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Deposition of zinc nanoparticles on woolen substrates in footwear

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In footwear, especially winter footwear, lining and insoles are made of woolen materials. Owing to to their organic origin and exposure to moisture and temperature, these materials are susceptible to microbial attack. It is known that ZnO nanoparticles exhibit excellent antibacterial activity.

In our study, several methods were applied to deposit zinc nanoparticles on the surface of wool substrates. The particles were synthesized *in situ* on a film of cross-linked collagen hydrogel. The wool samples were modified using three different methods, varying the sequence of component addition and reaction conditions to achieve uniform distribution and dispersion of the synthesized particles. In one method, ultrasonication was applied.

Microscopic analyses showed that ultrasonic treatment of the samples improves the distribution of zinc particles on the surface and within the substrate. It was found that for the physico-mechanical properties, a change in color fastness is observed compared to the control sample, i.e., darkening of the surface. In terms of vapor-air properties, the best results were again registered for the ultrasonically treated samples, in which an increase in water vapor absorption and a slight decrease in the vapor permeability coefficient were observed. The obtained experimental data are promising for antibacterial activity; however, for a more comprehensive characterization of the obtained composites, antimicrobial studies are necessary.

Keywords: wool, antibacterial modification, ZnO nanoparticles, footwear

INTRODUCTION

Footwear is a complex composite consisting of many different in nature details that must be connected, providing the shoe with reliability, durability, comfort, and elasticity. Of great importance is the choice of materials for the footwear, especially for the inner details. Insoles and lining details, especially in winter footwear, are often made of woolen materials. Prolonged wearing of shoes creates prerequisites for bacterial and fungal diseases. To protect the foot from microbial attack, it is also extremely important that the materials have undergone a certain antibacterial treatment.

The unique properties of wool include its complex morphology with multilayered cuticle and cortex, the keratin proteins in wool, which contribute to its chemical resistance, strength, moisture permeability, and porosity. Its diverse functional groups facilitate various binding mechanisms, making it suitable for nanoparticle functionalization and enabling the production of fabrics with antimicrobial, self-cleaning, and UV-protective properties. Additionally, as a protein fiber, wool contains active groups such as amide, disulfide, and carboxyl, which can reduce metal salts to form nanoparticles [1].

The treatment of wool with metal salt solutions gives rise to metal ions interaction. The attained wool properties depend on the type of interacting metal ions, the conditions of treatment, and the metal salt concentration. The free carboxyl groups of wool are considered to be the binding sites over a wide range of pH values. Several reactions of metal ions with wool effectively improved its antibacterial properties, flame resistance, shrinkage, abrasion, wrinkle recovery, dye ability, deodorizing [2].

ZnO is widely used as an antimicrobial agent. The antibacterial mechanisms of ZnO NPs are as follows: integrated cell destruction due to direct contact of ZnO with the cell wall, release of antimicrobial ions, mainly Zn^{2+} ions, ROS formation, and photoconductivity [3].

The surface layer of wool fibers has a scale structure that provides good insulation and hygroscopic properties. However, this structure also makes wool prone to absorbing moisture in humid environments, creating ideal conditions for mold and bacteria growth. Functional finishing of wool fabrics currently emphasizes on antimicrobial treatment, with Ag nano-ions, chitosan, lysozyme, and natural dyes being the most commonly applied agents to achieve antimicrobial effects [4].

Several physical and chemical methods including microwave-assisted, sol–gel, and hydrothermal methods have been established to prepare nano-ZnO.

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Zinc acetate, zinc sulfate, and zinc nitrate are the most common precursors used as a Zn source. The sol–gel consequential precipitates are usually requiring post-heat treatment to induce crystallization that frequently gives rise to particle agglomeration and grain growth. In contrast, ultrasound has been established as an accomplished and environmentally friendly technique for synthesis of novel materials at low temperature and short time. The effect of ultrasound in enhancing the kinetics of chemical reactions is related to the creation of highly reactive free radicals such as O^-, OH^-, HO_2^- due to the temporary cavitation bubble collapse caused by the ultrasound wave [5].

In situ synthesis of nano-ZnO is an alternative pathway towards durable finishing. The durability of the incorporated properties can also be improved by adding suitable chemicals, which will form covalent or ionic bonds between the host substrate (textiles) and the used NPs. This study employs a novel *in situ* approach to synthesize ZnO nanoparticles by utilizing wool, silane, and a capping agent [6]. These agents help binding ZnO nanoparticles more strongly to the wool surface.

Functional coatings aim to enhance the properties and performance of textile substrates, as well as to introduce new textile functions. To this end, different classical and contemporary organic, organic-inorganic hybrid, and inorganic compounds are used in application processes. Among the latter compounds, ZnO has already been established as a chemical agent for textile functionalization because of its unique physical and chemical properties, environmental friendliness, biocompatibility, and low price [7].

For improvement of the mechanical properties of medical textiles, polyethylene wax emulsion is added to nano-ZnO. It is an agent that forms a flexible film on the fabric surface, making the fabric soft with a smooth coating and improving its mechanical properties. Excellent and encouraging results have been found for antibacterial activity [8].

Amidoxime-functionalized wool fibers loaded with nano-ZnO were successfully prepared by *in situ* coprecipitation and radiation-induced copolymerization. Wool-AO@ZnO showed excellent antimicrobial properties to aerobic bacteria, anaerobic bacteria, and fungi [9].

The padding method has been used for the treatment of dyed cotton and wool fabrics with ZnO, TiO_2 and CuO to impart functional properties such as antimicrobial, self-cleaning, and UV blocking properties [10].

Antimicrobial finishing prevents microbial attack on wool and prolongs its useful life. There are different ways to prevent the attachment of microorganisms to fiber surface, using aromatic halogen compounds, organometallic salts, quaternary ammonium salts, iodophors, phenols, urea and its related compounds (i.e., formaldehyde derivatives), amines and silver nanoparticles. Natural products such as chitin derivatives and their protonated amino groups on the glucose ring are also used. The goal of these studies [11] is to develop an approach to imparting bioactive features to wool macromolecules by incorporating functional groups such as carboxylic groups, into fibers before the treatment with appropriate antibiotics. Carboxylic groups were incorporated into wool fabric by grafting acrylic acid chemically initiated with hydrogen peroxide and metallic ions (e.g., Cu^{2+}) and post-treatment with two antibiotics: neomycin (Ne) and tetracycline hydrochloride (Te) to obtain antibacterial fibers active to Gram-positive and Gram-negative microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). This was confirmed by measuring the zones of inhibition of wool fabric treated with the abovementioned antibiotics under various conditions. Higher temperature enhanced sorption rate to wool grafted with acrylic acid. There is a correlation between the sorption percentage and size of inhibition zone, depending on the type of added antibiotics [11].

In our previous studies, we synthesized and applied TiO_2 nanoparticles in coatings for leather materials [12]. Based on these investigations, in the present study, we decided to develop formulations for the synthesis and application of ZnO nanoparticles on wool substrates in a similar manner. Consequently, the present study aims to develop methods for modifying wool fabrics with ZnO for use in shoe linings by improving the functional properties and potentially provide antibacterial protection capabilities. For the first time, an *in situ* method was applied for depositing zinc oxide particles in a cross-linked gelatin hydrogel coating on a wool substrate. This method aims to improve the fixation of particles in the wool fabric. An ultrasonic approach was also applied to improve the dispersion and the uniform distribution of particles.

METHODS AND ANALYSES

Materials

Wool samples from a local upholstery materials producer - industrial wool, 100% wool felt sheet with specifications of 3.3 mm thickness and 250 g/mm³ density were used. Square samples, each measuring 50×50 mm in dimensions and weighing

approximately 2.6 g, were carefully prepared. The chemical reagents employed in the study consisted of zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$, CAS: 10196-18-6), sodium hydroxide (NaOH, Sigma-Aldrich, Darmstadt, Germany), a 25% aqueous solution of glutaraldehyde (Sigma-Aldrich, Darmstadt, Germany), and gelatin (CAS: 9000-70-8, Merck KGaA, Darmstadt, Germany). Distilled water was used as the solvent for all prepared solutions.

Methods for preparation of composite materials

ZnO nanoparticles were deposited and fixed to the wool substrate using a cross-linked gelatin hydrogel. The ZnO particles were synthesized *in situ* and deposited on the gelatin film. Modification of wool samples was performed with glutaraldehyde - cross-linked gelatin. The synthesis of ZnO nanoparticles was carried out by varying the components and processing conditions (Fig. 1).

The processing of the first sample PW ZnO_1 involved initial pre-treatment of the wool with 2% oxalic acid ($H_2C_2O_4$) followed by immersion in a 5% gelatin solution and final cross-linking with 1,5% glutaraldehyde. The next stage of the processing was with a solution of zinc nitrate hexahydrate (0.1M) and sodium hydroxide (1M), followed by heat treatment and dehydration. The complete processing sequence included: 30 min treatment at room temperature, 24 h conditioning at 23°C, heat treatment for 30 min at 85°C, 5 min treatment at 55°C, dehydration in a dryer for 2 h at 85°C and final drying phase of 96 h at room temperature.

Fot the second sample PW ZnO_2 a comparable methodology was used, starting with immersion of the wool sample in a gelatin solution, followed by treatments with $Zn(NO_3)_2 \cdot 6H_2O$, glutaraldehyde, and NaOH. The heat treatment and dehydration steps were the same as in the previous method.

The third sample PW ZnO_3 was treated in an ultrasonic bath. The wool samples were first immersed in a gelatin solution, then in the mixed solution of $Zn(NO_3)_2 \cdot 6H_2O$ and NaOH, followed by addition of glutaraldehyde.

ANALYSES

Analyses of specimen thickness, air permeability, absorption, abrasion resistance, microscopic observations, and photoluminescence were performed in the study to characterize the deposition of zinc oxide particles on wool fibers and study their influence on permeability and physico-mechanical responses of wool fibers after such modification.

Thickness and weight

The ASTM D1777 standard test was used to measure the thickness of a textile sample. A digital gauge was used for this test [13].

Vapor absorption and water vapor permeability

Equipment used for evaluating water vapor permeability was SATRA STM 473, England. Water vapor absorption and water vapor permeability were calculated by formulas 1 and 2, respectively [14, 15]:

$$W_1 = \frac{m_2 - m_1}{a} \quad (1)$$

where: W_1 – water vapor absorption [mg/cm^2]; m_1 - initial mass [g]; m_2 – mass after testing [g]; a - test surface [cm^2].

$$W_3 = \frac{m_2 - m_1}{a.t} \quad (2)$$

where: W_3 – water vapor permeability [mg/cm^2.h]; m_1 - initial mass [g]; m_2 – mass after testing [g]; a - test surface [cm^2]; t - test time [h].

Water absorption insoles [16]

$$W_A = (M_P - M_0)/A$$

where: W_A– absorption [g/m^2]; M_P –final mass [g]; M_0 –initial mass [g]; A - area [m^2].

Water desorption insoles [16]

$$W_p = \frac{M_F - M_R}{M_F - M_0} \times 100$$

where: W_p – desorption [%]; M_F –final mass [g]; M_R – mass after conditioning for 24 h [g].

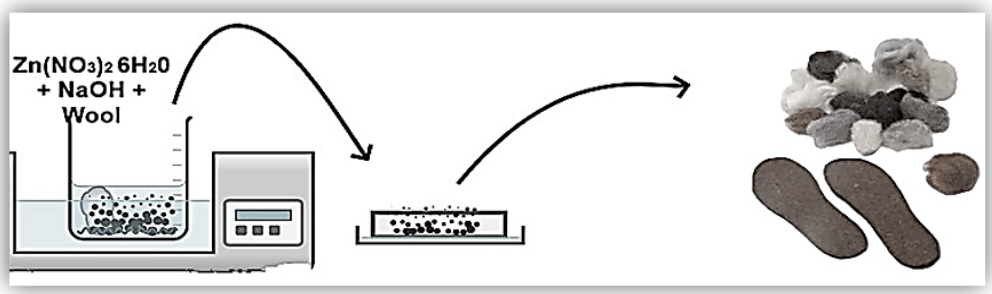

Fig. 1. Preparation of wool samples with ZnO coatings

Abrasion resistance

A test method was used to determine the abrasion resistance of insoles irrespective of the material. After 400 cycles of friction with the accompanying fabric, a visual evaluation was made against the uneraded material of the test specimen [17].

Color coordinates and color difference

The color changes of the samples, the intermediate values of L*, a* and b* and the color differences (E*) were determined using a reflectance spectrophotometer (UVA/VIS/NIR Lambda 750S Perkin Elmer, in the wavelength range λ=800 ÷ 340 nm) with a D 65 light source and a 10° viewing angle. Chromatogram of all colors visible to the human eye on an x/y grid and assigning a numerical value allows us to make uniform measurements and comparisons between colors. All measurements and calculations were made according to CIELAB: 1973.

Equipment used to assess the degree of damage and color transfer on the surface of the material during mild dry or wet abrasion was Satra STM 421. Grey scales corresponding to ISO 105-A02 and ISO 105-A03, respectively, were used to assess the color changes and the degree of staining, according to method B, EN ISO 17700 [18].

Microscopic and fluorescence analyses

Samples were observed using an inverted microscope Metaval (Carl Zeiss, Germany) operated in the dark-field mode with Planachromat-HD objectives (at magnification from 5× to 50×) and halogen light illumination (12V, 50W). A microfluorimeter was adapted to the microscope for fluorescence spectroscopy studies.

UVA – VIS - NIR transmittance spectral analyses

A spectrophotometer (UVA/VIS/NIR Lambda 750S, Perkin Elmer, USA) was used to perform the analysis which covered the wavelength range from λ 2000 to 250 nm.

RESULTS AND DISCUSSION

Thickness and weight

The results of the thickness ($\bar{t}$) and mass (m_2) measurements of the wool samples before and after coating are shown in Table 1. The values are presented as mean ± instrumental error.

Thickness and mass measurements before and after coating showed that ZnO-based layers were successfully deposited on the wool samples. While all samples showed an increase in mass and thickness, the effect was most pronounced for PW ZnO_2.

Table 1. Thickness and weight of the tested wool samples

Sample name	m_1, g	m_2, g	$\bar{t}$, mm
Zero sample	2.6 ± 0.1	2.6 ± 0.1	3.32 ± 0.12
PW ZnO_1	2.6 ± 0.1	3.2 ± 0.1	3.72 ± 0.12
PW ZnO_2	2.6 ± 0.1	3.5 ± 0.1	3.75 ± 0.13
PW ZnO_3	2.6 ± 0.1	3.1 ± 0.1	3.74 ± 0.13

For sample PW ZnO_3 the coating was the thinnest, which could be due to a more uniform and finer deposition. Such modifications are of particular importance, as they provide information on improved surface coverage and possible enhancement of functional properties, including UV protection, antibacterial activity, etc.

Water absorption, desorption, and abrasion resistance

Table 2 shows the experimental data on water absorption, desorption and abrasion resistance of the modified samples. The values are presented as mean ± instrumental error.

The samples PW ZnO_2 and PW ZnO_3 show the best results, for PW ZnO_2 probably due to the NaOH-induced partial hydrolysis of the wool cuticle, which increased hydrophilicity and absorption, and for PW ZnO_3 due to the ultrasonic modification with nanoparticles, maintaining the integrity of the fibers and causing moderate absorption.

A change in water absorption and water release properties was observed. It was found that the treatment using ultrasound did not significantly affect the newly obtained characteristics. All three methods also meet the requirements for use in safety shoes. The best water absorption was observed in PW ZnO_2. From the visual assessment of abrasion resistance, it followed that there is no decrease in the quality of the materials.

The analyses showed that all tested samples exceeded the requirements of the ISO 20345 standard for water absorption, confirming the presence of hydrophilic properties. The wool samples modified with ZnO showed higher values than the zero sample, and the sample PW ZnO_2 reached the maximum absorption (156.9 mg/cm²), indicating that the inclusion of ZnO improves moisture retention through increased surface polarity and interactions between nanoparticles and fibers [19]. While all samples in Table 2 met the permeability requirement, the zero sample showed the highest release (130.6%). In contrast, the samples treated with ZnO showed slightly lower permeability values (lowest in PW ZnO_1 at 89.7%),

probably due to the stronger binding of water in the modified fiber matrix. The abrasion resistance remained unchanged, demonstrating that the ZnO modification preserves the structural integrity under friction.

Color coordinates and color difference

The analysis of the color coordinates reveals distinct differences between the zero sample and the three PW ZnO specimens (see Table 3). PW ZnO_1 shows moderate darkening (L*= 79.52), slight shift toward red and increase in the yellow component, resulting in a visible color difference. PW ZnO_2 exhibits the strongest deviation, with substantial decrease in brightness, pronounced change in the red-yellow range and high color difference (ΔE=17.92). PW ZnO_3 occupies an intermediate position with a color difference of ΔE=11.75.

These analyses confirm that ZnO modifies the surface and the optical properties of the material, which may serve as an indicator of functional effects such as antibacterial activity and coating durability. The observed deviations from the zero sample indicate a modification of the optical properties, which, from a physical perspective, is directly related to the interaction of the material with light and its potential antibacterial activity. Surface modifications in the presence of ZnO promote the formation of reactive oxygen species and the discharge of Zn^{2+} ions, resulting in damage to bacterial cells [20].

Morphological analysis

Figure 2 presents microscopic images of the wool specimens treated with ZnO-NPs. It is observed that the changes in the surface depend on the treatment method used.

The micrographs clearly show the applied coating of cross-linked gelatin hydrogel containing ZnO. In the modified sample PW ZnO_1, the nanoparticles are finely dispersed and evenly distributed on the surface of the film. The distribution of ZnO nanoparticles is even more uniform and compact in PW ZnO_2, but is located in the film. The use of ultrasonication (in sample PW ZnO_3) allows the particles to penetrate the micropores of the fibers, resulting in a more stable film structure. These observations confirm that the ZnO particles are effectively incorporated into the wool fibers and suggest a potential for improved functional properties and antibacterial effectiveness.

Table. 2. Water absorption, permeability and abrasion resistance of analyzed wool samples

Sample	W_A, mg/cm^2	ISO 20345 requirements	W_p, %	ISO 20345 requirements	Abrasion resistance	ISO 20345 requirements
Zero sample	120.9 ± 4.3	> 70	130.6 ± 2.3	> 80	no defects	without visual defects
PW ZnO_1	137.7 ± 4.7	> 70	89.7 ± 1.3	> 80	no defects	
PW ZnO_2	156.9 ± 5.6	> 70	98.2 ± 1.5	> 80	no defects	
PW ZnO_3	152.8 ± 5.4	> 70	97.2 ± 1.4	> 80	no defects	

Table 3. Color coordinates and color difference of the tested wool samples

Sample name	Measurement conditions	Color coordinates			Color difference
		L*	a*	b*	Δ Eab
Zero sample	Illuminator D65 Viewing angle 100	83.06	0.84	9.87	-
PW ZnO_1		79.52	2.18	17.47	8.49
PW ZnO_2		70.81	8.48	20.49	17.92
PW ZnO_3		76.03	6.25	17.58	11.75

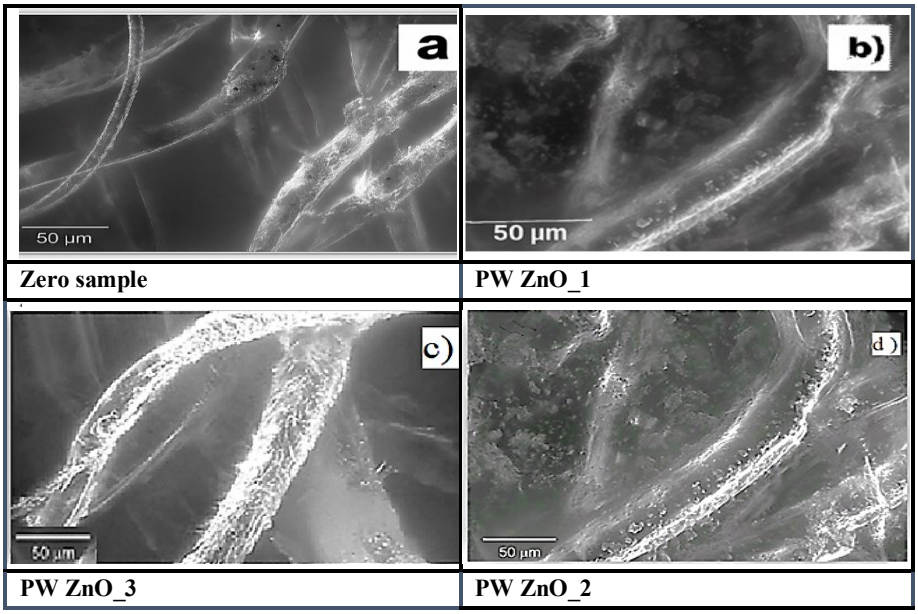

Fig. 2. Microscopic images of wool samples (optical microscope, ×50): a) Zero sample; b) PW ZnO_1; c) PW ZnO_3; d) PW ZnO_2

Fluorescence analysis

The fluorescence analysis of the three wool samples revealed clear differences in their optical properties (Figs. 3, 4). The zero sample of untreated wool exhibited only weak autofluorescence typical of keratin. PW ZnO_1 showed enhanced intensity and a distinct peak around 540 nm due to alkaline hydrolysis of the cuticle, which increases hydrophilicity and creates active binding sites for nanoparticles. The best results were observed for PW ZnO_3 (under ultrasound) - the most intense and uniform fluorescence indicating good nanoparticle dispersion and stable adhesion to the fibers [21, 22].

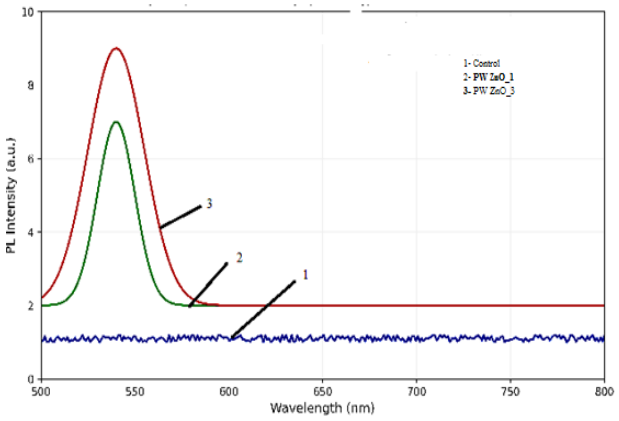

Fig. 3. Fluorescence analysis of wool samples treated with ZnO nanoparticles

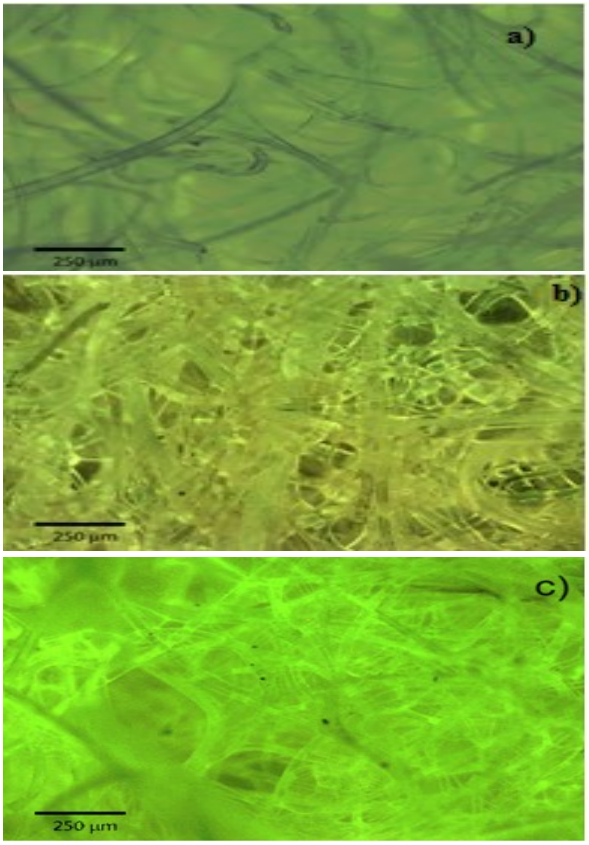

Fig. 4. Fluorescence images of the studied samples: a) Control; b) PW ZnO_1; c) PW ZnO_3

These data confirm that the combined chemical and physical modification of wool samples significantly improve the functionalization of wool fibers and the promotion of nanostructuring.

UV-Vis analysis

UV-Vis analysis was used to identify electronic transitions and bonding interactions between ZnO nanoparticles, gelatin, glutaraldehyde, and wool keratin, demonstrating how chemical modifications alter the optical properties of the fibres and reflect the strength and type of bonds formed [23]. Previous studies on cotton fabrics using similar modifications with gelatin-glutaraldehyde and ZnO nanoparticles have shown improved UV protection and antibacterial activity [24].

On Fig. 5 the characteristic UV peak for PW ZnO_2 at 423 nm corresponds to the absorption band of ZnO. NaOH hydrolyzes the wool cuticle, thereby exposing carboxyl groups that coordinately bind to Zn^{2+}, increasing the hydrophilicity and absorption compared to the control sample [23, 24]. The main peaks in the near-infrared region (1183 - 150 nm) for PW ZnO_1 are associated with ZnO stabilized by gelatin cross-linked with glutaraldehyde. In PW ZnO_3, the peaks at 441 nm indicate improved dispersion of ZnO nanoparticles.

CONCLUSION

Modification of woolen materials was carried out with a view to their application as lining details of footwear. A finish coating was successfully obtained by modifying woolen samples with cross-linked gelatin containing *in situ* synthesized ZnO particles. Microscopic studies showed that ZnO-NPs were impregnated into the structure of the gelatin hydrogel and were distributed into small film-forming structures. Hygienic parameters such as water adsorption and water permeability were also preserved and even improved in some of the samples. The obtained experimental data are promising for antibacterial activity, but for a more complete characterization of the obtained composites, antimicrobial studies are necessary.

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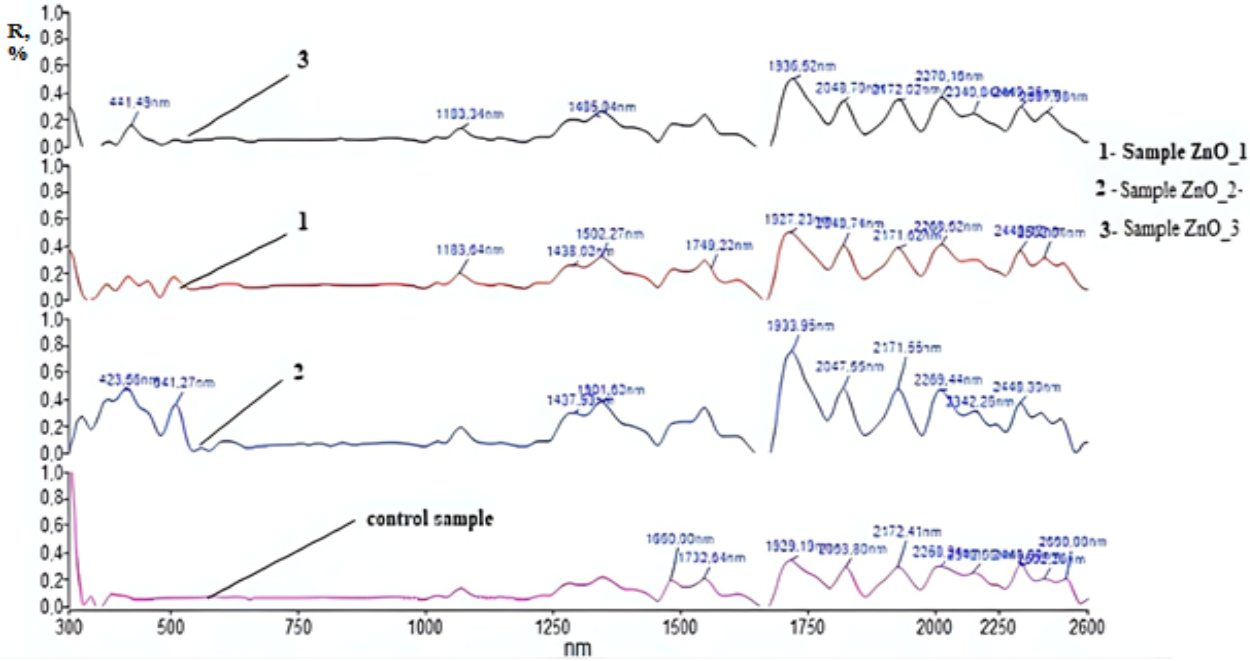

Fig. 5. UV-Vis spectra of wool samples: Control; PW ZnO_1; PW ZnO_2; and PW ZnO_3

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