antibiotic antimicrobial reagents. Therefore, materials with such nanoparticles are present everywhere.

Firstly, nanoparticles receive global interest since their very small diameter belongs below the range of the microorganisms' dimensions. Secondly, nanoparticles pose large specific surface area, which makes them bio-safe materials [1]. For example, nanoparticles ZnO interact both with bacterial surface and bacterial core where it enter inside the cell [2].

Moreover, ZnO nanoparticles show antimicrobial effects on different microorganisms, including Gram-positive and Gram-negative bacteria as well as against spores which are resistant to high temperature and high-pressure [3]. Activity of nanopartilces covers wide spectrum of bacterial species as was reported by many researchers [5 – 16]. Padmavathy and Vijayaraghavan investigated various particle sizes and reported that by decreasing particle size the antibacterial increased [5].

Cellulose is functionalized by applying nanoparticles on its surface. Functionalization of textiles with nanostructures enables producing reinforced textiles resistant to tear and wear antibacterial textiles, UV-blocking textiles and other interesting materials. By this, the sol-gel process is an important part of cellulose modification by being simple, practical and cost-effective approach [17]. It enables homogenous layer on large sample surfaces, easy control, lower sintering temperatures and other advantages [18 – 20]. The most important fact is that homogenous, unified and flat coating is achieved under temperatures below 100 °C. Cellulose materials are often modified with nanoparticles for broad application, from textile materials to biosorptive carriers and indicators of pollution [21]. Sol-gel process is influenced by numerous parameters such as temperature, precursor and catalizator, time and the temperature. This work presents the attempt to recognize the effects of several nanopartilces (Ag, ZnO and TiO2) after optimization of sol-gel modification of cellulose.

II. MATERIALS AND METHODS

A. Samples

Properties of tested cellulose samples are shown in table 1, together with the methods of their characterization.

Table 1: Investigated samples and methods of their testing

Property	Norm	Sample
Fiber composition [%]	HRN ISO 1833: 2003	100 % cotton
Mass per unit area [g/m2]	HRN EN ISO 2286-2:2008	72.26
Thickness [mm]	HRN EN ISO 5084: 2003	0.51
Number of threads in warp [threads/cm]	HRN EN 1049-2: 2003	54

B. Modification of cellulose with nanoparticles by sol-gel

Cellulose materials were modified by using 3-glycidyloxypropyltrimethoxysilane (GLYMO) as a precursor, with the speed of 1 mm/s. Sols were stirred magnetically and afterwards homogenized by ultrasound. Lastly, samples were left to gel at room temperature for 24 hours on room temperature and then heated for 60 minutes at 100 °C.

C. Spectroscopical and microscopical characterization

Cellulose modified products were characterized by SEM-EDX and FTIR-ATR. The structure and the morphology of samples before and after the modification with the ZnO nanoparticels were recorded under the SEM-EDX instrument. Scanning electronic microscope "TESCAN VEGA TS5136LS" with the EDS detector was applied for this characterization of modified cellulose coating, and the instrumental parameters are listed in Table2.

Table 2: SEM-EDS instrumental parameters

SEM-EDS instrumental parameters	
Working Distance	25 mm
Energy	20 eV
Magnification	1000 – 10 000 times
Detectors	1. back scatter detector 2. detector of secondary electrons
The collection time	30 seconds

D. Antibacterial testing

In order to assess the in vitro antimicrobial activity we have investigated following nanoparticles: Ag (19 and 40 nm), ZnO (100 nm) and TiO_2 (100 nm) on different model ATCC-microorganisms (Staphylococcus aureus- MSSA and MRSA) by serial micro-dilution, and “time-kill” methodology.

III. RESULTS AND DISCUSSION

Prior the antimicrobial testing of cellulose antibacterial layers, pure nanoparticles were investigated. Secondly the sol-gel process was applied on cellulose materials in order to modify them, and then antimicrobial effects of modified cellulose was performed.

A. Spectroscopical and microscopical investigations

SEM-EDX and FTIR spectroscopic methods were used to test modification of cellulose materials after optimization by Design of Experiment, in which the team has expertise [21-31]. Firstly the SEM-EDX was performed under the magnification of 80×, 500×, 2000× and 5000×. All samples were coated with the Au/Pd coating prior the investigation (Figure 1).

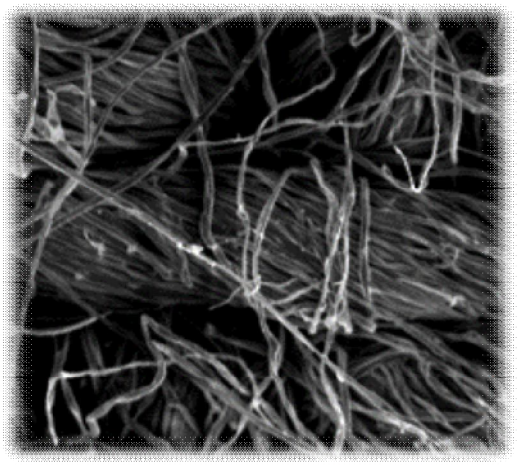


Fig. 1 SEM microphotograph of modified cellulose material recorded on samples after the dip-coating process, magnification 80×

The SEM-EDX results show that cellulose materials were successfully modified, and that uniform homogenous coating was created over sample surface with equal distribution of ZnO nanoparticles. Secondly, the samples were investigated by Fourier Transform Infra Red spectrometer (Spectrum 100 FT-IR, Perkin Elmer) before (Figure 2) and after (Figre 3) the modification with nanoparticles in order to prove that new chemical bonds were achieved.

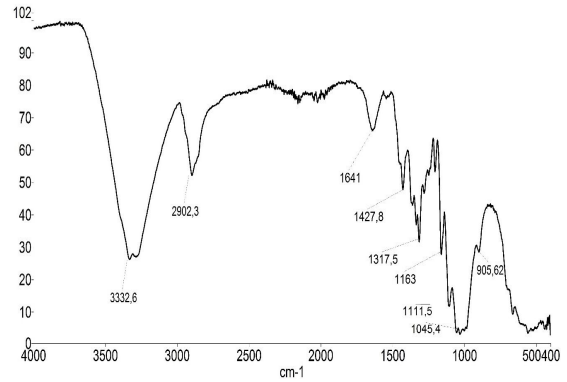


Fig. 2 Fourier Transform Infra Red spectra of cellulose sample before modification with the nanoparticles

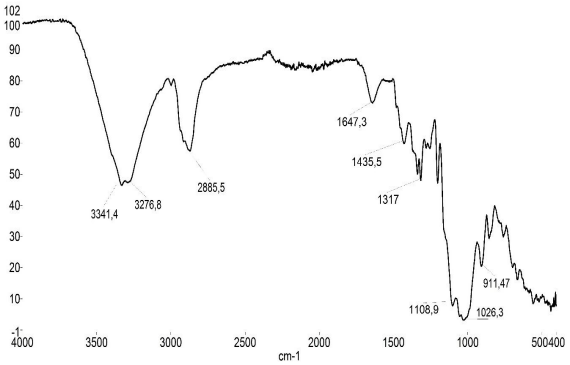


Fig. 3 Fourier Transform Infra Red spectra of cellulose sample after modification with the GLYMO precursor for modification with nanoparticles

B. Recovery angle

Since the modification of the surface of cellulosic materials was performed with a goal of making materials foreseen as medicine textiles, food packaging materials or other products with antimicrobial properties, the maximization of recovery angle is selected as the goal of optimization. Maximal recovery angle achieved was in range of 132 and 134.

C. Antimicrobial assays

Antimicrobial results performed by methods of microdilution (Table 3) using colloidal nanoparticles Ag, ZnO and TiO_2 showed that the efficiency depends on the nanoparticle used. The most efficient antimicrobial activity with the lowest MIC values was noticed with Ag and ZnO nanoparticles (testing MRSA and MSSA). Nanoparticles of TiO_2 showed the highest MIC/MBC values for both MSSA and MRSA obtaining results with 80x higher ppm (parts per million) values which indicate that Ag and ZnO are good samples for future use as preventive coatings of a vast range of medical textile and other pharmaceutical forms for prevention/treating wounds.

Tbl. 3. Results of microdilution methodology for investigation of antimicrobial effects of selected nanoparticles. Data showed as mean ± S. D. (N=3)

S. aureus ATCC 29213 (MSSA)		
	MBC/ppm	MIC/ppm
Ag	0,313 ± 0,271	0,085 ± 0,069
TiO_2	80,566 ± 0,000	40,283 ± 0,000
ZnO	0,137 ± 0,059	0,026 ± 0,000
S. aureus MFBF 10679 (MRSA)		
	MBC/ppm	MIC/ppm
Ag	2,500 ± 0,000	0,469 ± 0,271
TiO_2	93,994 ± 61,534	80,566± 0,000
ZnO	3,281 ± 1,894	3,281 ± 0,553

***ppm = part per million**

The size of nanoparticles used in antimicrobial testing was investigated and monitored by Nanoparticle Tracking Analyzer Nanosight which can determine the concentration of nanoparticles (Figure 4), as well as their distribution in the system (Figure 5).

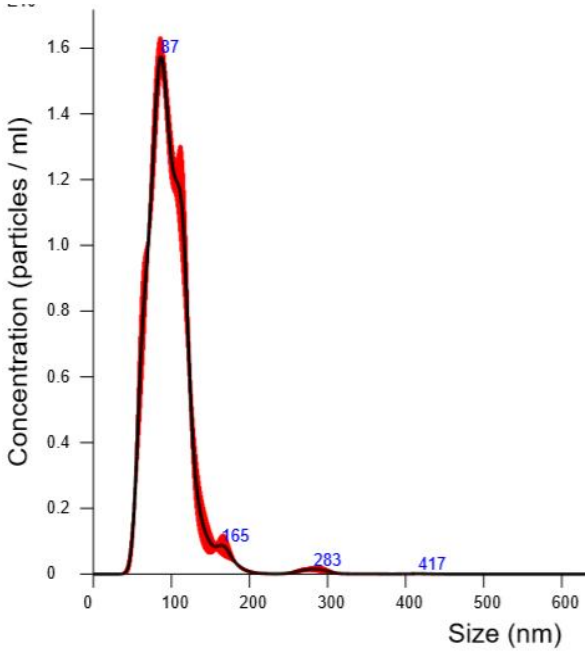


Fig. 4 Results of determination of ZnO nanoparticles size in samples determined by Nanoparticles Tracking Analyzer "Nanosight"

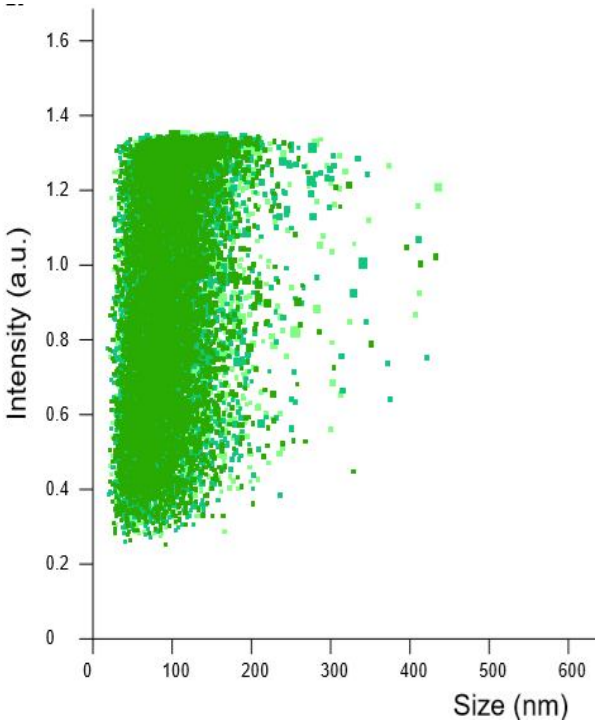


Fig. 5 Results of determination of ZnO nanoparticles concentration and distribution in samples determined by Nanoparticles Tracking Analyzer "Nanosight"

Final results obtained by "time-kill" methodology of nanoparticles after their distribution on cellulose materials are shown in Figure 6.

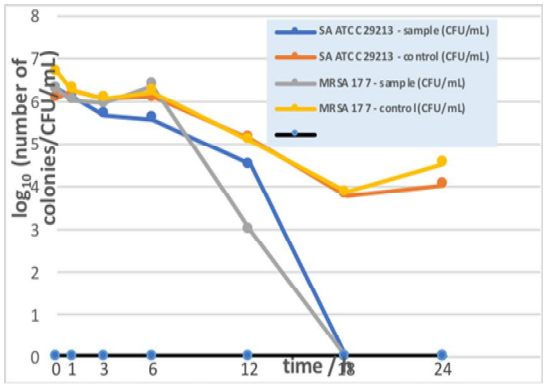


Fig. 6. Graphical presentation of "time-kill" efficiency of nanoparticles on MSSA & MRSA S. aureus during a 18h testing time

As can be seen from figure 6, in comparison with a control (untreated cells) antibacterial effect started after 6h of exposure From this results it can be concluded that nanoparticles may poses a threat to the environment, due to large-time exposure of microorganisms present in water, soil and air. Moreover, many industrial sights use microorganisms in wastewater treatment due to which we should carefully dispose materials impregnated with nanoparticles before releasing them into our environment.

CONCLUSION

Investigation of antimicrobial effects of commercially available nanoparticles (Ag, ZnO and TiO_2) on different model microorganisms MSSA and MRSA used for modification of cellulose materials has shown that nanoparticles has direct antibacterial effect in tine and thus influence environmental biota as well.

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