

# ZnO Nanoparticles for Antimicrobial Treatment of Leather Surface

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*The paper presents the antimicrobial performances of leather surfaces treated with ZnO nanoparticles as alternative to the use of volatile organic biocide materials. The hydrothermal synthesis of ZnO nanoparticles is described as an efficient and easy to control method compared to sol-gel methods. The characterization of crystallite morphology and size were measured by XRD, the nanopowder particles were assessed by TEM and the dispersion stability in water and in film forming polymers by dynamic light scattering (DLS). The ZnO nanoparticles were uniformly dispersed on leather surface by conventional technologies based on sprayed layers and were available for direct contact with bacteria and fungi. The treated leather surfaces were tested according to adapted diffusion standard methods and proved sensitivity against bacteria and resistance to fungi. The modification of dynamic contact angle of water on leather surface exposed to UV and visible light irradiation confirmed the photocatalytic generation of oxygen reactive species attributed to antimicrobial efficiency.*

**Keywords:** ZnO nanoparticles, antimicrobial activity, hydrophilic properties

There is an increasing interest in nanoparticles application as alternative to the extensive use of organic antimicrobial compounds. Many studies were devoted to the synthesis and characterization of TiO<sub>2</sub>, Ag, ZnO, CuO based nanomaterials with promising antimicrobial properties for purification facilities, house holding goods [1], medical devices [2] or nanotextile articles [3-5]. The treatment of collagen and keratin based materials with Ag, TiO<sub>2</sub> nanomaterials and their doped combinations showed a strong antimicrobial effect [6-8] and was not toxic below 370 ppm Ag concentration [9]. Some nanoscale oxides with antimicrobial properties have been identified as environmentally safe, among which MgO, CaO, TiO<sub>2</sub>, ZnO, Al<sub>2</sub>O<sub>3</sub>, and Fe<sub>2</sub>O<sub>3</sub> [10]. ZnO and Fe<sub>2</sub>O<sub>3</sub> nanoparticles are intensively studied for their high potential and efficiency as biomedical markers [11]. ZnO nanoparticles are included in many commercial products such as cosmetic and pharmaceutical creams, pigments, UV and fungicide protective coatings, electronic devices and catalysts for solar cells. Micron-size ZnO is well known as antibacterial with lower efficiency as compared to nanosize particles. The mechanism of antimicrobial action of ZnO is not completely understood but there are studies which showed that both mechanisms, the generation of reactive oxygen species and the accumulation of ZnO in cytoplasm and in outer membrane of cells can explain the higher efficiency of nanoparticles as antibacterial in comparison with bulk materials [12]. In other publications [13] the sterilization effect of Ag, TiO<sub>2</sub> and ZnO nanoparticles is due to the catalytic turning of oxygen from air or water into reactive oxygen [14, 15]. The antimicrobial leathers are very important for the protection of foot against fungi and bacteria inside footwear, especially since the incidence of foot mycosis is increasing due to the growing number of elderly and diabetic persons is already reported [16]. The ZnO nanoparticles have the advantage of non

volatility, high surface and efficiency in low concentration as compared to the organic biocides which are under toxicological restrictions. There are limited research studies on synthesis and application of nano ZnO on leather processing. A recent report [17] presents the synthesis of ZnO nanoparticles by wet chemical method using zinc nitrate, sodium hydrate and starch as stabilizer. The use of 2% of ZnO nanopowder in retanning processing of goat skins was an efficient bactericide against *Bacillus subtilis*, *Escherichia coli* and *Clostridium perfringens* strains as compared to untreated skins. Other authors [18] showed the possibility of preparing a vinyl polymer/ ZnO nano composite by free radical polymerization and ultrasound blending with ZnO nanoparticles for use in skin tanning with improved effect on tensile and tearing strengths of leathers. The application of nanoparticles for leather surface finishing is a new approach with high potential for multifunctional properties development [19, 20].

Our research focused on syntheses of ZnO nanoparticles with 20 nm particle size by hydrothermal method at high pressure, an ecological method as compared to sol-gel methods, with low energy consumption and improvement of chemical reactivity. The nanopowders were characterized and experimented for leather surface finishing with good results in bactericidal properties. The leather surface finishing with ZnO nanoparticles showed to be efficient against *Escherichia coli* ATCC 25922, *Stafilococcus aureus* ATCC 25923, *Candida albicans* ATCC 26790 and *Candida albicans* 1726 due to the more hydrophilic surface and easy access to high surface of nanoparticles.

## Experimental part

### Nanoparticles synthesis

The ZnO nanoparticles (ZnO NPs) were synthesized by hydrothermal method using Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O from Merck,

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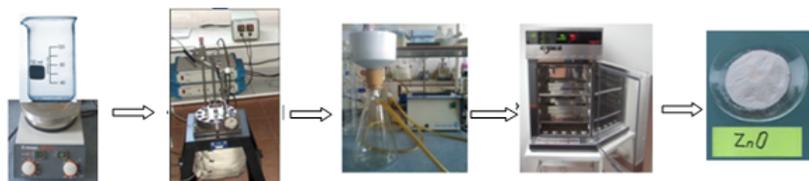


Fig. 1. ZnO nanoparticles synthesis stages: slurry preparation, hydrothermal syntheses in CORTEST autoclave, filtration/wash, oven dry and the obtained nanoparticles (right)

$\text{Na}_2\text{SiO}_3$  solution with density =  $1.37 \text{ g/cm}^3$ , Scharlau Chemie product,  $\text{NH}_4\text{OH}$  solution of 25% and  $\text{HNO}_3$  solution from Chimopar Bucharest. The  $\text{Na}_2\text{SiO}_3$  solution was continuously stirred with the  $\text{HNO}_3$  and  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . After the precipitation of ZnO, the solution was mixed with  $\text{NH}_4\text{OH}$  for alkaline environment and then the suspension was transferred into an autoclave type CORTEST (USA) for synthesis in nano state at  $T > 250^\circ\text{C}$  and  $P > 100 \text{ kPa}$ . The obtained slurry was filtrated, washed and dried in an oven. The synthesis flow chart is presented in figure 1.

### Nanoparticles characterization

The characterisation of ZnO nanoparticles was done by TEM (Philips EM 410), dynamic light scattering-DLS (Zetasizer NANO ZS, Malvern) and X-ray diffraction (BRUKER D8 ADVANCE diffractometer equipped with DIFFRAC plus BASIC Evaluation Package) through Bragg-Brentano diffraction method for crystal composition and particles size determination by Scherrer equation.

### Leather surface functionalization with ZnO NPs

Polyethylene glycol (PEG 600) was supplied by Romaqua SA and sodium polyacrylate (Na-PAA) with MW of 2100 from Sigma-Aldrich Chemie GmbH. The film forming polymers were commercial products supplied by SC Europlastic Ltd: Epacril, an aqueous dispersion based on acrylic copolymers with 30% dry substance content, pH-7-9, density of  $1.050 \text{ g/cm}^3$ , and Epafix, an emulsion of nitrocellulose lacquer, soluble in water with 15% dry substance. The leathers were sheepskins, processed as crust leathers (without finishing coat) in Leather Research Department following standard technologies available on the market. The technology for leather surface finishing includes the mechanical and ultrasound stirring of ZnO NPs dispersed in polyethylene glycol (PEG) or sodium polyacrylate (Na-PAA) with acrylic based film forming polymers and base coat layer deposition [21] on leather surface by spraying in conventional technologies followed by nitrocellulose top coat layer deposition according to the scheme presented in figure 2.

### Leather surface characterization

**SEM-EDX**, Quanta 200 FEI instrument was used to evaluate the leather coating appearance and nanoparticle uniform dispersion. The identification of Zn on leather surface by EDX confirmed the availability of nanoparticles for interaction against bacteria and fungi.

The contact angle of water drop on leather surface was measured after 1h, 2h and 1h rest after the UV and visible light exposure, with VGA Optima XE device. The aim of the contact angle measurement in dynamic conditions was the identification of hydrophilic properties generated through photocatalytic decomposition of water in hydroxyl radical, superoxide and singlet oxygen [22].

### Antimicrobial activity

The antibacterial tests were performed against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, using adapted diffusion method (SR EN ISO 20645). To test their sensitivity to the direct action of leather samples, 10 mL agar agar medium (1.5%), liquefied and cooled at  $45^\circ\text{C}$ , were inoculated with  $10 \mu\text{L}$  bacterial suspension at standard Mc Farland density of 0.5 and cast over a ground layer of agar agar 2%. After solidification, fragments of leather finished with film-forming polymers additivated with nanomaterials were placed on the surface of the medium, making sure their entire surface is in direct contact with the inoculated medium.

The dishes processed as above were incubated at  $37^\circ\text{C}$  for 24 hours, and subsequently results were interpreted by noticing the emergence of a growth inhibition area in the close proximity of leather samples. The leather fragments were then removed from the medium surface in a sterile manner, using the microscope to view the appearance of bacteria colonies developed both in the area of direct contact and in the close proximity area. Microscopic analysis were performed using a CETI inverted microscope with 10X dry objective.

The quantitative method for testing the sensitivity of yeast strains (SR EN ISO 20743) to the action of ZnO nanomaterials on the surface of leathers diffusive in sterile water was performed for *Candida albicans* ATCC 26790, *Candida albicans* ATCC 1726 and *Candida albicans* 1760.

For this method 24-hour yeast cultures were seeded onto a solid medium, of which cell suspensions were made by transferring an isolated colony into a tube of sterile water, until reaching the standard Mc Farland density of 0.5 (corresponding to a density of  $1.5 \times 10^8 \text{ CFU/mL}$ ) determined by means of nephelometry. Subsequently, to test their sensitivity to the action of nanomaterials diffusible in sterile water, sterile Petri dishes were used in which samples to be analysed were distributed, treated side up.

On the surface of each sample were distributed  $100 \mu\text{L}$  of yeast suspension at standard density. The samples were

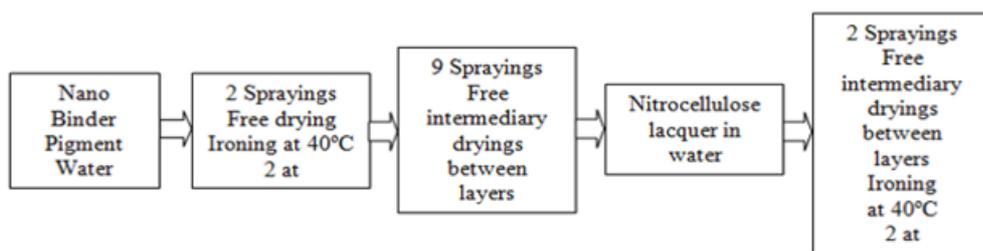


Fig. 2. The scheme of leather surfaces finishing with ZnO NPs embedded in film forming polymers



subsequently incubated at 37°C for 6 h in a moist atmosphere. After the incubation period, of the 100  $\mu$ L of suspension, 30  $\mu$ L were taken and decimal dilutions were made to quantitatively determine the number of yeast cells expressed in CFU/mL.

## Results and discussions

Hydrothermal synthesis is a process where numerous reactions take place in homogeneous or heterogeneous systems, in aqueous solution, at relatively low temperatures ( $T > 250$  °C) and high pressures ( $P > 100$  kPa) [23]. Hydrothermal reactions among dissolved species and solid species lead to the formation of multi-component oxides in a single stage, in closed systems, starting from low-cost and simple precursors from the category of water soluble salts, hydroxides, oxides [24]. The hydrothermal crystallisation process enables excellent control of morphology (spherical, fibre, cubic, flat particle shape), size (from several nanometers to tens of microns) and the degree of agglomeration, leading to polycrystalline powders, thin films or monocrystals. These characteristics can be controlled during long intervals with the help of thermodynamic variables such as reaction temperature, type and concentration of reagents, pressure, as well as by means of kinetic variables. The chemical composition of the resulting powders can be easily controlled based on stoichiometry and formation of solid solutions. The hydrothermal process also enables technology transfer on a large scale and commercial production of resulting powders.

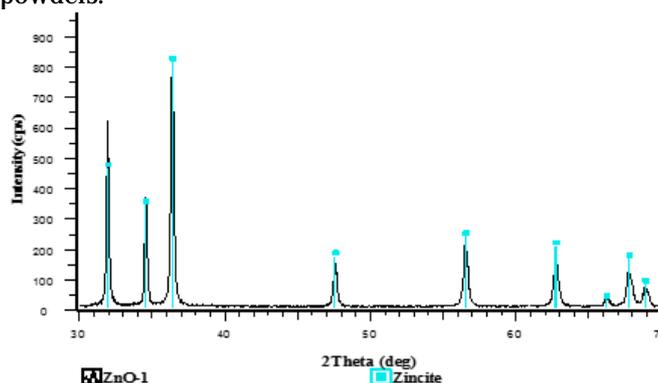


Fig. 3. X-ray diffractogram of hydrothermally synthesized ZnO nanopowder

X-ray diffraction spectra of hydrothermally synthesized ZnO powders depicted in figure 3 showed that there is a single crystalline phase of 100% zincite and the crystallite size calculated by Scherrer method is of 40 nm.

The nanopowders were dispersed in ethanol by mechanical stirring for 15 min and sprayed onto copper EM grid percoated with carbon for particle size measurement by *Transmission Electron Microscopy* at 320 000 x magnification and 80 kV. The TEM micrograph showed in figure 4 evidenced well dispersed nanoparticles, with average size of 30.6 nm.

As ZnO nanopowder is embedded in film forming polymers in water the study of particle size in water dispersion and in polymer composite was done by dynamic light scattering to evaluate the best conditions and stability for nanoparticle dispersion on leather surface.

ZnO powder of 0.1% concentration was dispersed with 0.01% sodium polyacrylate in deionised water by mechanical and ultrasound mixing for 20 min and 5 min, respectively. The same dispersion was mixed in the same conditions with Epacril binder in 3:1 ratio of water dilution. The nanoparticle size distribution and Zeta potential ( $\xi$ ) of ZnO NPs dispersion show average values of 60 nm (fig. 5, left) and -59.4 mV (fig. 5, right). The Zeta potential of ZnO NPs in water dispersion with acrylic polymer dropped at -32.6 mV value (fig. 6), which still remains in the range of stable systems. The results are consistent with literature data [25] on Zeta potential measurement of base coat polymers which showed that the addition of oxide pigments decreased the Zeta potential value.

Leather surface treated with ZnO NPs was analyzed in view of uniform distribution (SEM-EDX) and nanoparticles identification. The analyses showed that the ZnO NPs are well distributed on leather surface (fig. 7) and the

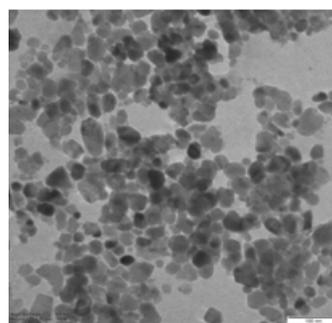


Fig.4. TEM micrograph of ZnO nanopowder

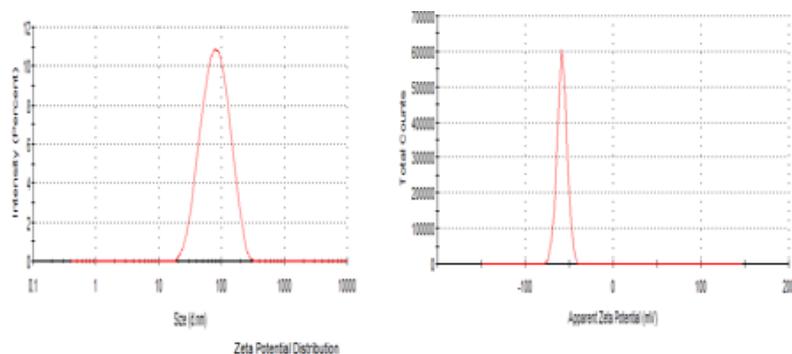


Fig.5. Particle size (left) and Zeta potential (right) of ZnO NP in water dispersion

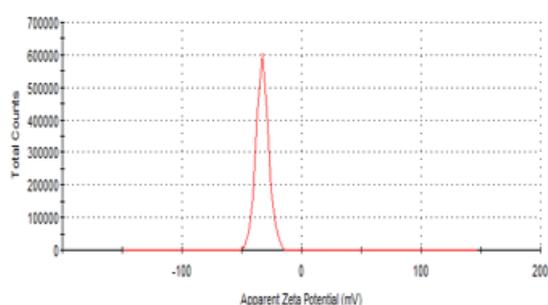


Fig.6. Zeta potential of ZnO NPs in water dispersion with acrylic polymer

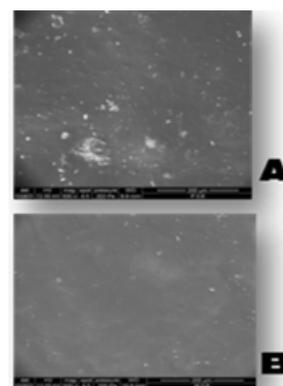


Fig.7. SEM image (500x) of leather surface with ZnO NPs (A) and without (B)

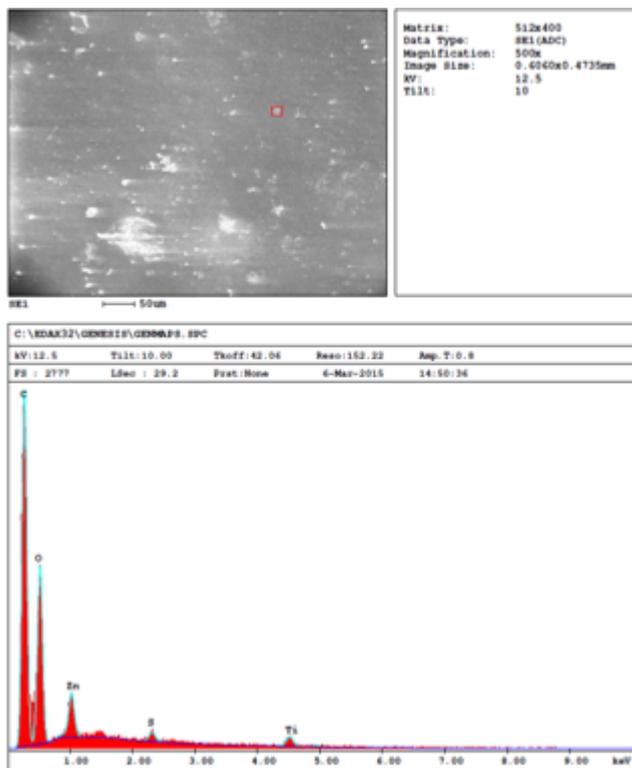


Fig.8. EDX analysis of leather surface and Zn identification

concentration of Zn (fig. 8) is 4.77 % wt (EDX). The results suggest that the ZnO on leather surface is available for the direct contact of bacteria and fungi.

The antibacterial sensitivity tests of leather surface treated with ZnO NP showed an inhibitory effect on contact area and around the contact of leather surface with media inoculated with *Escherichia coli* ATCC 25922 (fig. 9a) and *Staphylococcus aureus* ATCC 25923 (fig. 9b).

The fungitoxic properties of leather surface treated with ZnO NPs against *Candida albicans* ATCC 26790, *Candida albicans* 1726 and *Candida albicans* 1760 were clearly shown on qualitative tests (table 1) and quantitative tests



Fig. 9 a. Optical microscopy images of *E. coli* ATCC 25922 colonies: A) in the depth of the growth medium, outside the area of contact with the treated leather, and B) in the depth of the growth medium, in the area of contact with the treated leather.

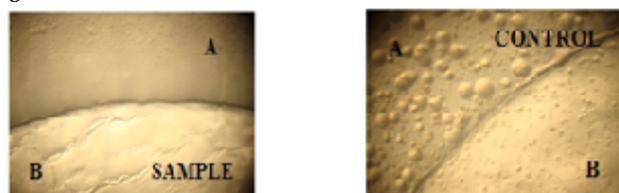


Fig. 9 b. Optical microscopy images of *S. aureus* ATCC 25923 colonies: A) in the depth of the growth medium, outside the area of contact with the treated leather, and B) in the depth of the growth medium, in the area of contact with the treated leather

(table 2). The control samples (untreated leathers) were not sensitive to the direct contact with inoculated media with fungi and in diffusible solutions.

The measurements of water drop contact angle on leather surface treated with ZnO NPs and irradiated with UV (VL 204 with irradiation at 365 nm) and visible light (500W halogen lamp) in comparison with untreated leather surface after 1h, 2h exposure and 1h rest are presented in figure 8. The results showed that leather surface treated with ZnO NPs is more hydrophobic (14 degrees difference as compared to untreated leathers) at time 0 and became hydrophilic under UV irradiation (19 degrees difference) as compared to untreated leather surface. The effect is lower under visible light (14 degrees difference) as compared to UV irradiation. The behavior of leather surface treated with ZnO NPs under UV and visible light irradiation is due to the photocatalytic increased activity and reactive oxygen species (ROS) generation with antimicrobial effect.

Direct contact of treated leathers with inoculated media with standardized <i>C. albicans</i> ATCC 26790 and the inhibitory activity.	Direct contact of treated leathers with inoculated media with standardized <i>C. albicans</i> ATCC 1726 and the inhibitory activity.	Direct contact of treated leathers with inoculated media with standardized <i>C. albicans</i> ATCC 1760 and the inhibitory activity.

**Table 1**  
QUALITATIVE TEST ON DIRECT CONTACT OF LEATHER SURFACE WITH THE MEDIA INOCULATED WITH FUNGI STRAINS (LEFT IMAGES ARE LEATHERS AND RIGHT IMAGES ARE MEDIA UNDER LEATHER)

Sample	(CFU/mL)		Reduction, %
	T <sub>0</sub>	T <sub>24</sub>	
<b><i>Candida albicans</i> ATCC 26790</b>			
L*-Control	6.6x10 <sup>3</sup>	6.6x10 <sup>3</sup>	0
L-ZnO -13	6.6x10 <sup>3</sup>	0	100
L-ZnO -14	6.6x10 <sup>3</sup>	0	
<b><i>Candida albicans</i> 1726</b>			
L-Control	173x10 <sup>3</sup>	173x10 <sup>3</sup>	0
L-ZnO -13	173x10 <sup>3</sup>	0	100
L-ZnO -14	173x10 <sup>3</sup>	0	100
<b><i>Candida albicans</i> 1760</b>			
L-Control	56x10 <sup>3</sup>	56x10 <sup>3</sup>	0
L-ZnO -13	56x10 <sup>3</sup>	0	100

\*L-Leather

**Table 2**  
QUANTITATIVE TEST OF INHIBITORY ACTION OF LEATHER SURFACE TREATED WITH ZnO NPs

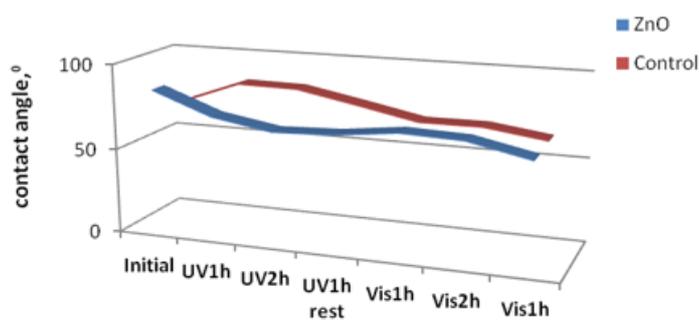


Fig.10. Contact angle of water drop with leather surface treated with ZnO NPs at initial time and after 1h, 2h exposure and after 1h rest as compared to control leather surface

Both effects, the ROS generation and the accessibility of ZnO NP to bacteria and fungi membranes can explain the antimicrobial efficiency of leather surface.

The hydrothermally synthesized ZnO NPs of zincite crystallites with 40 nm size were successfully deposited on leather surface with high efficiency on antimicrobial sensitivity and resistance. The originality of the results are connected to the hydrothermally synthesized ZnO NPs, efficient and uniform distribution of NPs on leather surface, the availability of Zn in concentration of 4.77 % wt with effect against bacteria and fungi. The photocatalytic generation of oxygen reactive species under UV and visible light irradiation was demonstrated by contact angle measurements and hydrophilic modification of leather surface as compared to untreated samples. The antimicrobial leathers are important for foot protection of healthy persons, diabetic patients or in relation to sport or professional shoes.

## Conclusions

The synthesis of ZnO nanoparticles was done by hydrothermal method under high pressure and relatively low temperature with good results regarding the pure crystallite morphology of zincite and 40 nm size according to XRD analyses. The particle size of ZnO nanopowder analysed by TEM was of 30.6 nm and the water dispersion proved to be stable with Zeta potential of -59.4 mV and of -32.6 mV in film forming polymer according to DLS measurements. Leather surface with ZnO nanoparticles embedded in film forming polymers and applied by spraying showed to be uniformly covered (SEM) with 4.77 % wt Zn (EDX). Leather surfaces treated with ZnO nanoparticles were sensitive with inhibitory activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 strains. Inhibitory activity of leather treated with ZnO nanoparticles was proved against *Candida albicans* ATCC 26790, *Candida albicans* 1726 and *Candida albicans* 1760 according to quantitative standardized methods. The modification of contact angle of water drop under dynamic conditions after leather surface exposure to UV and Vis light irradiation showed hydrophilic properties due to photocatalytic generation of oxygen reactive species. Both factors, surface availability of ZnO nanoparticles and photocatalytic reactive leather surface were attributed to antimicrobial efficiency.

Antimicrobial activity exhibited by the leather surfaces treated with ZnO nanoparticles embedded in film forming polymers may represent an improvement in the quality and life safety with very good application in leather industry, as alternative to organic biocides.

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