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Research Article

Preparation of Antibacterial Nanofibre/Nanoparticle Covered Composite Yarns

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The antibacterial efficiency of nanofibre composite yarns with an immobilized antibacterial agent was tested. This novel type of nanofibrous composite material combines the good mechanical properties of the core yarn with the high specific surface of the nanofibre shell to gain specific targeted qualities. The main advantages of nanofibre covered composite yarns over the standard planar nanofibre membranes include high tensile strength, a high production rate, and their ability to be processed by standard textile techniques. The presented paper describes a study of the immobilization of an antibacterial agent and its interaction with two types of bacterial colonies. The aim of the study is to assess the applicability of the new composite nanomaterial in antibacterial filtration. During the experimental tests copper(II) oxide particles were immobilized in the polyurethane and polyvinyl butyral nanofibre components of a composite yarn. The antibacterial efficiency was evaluated by using both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus gallinarum* bacteria. The results showed that the composite yarn with polyvinyl butyral nanofibres incorporating copper(II) oxide nanoparticles exhibited better antibacterial efficiency compared to the yarn containing the polyurethane nanofibres. The nanofibre/nanoparticle covered composite yarns displayed good antibacterial activity against a number of bacteria.

1. Introduction

Nanofibrous materials have attracted a huge amount of interest during the last few decades mainly in the framework of research and innovation studies. Their high surface area, high porosity, small pore size, and compatibility with functionalizing additives mean that they are promising for various applications including filtration, membranes, medical applications, sensors, catalysts, and enzyme carriers [1–10]. Many scientists focus on evaluating the activity and application of nanofibres and nanoparticles [3, 11–13]. However, most of their work is done on a laboratory scale and future developments in technology are anticipated.

In addition to the laboratory scale, there are several companies which produce nanofibres on an industrial scale using various production methods. The most commonly used methods include melt blowing, centrifugal spinning, island-in-the-sea splitting, and needleless electrospinning [14–16]. Each technology has its own advantages and disadvantages. For instance, melt blowing is an environmentally friendly technology with a high production rate; however, the fibre diameter is usually high and the diameter distribution is very wide. This method has an additional problem with die clogging. Island-in-the-sea bicomponent fibre splitting is another technology suitable for the production of nanofibres with relatively high productivity; however, the resultant fibres

are tangled and it is difficult to separate them from each other. In the case of centrifugal spinning, production rate is high but with a wide fibre diameter distribution.

In this work, the needleless electrospinning process was used to eliminate the disadvantages of productivity and wide fibre diameter distribution. Specifically, the needleless roller electrospinning method developed by Jirsak et al. was used [17]. The disadvantage of the RES is the fact that not all polymer solutions can be spun into nanofibres. The reason for this has been explained in previous studies [18, 19]. On the other hand, the RES system was found to be suitable for many polymer-solvent systems [18–21].

A modified RES system for the production of nanofibre/nanoparticle covered yarn was recently developed by Jirsak et al. [22]. In this system, instead of using a flat sheet collecting material, a textile yarn was used. The principle of the roller electrospinning system has been explained in more detail in previous works [20, 21]. This method was used to cover the core yarn with nanofibres. Afterwards, a supporting yarn was covered around the composite yarn to improve the abrasion resistance of the nanofibre cover during further processing, for example, weaving.

Due to the intended use of the material in antimicrobial filters, the suitable antibacterial agent had to be immobilized in the nanofibre component of the composite yarn. There are many antibacterial agents available on the market but copper(II) oxide was chosen due to its long life and low cost. Two types of polymers, polyvinyl butyral (PVB) [20, 21] and polyurethane (PU) [23, 24], were tested in this study to form the nanofibre component of the system.

Compared to the antibacterial test where both PU and PVB were used, the PVB polymer solution was selected for the next step of the experiment. In the next step, the effects of the amounts of copper(II) oxide (CuO) on the antibacterial efficiency were determined by changing its amount in the solution and changing the linear weight of nanofibres on the produced yarn.

For this paper it was important to produce the lowest possible efficient amount of nanofibres with an antibacterial agent at a high nanofibre composite yarn production speed, which is promising for industrial application.

2. Materials and Methods

PVB was purchased from Kuraray America Inc. (Mowital B 60 H, 60,000 g/mol). PU Larithane LS 1086 (Novotex, Italy), which is an aliphatic elastomer composed of 2,000 g/mol linear polycarbonate diol and isophorone diisocyanate and extended by isophorone diamine, was selected as the second polymer.

DMF (Fluka, Switzerland) was used as the solvent for the PU, whereas acetic acid (Penta, Czech Republic) was used as the solvent for the PVB. CuO nanoparticles were purchased from Penta (Czech Republic). The surfactant Triton X-100, obtained from Sigma Aldrich, was used to provide a uniform distribution of the CuO nanoparticles in the PVB and PU polymer solutions.

The principle of a modified RES system based on a rotating roller electrode was used. The electrode roller is immersed in a polymer solution tank and connected to a high voltage source. As mentioned above, the modified RES system was used to cover the base yarn (Figure 1).

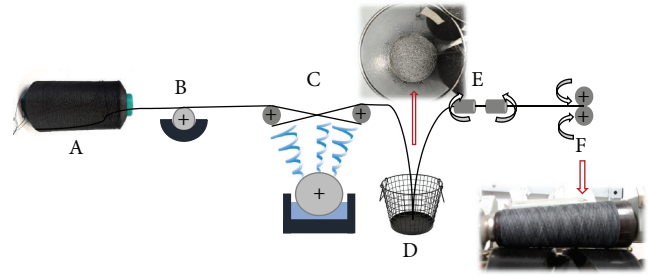


Figure 1: Schematic diagram of a continuous production device. A: black core yarn, B: application of a conductive solution, C: roller electrospinning system, D: collector, E: application of protective yarn(s), and F: take-up mechanism.

The morphology of the nanofibre covered yarns with CuO particles was evaluated using a Tescan Vega3 SB scanning electron microscope (SEM). The zero-shear viscosity of the polymer solutions was measured using a HAAKE Roto Visco rheometer at 25°C. The antibacterial efficiency of the CuO agent immobilized in the composite yarns against Gram-positive *Staphylococcus gallinarum* (*S. gallinarum*) and Gram-negative *Escherichia coli* (*E. coli*) was tested by the modified AATCC test method 100-2004.

3. Experimental

3.1. Fabrication of Composite Nanofibrous Yarns

3.1.1. Preparation of the Polymer Solutions. Two polymers, PVB and PU, were tested for the production of nanofibre composite yarns in order to compare their antibacterial effect. PVB was selected because of its low cost, good mechanical properties, and nontoxicity of the solvent. PVB was dissolved in concentrated acetic acid to form an 11% wt. solution. A total of 10% wt. CuO was added to the PVB solution. PU was selected because of its high elasticity and the relatively good abrasion resistance of the nanofibre part of the composite. PU was dissolved in DMF to form a 15% wt. of solution and stirred overnight and then 10% wt. of CuO was added to the PU solution and dispersed using an ultrasonic disperser for 5 minutes. Finally, 1% wt. of Triton X-100 surfactant was added to minimize the aggregation of the CuO nanoparticles. The bulk viscosity of the polymer solutions was measured to characterize the flow behaviour.

3.1.2. Electrostatic Spinning. A textured polyester base yarn (dtex 167f 36 × 1 × 3) was transported to the roller electrospinning system and used as a collector. After being covered by the nanofibres, the yarn was transported into the collector. Stable electrospinning conditions were used for both solution systems (Table 1).

To compare the antibacterial efficiency of the two solution systems, the speed of the yarn was set to 134 m/min.

Table 1: Process parameters of the electrostatic spinning.

Applied voltage (kV)	Distance between electrodes (mm)	Speed of yarn on the collector (m/min)	Humidity (% RH)	Temperature (°C)	Width of roller (mm)
60	170	80-120-134-160-200	18 ± 1	24 ± 1	470

Table 2: Viscosity of PVB polymer solutions with 0, 5, and 10% wt. of CuO nanoparticles.

Sample and abbreviations	Viscosity (Pa·s)
(P0) Content: 11% wt. PVB60H in 4% wt. H_2O and 85% wt. acetic acid mixture	0.30
(P5) Content: 11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 5% wt. CuO, and 79% wt. acetic acid mixture	1.59
(P10) Content: 11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 10% wt. CuO, and 74% wt. acetic acid mixture	2.47

To optimize the concentration of CuO, 11% wt. of the PVB polymer solution with 0, 5, and 10% wt. of CuO was used during fabrication of the nanofibres covered yarns, and 1% wt. of surfactant was used to prevent aggregation. The viscosity of the solutions was measured and is included in Table 2.

The descriptions of the samples are listed in Table 3. The symbol "P" is the abbreviation for PVB, the number next to P is the amount of CuO particles (% wt.) in the polymer solution, and the last number indicates the speed of composite yarn production.

3.1.3. Characterization of Amounts of Nanofibres in the Product. The amounts of PVB/CuO nanofibres for different production speeds were evaluated by dissolving the specific nanofibre material in ethanol. Ethanol is a good solvent for PVB [25]. The 1 m samples were cut, weighed, and immersed in ethanol overnight. After dissolution of the nanofibrous component, the samples were washed with distilled water, dried, and weighed again. The results were calculated according to the following equation:

$$\text{Amount of nanofibres (\%)} = \frac{(W_0 - W_1)}{W_0} * 100, \quad (1)$$

where W_0 is the weight of composite yarn before nanofibre dissolution and W_1 is the weight of the yarn without nanofibres.

3.2. Antibacterial Test. *Escherichia coli* (*E. coli*) and *Staphylococcus gallinarum* (*S. gallinarum*) bacteria were purchased from the Czech Collection of Microorganisms, Masaryk University. Incubation of the bacteria was performed on a sterile agar with a broth agar medium from Oxoid Ceska Republika.

The antibacterial activities of the fabric systems were evaluated quantitatively in accordance with ASTM E 2149-01 and AATCC test method 100 (standard test method for determining the antibacterial activity of immobilized antibacterial agents under dynamic contact conditions).

The samples were sterilized in an oven at 80°C for 60 min before conducting the test. A blank sample was prepared without any antibacterial agent (CuO). The antibacterial test against Gram-negative (*E. coli*) and Gram-positive (*S. gallinarum*) was performed as the first step. The microorganisms were cultivated in a sterilized LB broth medium and then incubated overnight at 37°C in a shaking incubator. The bacterial suspensions employed for this test contained between 10^2 and 10^3 colony forming units (CFU).

Sterilized samples of nanofibre covered yarns were individually placed into a sterilized test tube and inoculated with 30 mL of *E. coli* or *S. gallinarum* bacterial suspension. At "0" contact time and after 1, 2, 3, 4, and 24 hours, 600 μL of bacterial suspension was extracted and quickly spread on tryptic soy agar plates. The number of viable *E. coli* or *S. gallinarum* was determined by plating the extracted solution onto the Tryptic Soy agar plates and counting the colonies after 24 hours of incubation at 37°C.

Three selected yarns P0-200, P5-200, and P10-200 (each around 1 ± 0.01 g) were used for the disinfection test. The disinfection test was performed on the selected samples against Gram-negative (*E. coli*) and Gram-positive (*S. gallinarum*) bacteria. The bacterial suspensions contained 10^6 colony forming units (CFU).

The percentage reduction of test microorganisms in the test tubes with the nanofibre membranes was calculated using the following equation:

$$\text{Reduction \%} = \frac{(A_0 - A_1)}{A_0} * 100, \quad (2)$$

where R is the percentage reduction of test microorganism; A_1 is the number of bacteria recovered from the inoculated nanofibre membrane with the nanoparticles in the test tube after specified contact time, and A_0 is the number of bacteria recovered from the inoculated nanofibre membrane with the nanoparticles in the test tube at "0" contact time.

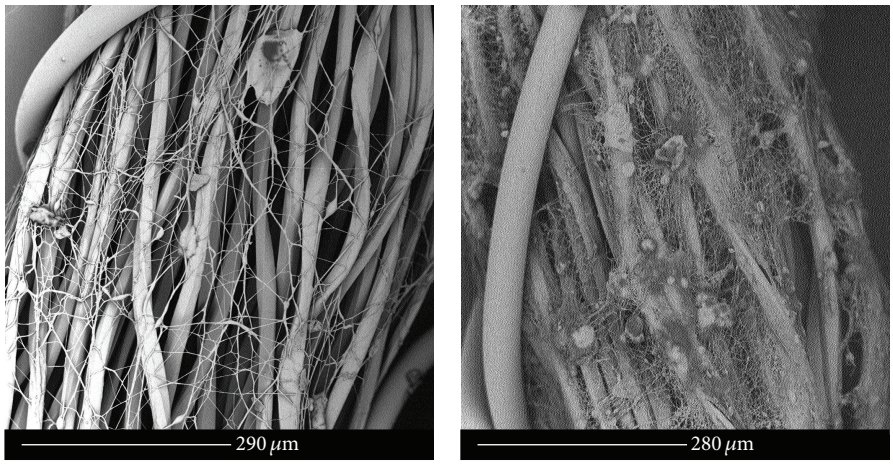
4. Results and Discussion

4.1. Morphology Characterization. A polyester base yarn was covered with nanofibres/NPs and then a polyamide filament was used to protect against mechanical abrasion. The SEM images of the nanofibre/NP covered yarns made of 15% wt. PU with 10% wt. CuO and 11% wt. PVB with 10% wt. CuO are shown in Figure 2.

Figure 2 shows the nanofibres/NPs on the surface of the core yarn. Beads are present on the surface of the nanofibres. The number of beads on the PVB/CuO yarns is higher than the number on the PU/CuO yarns. These beads can be caused by aggregation of 10% wt. CuO or the low concentration of the PVB polymer solution compared to the PU solution.

Table 3: Abbreviations of the second group of samples.

Abbreviation	Content solution (% wt.)	Speed of yarn (m/min)
P0-80	11% wt. PVB60H in 4% wt. H_2O and 85% wt. acetic acid mixture	80
P0-120	11% wt. PVB60H in 4% wt. H_2O and 85% wt. acetic acid mixture	120
P0-160	11% wt. PVB60H in 4% wt. H_2O and 85% wt. acetic acid mixture	160
P0-200	11% wt. PVB60H in 4% wt. H_2O and 85% wt. acetic acid mixture	200
P5-80	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 5% wt. CuO, and 79% wt. acetic acid mixture	80
P5-120	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 5% wt. CuO, and 79% wt. acetic acid mixture	120
P5-160	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 5% wt. CuO, and 79% wt. acetic acid mixture	160
P5-200	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 5% wt. CuO, and 79% wt. acetic acid mixture	200
P10-80	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 10% wt. CuO, and 74% wt. acetic acid mixture	80
P10-120	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 10% wt. CuO, and 74% wt. acetic acid mixture	120
P10-160	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 10% wt. CuO, and 74% wt. acetic acid mixture	160
P10-200	11% wt. PVB60H in 4% wt. H_2O, 1% wt. surfactant, 10% wt. CuO, and 74% wt. acetic acid mixture	200



(a) (b)

Figure 2: SEM images of (a) PU/CuO, (b) PVB/CuO.

4.2. Antibacterial Efficiency

4.2.1. Dependence of the Antibacterial Efficiency on the Type of Nanofibre Polymer. The inhibition efficiency (% reduction of bacteria colony) was calculated according to (2) and the results are shown in Figure 3. The results show that, even after 1 minute of contact of the bacteria with the PVB/CuO, the antibacterial efficiency is over 90%. For each polymer, a blank sample without CuO was tested and a comparison showed that the bacterial colonies started to die after two hours.

This experiment shows that the antibacterial efficiency of nanofibre/NP covered yarns made from PVB/CuO is much higher than nanofibre/NPs covered yarns made from PU/CuO. This could be explained by the formation of cupric acetate, which distributes the Cu^{2+} ions more homogenously than in the case of the nanoparticles. Acetic acid reacts with copper oxide to form cupric acetate. The reaction between CuO and acetic acid is shown in the following equation:

$$2CH_3COOH + CuO \longrightarrow (CH_3COO)_2\,Cu + H_2O \qquad (3)$$

4.2.2. Dependence of the Antibacterial Efficiency on the Amount of Nanofibres in the Product. Three polymer solutions, listed in Table 3 as P0-200, P5-200, and P10-200, were prepared with a constant PVB polymer concentration. From the previous experiment it was determined that the PVB dissolved in acetic acid with the CuO particles evinced an enormous antibacterial efficiency. It was determined that more than 90% of the bacteria died at "0" contact time of the nanofibre/NP covered yarns with the tested microorganism. This excellent antibacterial effect was observed after 1 hour. For that reason, the concentration of CuO was optimized (decreased) and various yarn speeds were used to improve productivity. To prevent aggregation of CuO particles, 1% wt. of nonionic Triton X-100 surfactant was used. PVB with 10% wt. of CuO has a very high bulk viscosity compared to the other samples. The viscosity results are shown in Table 2. High viscosity restricts the ability of the polymer solution to be electrospun into submicron fibres. As a result, a lower amount of nanofibres is collected on the yarn. SEM images show that the amount of nanofibres is lower but the amount

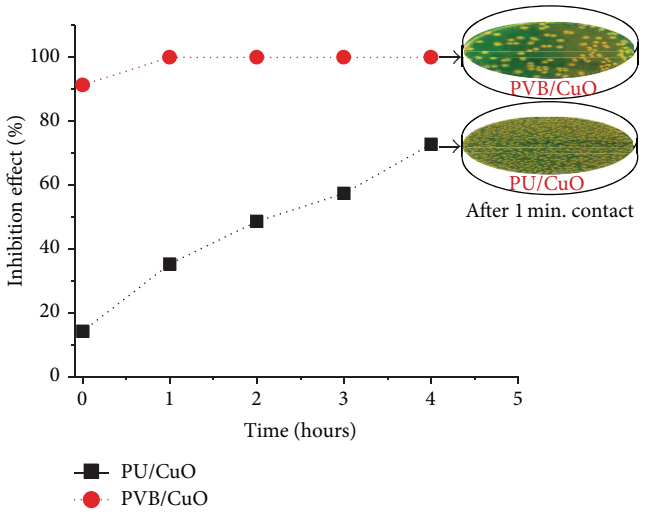


Figure 3: Reduction of bacteria (*E. coli*) overtime.

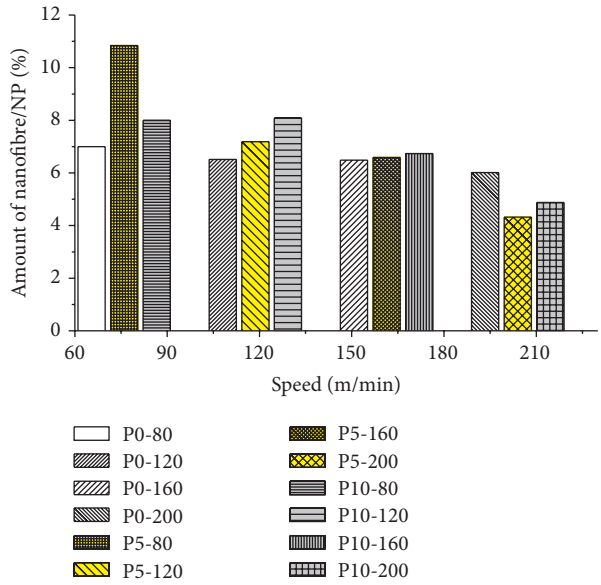


Figure 4: Amount of PVB/CuO nanofibres versus production speed of yarn. The amount of nanofibres on the yarn slightly decreased with an increase in speed due to a lower nanofibre collecting time.

of immobilized particles is higher in the case of 10% wt. of CuO.

The percentage amounts of nanofibre covered base yarn were calculated according to (1) and are shown in Figure 4.

Figure 4 indicates that the change in the amount of nanofibre/NPs depends on the speed of production. P0 indicates that no CuO is immobilized in the nanofibre component, while P5 has 5% wt. and P10 has 10% wt. of nanoparticles, respectively. When the speed of yarn production increases, the amount of nanofibres/NPs decreases on the surface of the composite yarn.

The aim of the experiment was to determine the lowest possible efficient amount of nanofibres and antibacterial agent. The results of the antibacterial efficiencies are shown in Figure 5. It was determined that PVB with CuO exhibits good antibacterial efficiency against *E. coli* and *S. gallinarum*. The amount of 5% wt. of CuO immobilized in PVB nanofibres showed an antibacterial efficiency of 99.99% at a production rate of 200 m/min. At "0" contact time more than 50% of *E. coli* and *S. gallinarum* bacteria were dead. CuO has a better antibacterial efficiency against *E. coli* at "0" contact time. On the other hand, after one hour contact time, no bacteria remained alive.

It can be seen that, even at high speeds and low amounts of nanofibres/NPs, CuO showed remarkable antibacterial properties against *E. coli* and *S. gallinarum*. It is possible to conclude that the production rate of the equipment can be increased with the same amount of CuO or the concentration of CuO can be decreased at a low yarn production speed. Both methods will show good results. For mass production, a higher yarn speed is more desirable.

Because the inhibition effect was found to be excellent even for nanofibre covered yarn with a lower concentration of CuO, a higher (10^6 CFU/mL) bacteria inoculum was prepared as explained in Section 2. Three yarns, P0-200, P5-200, and P10-200, were selected for subsequent testing. These samples were produced at the highest production rate, which means that the amount of nanofibres or nanofibre/NPs is the lowest compared to the others. The antibacterial test was performed against Gram-positive and Gram-negative microorganisms and the results are shown in Figure 6.

The results of the experiment show that more than 50% of *S. gallinarum* bacteria are killed by the immobilized agent at "0" contact time. In one hour, PVB with immobilized CuO has a disinfection effect in accordance with the high concentration of the tested bacteria inoculum (10^6 CFU).

The results in Figure 6 show that the initial antibacterial efficiency of CuO against *S. gallinarum* is higher than that against *E. coli* at "0" contact time. CuO nanoparticles are more effective against Gram-positive microorganisms. After 1 hour contact time, it was observed that both Gram-positive and Gram-negative bacteria were successfully disinfected. It can be concluded that the CuO agent in PVB nanofibre composite yarn exhibits a 99.99% disinfection efficiency against *E. coli* and *S. gallinarum* bacteria even at high CFU.

5. Conclusion

In this work, composite yarns with a nanofibre cover were prepared by a modified needleless electrospinning method. The testing comprised two types of polymers (PU and PVB). The process properties affecting the quality of the nanofibre compound, for example, the solution concentration, viscosity, spinning distance, voltage, and air humidity, were empirically optimized. The effect of the production rate on the linear weight of the nanofibre cover was evaluated. An inversely proportional effect of the production speed on the linear weight of the nanofibre cover was confirmed.

In accordance with the intended use of the composite yarn in antimicrobial filtration cartilages, the antibacterial agent CuO was incorporated into the nanofibrous layers and the antibacterial performance of the materials was studied.

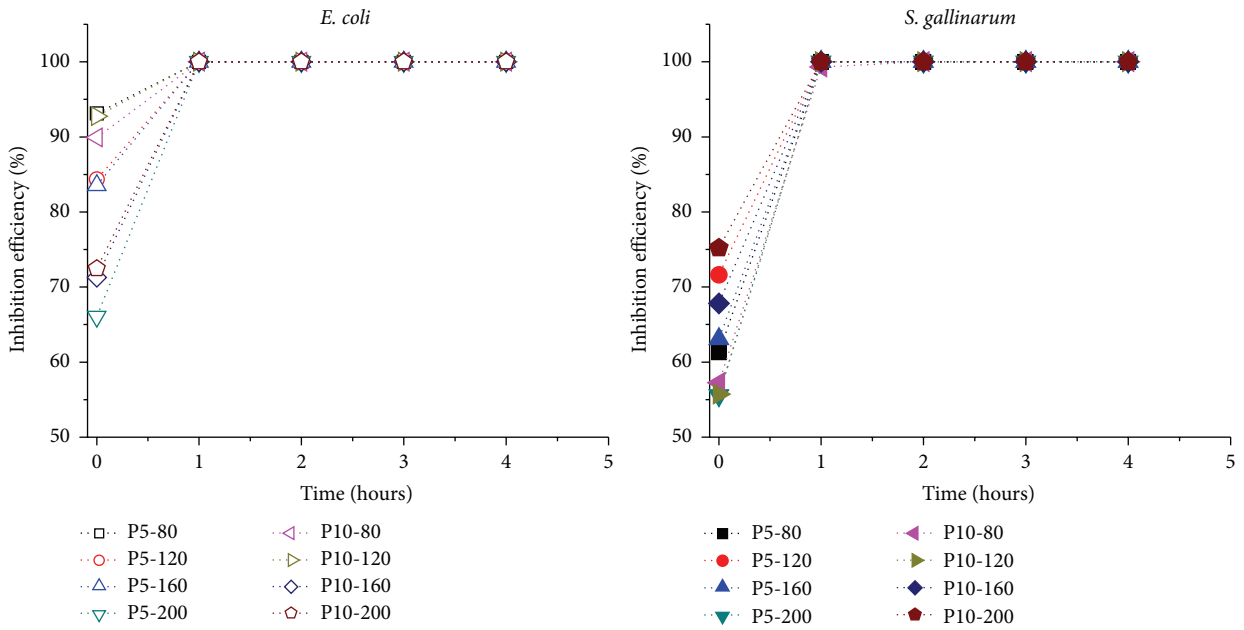


Figure 5: Antibacterial efficiency of nanofibre covered yarn with different concentrations of CuO agent.

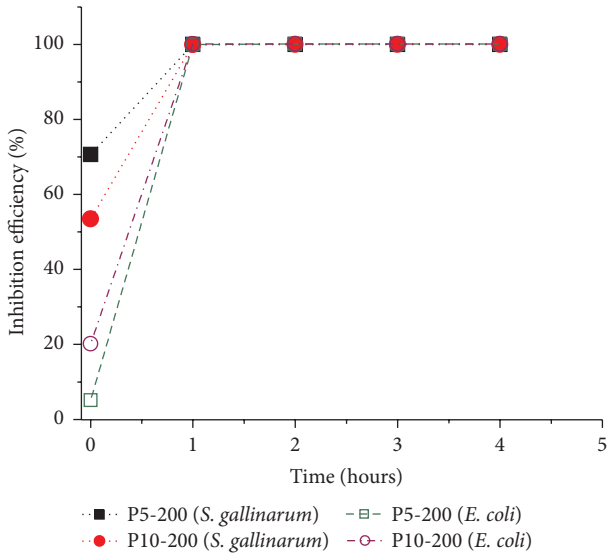


Figure 6: Antibacterial efficiency against 10^6 CFU bacterial concentrations.

The effect of the CuO concentration in the solution and the linear weight of the nanofibre cover on the antibacterial performance were measured.

Testing procedures comprised studies of the interaction of the prepared material with two bacterial types, that is, *E. coli* and *S. gallinarum* (Gram-positive and Gram-negative).

It was concluded through an evaluation of the sets of results that materials prepared by covering the core yarn with PVB/nanofibres with a CuO antibacterial agent generally show significantly higher antibacterial efficiency compared to yarns covered with PU nanofibres. This can be attributed to the better uniformity of the antibacterial agent distribution caused by the reaction of CuO with acetic acid creating copper acetate. Copper acetate then dissociates in the abundant acetic acid which helps to distribute the Cu^{2+} ions in the nanofibre mass. Better distribution of the Cu^{2+} ions enhances the probability of the contact between the antibacterial agent and the bacteria improving the overall antibacterial efficiency. The results showed that an antibacterial efficiency of 99.99% is achieved at 5% wt. of CuO additive and a production rate of 200 m/min. One hour contact is sufficient to disinfection all of the bacterial colonies.

The experimental work showed that the new type of composite nanofibrous yarns with immobilized antibacterial agents can be utilized to design antibacterial filtration cartridges for air and water purification.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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