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Development of Cuprous Oxide based Antipathogenic Textiles

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SUMMARY OF THE DOCTORAL THESIS

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ABSTRACT

The present work deals with the development of an environmentally friendly, low-price, easy, and fast method for developing antibacterial cuprous oxide-coated multifunctional cotton fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Subsequently, the cuprous oxide particles were deposited on cotton fabrics. Three different types of reducing agents with different concentrations were selected to make the Cu_2O particles. Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antibacterial effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite. Furthermore, the utility of hygienic antimicrobial-developed fabrics was analyzed for comfort properties regarding air permeability and stiffness. In the end, the durability of the coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.

In the second part of thesis a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance was developed**.** The objective of the second part was to develop an environmentally friendly, low-price, easy, and fast method for developing antipathogenic (antibacterial, antifungal, and antiviral) cuprous oxide-coated multifunctional fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. For sensitization and Cu_2O particles formation the same procedure was used. The most suitable reducing agent with optimum concentration was selected from the aforementioned study**.** Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. In the second step, a reactive antibacterial dye was made (by reacting Reactive Blue 4 with triclosan). The molecular structure of the modified dye was confirmed through FTIR and ^{13}C-NMR. The resultant antibacterial dye was applied on copper-treated cotton fabrics through exhaust dyeing protocol. The dyed fabric was characterized through colorimetric data (L*, a*, b*, C, H, and K/S), levelness of dye, fastness properties as well as exhaustion and fixation rates. The antipathogenic activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antipathogenic effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite. Furthermore, the utility of hygienic antimicrobial developed fabrics were analysed for the comfort properties regarding air permeability and stiffness. At the end, durability of coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.

Keywords: antimicrobial; hospital-acquired infections; medical textiles; cuprous oxide particles; color analysis

ABSTRAKT

Tato práce se zaměřuje na vývoj ekologického, nákladově efektivního, snadného a rychlého způsobu výroby antibakteriálních bavlněných tkanin potažených oxidem měďným. Nejprve byly tkaniny senzibilizovány pomocí kyseliny citrónové, poté následovala syntéza částic Cu_2O z Fehlingova roztoku. Následně byly částice oxidu měďného naneseny na bavlněné tkaniny. Pro výrobu částic Cu_2O byly vybrány tři různé typy redukčních činidel v různých koncentracích. Morfologie povrchu a přítomnost atomů kovů byly zkoumány pomocí rastrovací elektronové mikroskopie, dynamického rozptylu světla, FTIR, EDS a XRD. Antibakteriální účinnost tkanin potažených oxidem měďným byla hodnocena pomocí kvalitativních a kvantitativních analýz. Nejsilnější antibakteriální účinek byl pozorován u tkanin potažených částicemi oxidu měďného redukovanými pomocí hydrogensiřičitanu sodného. Dále byla analyzována vhodnost těchto vyvinutých hygienických, antimikrobiálních tkanin z hlediska komfortních vlastností, konkrétně prodyšnosti a tuhosti. Nakonec byla trvanlivost povlaku ověřena posouzením antibakteriálních vlastností a provedením SEM analýzy po praní.

Druhá část této práce představuje nový přístup k vytváření esteticky příjemných antibakteriálních bavlněných tkanin potažených oxidem měďným. Cílem této části bylo vyvinout ekologicky šetrnou, nákladově efektivní, snadnou a rychlou metodu výroby multifunkčních tkanin s antipatogenními vlastnostmi (antibakteriálními, antifungálními a antivirovými) potaženými oxidem měďným. Podobně jako v první části prošly tkaniny senzibilizací kyselinou citrónovou a syntézou částic Cu_2O pomocí Fehlingova roztoku. Na základě předchozí části práce bylo určeno nejvhodnější redukční činidlo s optimální koncentrací. Morfologie povrchu a přítomnost atomů kovu byla analyzována pomocí rastrovací elektronové mikroskopie, dynamického rozptylu světla, FTIR, EDS a XRD.

V následném kroku bylo syntetizováno reaktivní antibakteriální barvivo reakcí Reactive Blue 4 s triclosanem. Molekulární struktura modifikovaného barviva byla potvrzena pomocí FTIR a 13C-NMR. Toto antibakteriální barvivo bylo poté aplikováno na bavlněné tkaniny ošetřené mědí pomocí protokolu barvení vytahovacím postupem. Obarvená látka byla charakterizována pomocí kolorimetrických parametrů (L*, a*, b*, C, H a K/S), egality vybarvení, stálosti, vytažení z lázně a rychlosti fixace.

Antipatogenní účinnost tkanin potažených oxidem měďným byla hodnocena kvalitativně a kvantitativně. Nejvýraznější antipatogenní účinek vykazovaly tkaniny potažené částicemi oxidu měďného redukovanými pomocí hydrosiřičitanu sodného. Komfortní vlastnosti těchto hygienických, antimikrobiálně vyvinutých tkanin byly navíc hodnoceny z hlediska propustnosti vzduchu a tuhosti. Nakonec byla trvanlivost povlaku potvrzena vyhodnocením antibakteriálních vlastností a provedením SEM analýzy po umytí.

Klíčová slova: antimikrobiální; infekce získané v nemocnici; lékařské textilie; částice oxidu měďného; analýza barev

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1. Introduction

Hospital-acquired infections (HAIs) are on the rise despite efforts to decrease them. These infections develop in patients during their stay and continuous concern with the hospital. They are not only costly to treat, but more importantly, cause human suffering and even death [1]. A major source of cross-infection is contaminated (bacteria and viruses) fabrics in hospitals. The most common textile coverage used in a specific hospital area (surgical, ICUs, and patient wards and rooms) included surgical gowns, drapes, curtains, panel covers, wall papers/sheets coverage, shoe mats, outlet covers, seat chair covers, table covers, patient and doctors' socks, etc. [2]. The patients, medical and common staff all are carriers of hospital-acquired infections [3]. The current interest has focused on high-touch textile-based surfaces and their ability to serve as reservoirs for pathogenic microorganisms, including *Staphylococcus Aureus*, *Clostridium difficile*, and *vancomycin-resistant Enterococci* [4, 5]. Instead of bacteria, among most pathogenies, the human infected viruses also contribute massively to hospital-born infections. Adding fuel to the fire, bacteria (after *Ethicillin* now *Methicelline*-resistant *Staphylococcus Aureus*) and viruses (a new strain called SARS-CoV-2) increasingly are becoming resistant to last-resort drugs. The rate of transmission of infection is very high and mainly spread during close contact and via respiratory droplets discharge and by touching a contaminated surface [6]. The risk can be increased when individuals have continuous and close contact with animals; also, climate change. The tragedy is that the infected person has been visiting the places and remain in touch with surfaces and people. It becomes too late when a person realized about infection and till that time infection has been delivered to many places, communities, or even to family members [7][8][9]. The only way to avoid life-threatening pathogens is to kill/inhibit them before transmitting them inside any human body. That is why the selection of antibacterial/antiviral common material used in daily life is necessary. A very quick and fast option to inactive the viruses and bacteria within a minute is the use of surface disinfectants with 62-71% alcohol, or bleaching agents containing 0.5% hydrogen peroxide and 0.1% sodium hypochlorite [9]. The alkali soaps (pH over 12) are working as well very efficiently. Hygiene standards for surface cleanliness, based on food processing industry standards also proposed [3]. Some new technologies, such as the use of UV light units and various hydrogen peroxide (HP) systems [10], can effectively decontaminate hospital rooms. However, even when improved hand washing compliance and diligent surface hygiene disinfection are combined, hospital-acquired infections (HAIs) are still serious health issues. All the above approaches including hygiene hand washing, UV light, surface disinfection, and HP systems have one thing in common, they are episodic. or one-time approaches. Therefore, as soon as the decontamination process ends, the microbial contaminants can again begin to accumulate [11]. The researchers have been using different types of antimicrobial finishing on hospital textiles based on the coating of inorganic metal oxides [12][13].

2. Purpose and aim of thesis

The main aim of the thesis is to investigate preparation properties and selected applications of bioactive textiles having the antimicrobial ability, antiviral, antifungal, and durability. To develop the copper-coated bioactive textiles a sufficient amount of copper ions is required over the fabric structure. This thesis is focused on the copper deposition on cotton fabric and subsequently dyeing it with antimicrobial dye (modified reactive blue 4 dye). The fabric used in this study was plain-woven cotton fabric with an areal density of "150 g/m^2."

At first, the formation of Cu_2O particles was done by the Fehling solution method and studied the effect of three different reducing agents to make the Cu_2O particles.

In the second step, the deposition of cuprous oxide particles was done on woven cotton fabric, and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties. The purpose of deposition of the cuprous particles over cotton fabric was to find a suitable application in hospital areas (surgical, ICUs, and patient wards and rooms) including surgical gowns, drapes, curtains, panel covers, wallpapers/sheets coverage, shoe mats, outlet covers, seat chair covers, table covers, patient and doctors' socks, etc [17][18].

The third step was to dye the already cuprous oxide-coated fabrics with antibacterial dye. In some studies, dyeing of the coated textiles has been performed to overcome discoloration and staining but their antibacterial effectiveness is compromised [19].

3. Overview of current state of problem

The most common and particular interest of metal particles are of Ag, Cu, TiO_2, ZnO, MgO, CuO, Cu_2O, etc. [7][14]. They are not only stable under harsh process conditions but also generally regarded as odorless work wear. Copper-based materials are of most reliable because they are not only effective against microbes (within two hours) but are the only material that is most effective against the viability of pathogenic viruses (coronavirus within four hours). Copper, its ions, and alloys have demonstrated excellent antiviral, antibacterial, and antifungal activity against a wide range of pathogens [7][8][9]. The antibacterial properties of coper-based finishes are especially dependent on their shapes and sizes to assure a uniform size distribution of particles over the textile structure. In recent years, the copper and cuprous oxide particles have attracted so much attention in many potential applications in catalysis, cooling fluid or conductive inks, heat transfer systems, and antimicrobial, antifungal, and antiviral agents [12]. Ali et al. [13] coated the copper oxide nanoparticles on cotton fabric to achieve antimicrobial properties. The copper oxide particles have very low stability and antimicrobial properties are affected. They can easily convert to different copper-based compounds like copper carbonate (greenish), copper sulphate CuS, etc. in different environment conditions. The resulting compounds formed by copper oxide particles are more toxic and cause acute poisoning some time[13]. Among copper-based (CuO, Cu, Cu_2O) anti-microbial agents the cuprous oxide particles are extremely regarded due to their optical, catalytic, mechanical, and, low-cost preparation. The cuprous oxides are more stable, effective, and beneficial regarding antibacterial properties. They are easily reducible and soluble in alkaline conditions. The antibacterial properties of copper-based finishes are especially dependent on their shapes and sizes to assure a uniform size distribution of particles over the textile structure. CuO and Cu_2O particles have been used to develop antimicrobial substrates. The CuO has various limitations because of low stability, and can easily convert to different copper-based compounds like copper carbonate (greenish), copper sulphate, CuS, etc. in different environment conditions. The produced compounds are more toxic and cause acute poisoning. With the above background information work was focussed on the development of durable, stable, and evenly distributed cuprous oxide (Cu_2O) particles on the textile structure.

In this developed study, we report a special technique to develop the Cu_2O particles [15]. Excellent antibacterial activities have been attained through the deposition of Cu_2O particles nanoparticles by employing these technologies. However, such treatments have led to some undesirable effects such as discoloration or staining on the coated fabrics which affect the aesthetic properties of textiles. In some studies, dyeing of the coated textiles has been performed to overcome discoloration and staining but their antibacterial effectiveness is compromised [16]. Thus, the development of highly effective antimicrobial textiles with improved aesthetics is yet challenging. Therefore, a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with excellent aesthetic appearance was investigated in detail. Considering the above-mentioned problems, the current study has proposed a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Then, the cuprous oxide particles were deposited on cotton fabrics. In the second step, a reactive dye was selected and functionalized as active against pathogens. The functionality was induced by reacting the Reactive Blue 4 dye with triclosan (antibacterial agent). Subsequently, the cuprous oxide particles coated fabric were subjected to exhaust dyeing through the solution of functional bioactive dye.

4. Methods used and study materials

In this study, a plain-woven cotton fabric with an areal density of 220 g/m^2 was employed as the substrate for producing antibacterial fabrics. The chemicals used for the synthesis and deposition of cuprous oxide (Cu_2O) had 99.99% purity. Reactive blue 4 (35% dye content) was purchased from Sigma Aldrich. Triclosan (97%) was procured from TCI Japan. While Sodium Potassium tartrate, Copper sulfate pentahydrate, $Na_2S_2O_4$ (Sodium dithionite) and Glucose were of ACS reagent grade.

4.1 Preparation of cuprous oxides particles

Cuprous oxide particles Cu_2O were prepared by combinations of two Fehling (A & B) solution by using three different reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite). Fehling solution A and Fehling solution B were prepared separately. For the preparation of Fehling A, 34.64 grams of $CuSO_4.5H_2O$ was dissolved in 500 ml of distilled water and stirred continuously. The Fehling B solution was prepared by dissolving 70 grams of NaOH and 175 grams of Sodium Potassium tartrate were dissolved in 500 ml of water. Subsequently, we took 100 ml of each Fehling A and Fehling B in a cleaned round bottom flask and heated it to 95°C with continuous stirring. Then 10 grams of reducing agent (glucose) was dissolved in 100 ml of water and added into the above solution (Fehling A and Fehling B in a cleaned round bottom flask and heated to 95°C). The color of the solution turned from blue to red and a large amount of precipitate was formed in the bottom of the flask. The precipitate was centrifuged and washed 3- 4 times with deionized water. The same procedure was also repeated with the other two reducing agents (ascorbic acid, sodium hydrosulphite). Hence, we have three types of Cu_2O particles, prepared with three different types of reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite).

4.2 Deposition of Cu2O particles on cotton fabric

Before the deposition of cuprous oxide nanoparticles substrate was pre-treated. Pre-treatment was done with citric acid. A solution of 20 g/L citric acid was made and fabric was dipped in it at 80°C for 2 hours, then washed and dried at 90 °C for 50 minutes. After pre-treatment citric acid is not a part of cotton fiber. Citric acid is there as an ion exchanger. During pre-treatment, the sodium and other ions were removed from fibers and replaced by H^+ ions from the citric acid solution. By this pre-treatment are the fibers activated by a change of zeta potential. Cu particles are attracted after this preparation from solution effectively to fiber surfaces by electrical forces. As mentioned earlier, we have prepared three different types of Cu_2O particles with three different types of reducing agents (Glucose, Ascorbic acid, and Sodium hydrosulphite). In the next step, three different concentrations (1g, 0.5g, 0.25g) of each type of Cu_2O were applied to pre-treated cotton fabric. The concentrations (1g, 0.5g, 0.25g) of Cu_2O centrifuged particles were dispersed in 200 ml of water**.** Cotton fabric was dipped in each solution for 30 minutes then pad and dry at 90°C for 20 minutes. We made 3 samples against each selected reducing agent. The same procedure was adopted for each reducing agent (Glucose, Ascorbic acid, and Sodium hydrosulphite)**.** Hence, we developed a total of 9 samples for three reducing agents as shown in Table 4 below.

Table 1: Design of experiments for the developed samples.

No of samples	Reducing agent	Code of reducing agent	Concentration of Cu_2O particles
1	Glucose	G1	1g/200ml
2	Glucose	G2	0.5g/200ml
3	Glucose	G3	0.25/200ml
4	Ascorbic acid	A1	1g/200ml
5	Ascorbic acid	A2	0.5g/200ml
6	Ascorbic acid	A3	0.25/200ml
7	Sodium hydrosulphite	S1	1g/200ml
8	Sodium hydrosulphite	S2	0.5g/200ml
9	Sodium hydrosulphite	S3	0.25/200ml

4.3 Preparation of cuprous oxides particles and deposition on cotton for the second part

Cuprous oxide particles (Cu_2O) were synthesized by combinations of two Fehling (A & B) solutions and three separate reducing agents, namely Glucose, Ascorbic acid, and Sodium hydrosulphite. Separate preparations of Fehling solutions A and B were made. For the preparation of Fehling A, 69.28 grams of $CuSO_4.5H_2O$ was dissolved in 1 liter of distilled water. Then added a few drops of H_2SO_4 (we added 5 drops). 350 g of Sodium Potassium tartrate and 140 g of NaOH were mixed in 1 liter of distilled water to prepare the Fehling B solution. Fehling A and B solutions were mixed in a 1:1 ratio. Then reducing agent (Glucose) was immediately added to the mixed solution (2.5% of the total weight of the mixed solutions - Fehling A + Fehling B). The solution of fehlings and glucose was applied to cotton fabric using the padding method and the wet allowance was 75 %. After squeezing the fabric is placed in a heat press for 4 minutes at 50 °C. The same process was conducted for the other two reducing agents i.e., sodium hydrosulphite and ascorbic acid.

4.4 Functionalization of Reactive Blue 4 dye with Triclosan

Functionalization of Reactive Blue 4 **(2)** dye with triclosan **(1)** was carried out in a single-step reaction. An equimolar amount (20 mM) of both reactants was taken. In a round bottom flask, 13.6 g of reactive blue 4 dye was added and dissolved in 100 mL of distilled water (solution A). Solution A was refluxed and the temperature was maintained at 40-45°C. In a 100 mL beaker containing 20 mL of methanol, 5.79 g of triclosan (antibacterial agent) was added and stirred (solution B). The solution B was slowly added to solution A. The pH of the solution was kept neutral using sodium carbonate. The progress of the reaction was monitored through Thin Layer Chromatography (TLC) using ethyl acetate and petroleum ether (70:30) solvent system. The reaction mass was stirred at 45°C until the disappearance of triclosan spot on TLC plate which confirmed the successful completion of reaction and product formation. Functionalized reactive dye **(3)** was filtered and dried in the oven at 40°C.

4.5 Application of functionalized dye on Fabric

The functionalized reactive dye was applied to copper-coated cotton fabric through the exhaust dyeing method. For this purpose, an H-T dyeing machine was used. 3% dye shade (o.w.f) was applied on the fabric with a material-to-liquor (M: L) ratio of 1:50. Dyeing of the fabric was started at room temperature which was gradually increased to 70-80 °C. Electrolytes (sodium sulfate, 40 g/L) and alkali (1 g/L sodium hydroxide and 15 g/L sodium carbonate) were added portion-wise in the dyebath at different time intervals for proper exhaustion and fixation of dye. The dyeing of the fabric was continued for 60 minutes. After that, the dyed fabric was removed from the dyebath and rinsed with tap water followed by hot was at 90 °C for 10 minutes to wash away the unfixed dye molecules from the fabric. The possible formation of a covalent bond between the chlorine atom of the triazine reactive system of dye and hydroxyl groups of cotton fabric. Hence, we developed a total of 6 samples for three reducing agents (3 are dyed and 3 undyed). The plan of the experiments is given in Table 5.

Table 2: Plan of experiments for the developed samples.

No of samples	Reducing agent	Applicant of Dye	Sample code
1	Glucose	No	G
2	Glucose	Yes	DG
3	Ascorbic acid	No	A
4	Ascorbic acid	Yes	DA
5	Sodium hydrosulphite	No	S
6	Sodium hydrosulphite	Yes	DS

4.6 Surface characterizations

The morphological characteristics of cuprous oxide nanoparticles coated on the surface of cotton fabric were examined through scanning electron microscope (SEM) from FEI Quanta 50, while XRD analysis was performed with a diffractometer equipped with a conventional X-ray tube Cu Ka1 radiation (1.54 A°) power condition (40 kV/30 mA). The XRD pattern was measured in the 2θ range 10–80° with a step size of 0.02°. The dynamic light scattering (DLS) theory from Malvern Zetasizer, from Pan Analytical X'pert PRO tools. ^{13}C-NMRspectra were recorded using Bruker Advance Spectrophotometer which operates at 600 MHz and tetramethylsilane as an internal reference. The identification of functional group-modified reactive dye was done using FTIR spectra recorded on the FTIR Perkin Elmer spectrophotometer.

4.7. Dye exhaustion, fixation, and total fixation measurement

Exhaustion, fixation, as well as total fixation amount for the modified dye were determined using the method described in [94], following equations were employed for calculations of exhaustion

$$\%E = [(C_1 - C_2)/C_1] * 100 \quad (2)$$

Here, C_1 [94] and C_2 [94] represent the concentration of dye in solution before and after dyeing. Let C_3 [94] shows the concentration of extracted hydrolyzed dye from cotton fibres to the bath. Portion of covalently fixed dye is then calculated as

$$\%F = [(C_1 - C_2 - C_3)/(C_1 - C_2)] * 100 \quad (3)$$

Portion of hydrolyzed dye on fabric is then calculated as

$$\%H = [C_3/(C_1 - C_2)] * 100 \quad (4)$$

4.8 Fastness properties

The light, rubbing, and washing fastness properties of the copper-treated dyed fabric samples were assessed. ISO 105-X12, ISO 105-C06, and ISO 105-B02 standards were followed for the evaluation of rubbing fastness and washing fastness, and lightfastness, respectively.

4.9 Colorimetric data measurement

CIELAB values (a*, b*, h*, L*, C*) and K/S for copper-treated undyed and copper-treated dyed fabric sample was determined using a reflectance spectrophotometer. Negative and positive values of b* indicate the degree of the blueness and yellowness of the dye, respectively, whereas negative values of a* indicate the extent of the greenness and positive values the degree of its redness. Chroma is represented by C*, brightness by L* (values between 0 and 100, where 0 represents the pure black color and 100 pure white), and h* represents the hue angle (00–3600). K/S values of cotton-dyed cloth were estimated employing Kubelka-Munk Equation (equation 5).

$$K/S = (1 - R)^2/ 2R \quad (5)$$

Here, R indicates the percentage reflectance, K is the absorption coefficient and S is the scattering coefficient.

4.10 Assessment of dye levelness

Both visual and objective techniques were employed to evaluate the levelness of the modified reactive dye on the dyed fabric. Visual examination involved observing the fabric from different angles and assigning grades from 1 to 5, with 5 indicating excellent levelness and 1 indicating poor levelness. To achieve more precise results, an objective method was also used. In this method, the fabric was scanned at 12 different points using a reflectance spectrophotometer, and K/S values were calculated. The calculated standard deviation for each K/S measurement was used to assess dye levelness. Lower standard deviation values corresponded to higher dye levelness; values within the range of 0.20 indicated excellent dye levelness, while values greater than 1.0 indicated poor dye levelness.

4.11. Evaluation of Comfort Properties

Air permeability is defined as the rate of air flowing perpendicularly through a known specific area. The flow of air is maintained under a prescribed air pressure differential between the two surfaces of a material. The test was performed as per ISO9237 by using an SDL air permeability tester. The air pressure difference between the two surfaces of the substrate was 100 Pa. Furthermore, the stiffness of untreated and treated with cuprous oxide particles cotton fabric samples was measured by TH 4 bending rigidity tester.

4.12 Assessment of antibacterial properties

The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements.

4.12.1. Zone of inhibition test (qualitative measurements)

Preparation of bacterial strain. The bacterial strains used in this study, *Escherichia coli* (Gram-negative, CCM 3954) and *Staphylococcus aureus* (Gram-positive, CCM 3953), were sourced from the Czech Collection of Microorganisms at Masaryk University in Brno, Czech Republic. Fresh bacterial suspensions were prepared by growing single colonies overnight at 37°C in nutrient broth. The turbidity of the bacterial samples was adjusted to an optical density of 0.1 at 600 nm (OD600) before conducting antibacterial experiments. Agar plates were prepared freshly for each antibacterial test. A

sterilized cotton swab was immersed in the bacterial culture suspension and evenly spread across the agar plates. These plates were immediately used for the antibacterial activity assessments.

Determining Zone of Inhibition. The antibacterial activity assessment was conducted following the procedure described in detail in references [17][96]. For this study, squares of cuprous oxide particles coated cotton fabric measuring 6 x 6 mm were placed directly onto agar plates that had been inoculated with bacteria. Concurrently, untreated virgin cotton fabric was used as a control. The samples and inoculated agar plates were then incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was calculated as the combined diameter (mm) of the cuprous oxide particles coated textile sample and the clear halo zone where bacterial growth was impeded. All measurements were performed in triplicate to ensure accuracy and reliability.

4.12.2. Reduction factor (quantitative measurements)

ISO 20743:2013 transfer method was used for the quantitative antibacterial analysis of dyed cotton. Agar plates were prepared and inoculated with 1 mL of inoculum as mentioned in the method. The control (2 specimens) and dyed samples (2 specimens) of 3×8 cm in dimensions were placed on prepared agar plates and samples were pressed down by applying 200 g weight. One specimen of each sample was detached from the agar plates and was placed on separate Petri dishes keeping the transferred surface faced upside. The samples were then incubated at 37°C for 24 h. The second specimen of control and dyed samples were transferred immediately to 2 reagent bottles separately containing 30 mL of saline solution (0.85% NaCl) to get bacterial colony counts at 0 hr. After shaking bottles for 15 minutes, 8 serial dilutions of this saline solution were prepared and plating of all dilutions was performed on agar growth media as described in ISO 20743. The same procedure was repeated for samples placed in an incubator for 24h to get bacterial colony count after 24 h. The antibacterial activity (A) of the dyed cotton sample was determined using the formula given in Equation 6. Each sample was performed in triplicate for confirmation of results.

$$A=F-G \quad (6)$$

Where $F= (\log C_t - \log C_0)$ and C_0& Ct is the bacterial count of control cotton fabric at 0 and 24 h and $G= (\log T_t - \log T_0)$ and T_0 & Tt is the bacterial count of control fabric at 0 and 24 h.

4.13 Weight Gain

During the Cu_2O deposition, the weight gain percentage was examined according to the following equation:

$$w = \frac{m-m_0}{m_0} \times 100 \quad (7)$$

Where m is the final mass, m_0 is the original mass of the substrate and w is the total weight gain percentage.

4.14 Antifungal activity assessments

The antifungal property of the coated and dyed fabric sample was assessed using the AATCC 100-2004 standard testing method. A. Niger, a fungus species, was used for this test. Equation 10 was used to determine the antifungal effectiveness in terms of the percent change.

$$\text{Percentage reduction } R(\%) = \frac{(A-B)}{A} * 100 \quad (8)$$

Here, A and B indicate the number of spores for untreated control and treated cotton fabric samples, respectively.

4.15 Antiviral activity

The determination of virus titer reduction from the initial viral titer of infectivity (107) titer was done using Behrens and Karber's method. Vero-E6 cultures were maintained in Dulbecco's Modified Eagle Medium (DMEM), which contained 2% penicillin-streptomycin and 9% fetal-bovine serum (FBS) (PSA). Vero-E6 cultures were infected with the coronavirus at a ratio of 1:3 in polyethylene pots, and virus strains developed after one day. The virucidal impact of created viral stocks was investigated under a microscope. 10% FBS was added to the cell line, which was then frozen at 90 °C. Moderate centrifugation was used to filter the supernatant for 30 minutes at 5 to 7 °C and 3700 rpm. The

supernatant was used as the viral stocks in the experiment, and all macro residual was eliminated. Vero-E6 cell lines were deposited at a concentration of 2 x 10^5 in 96-well plates and cultured under normal conditions (24 hrs at 37ºC in 6% CO_2) to determine the virus titer. Each sample was diluted ten times from 10^1 to 10^8. Every dilution was injected into cell lines, where they were cultured for 3 days at 6% CO_2. The procedure established by Behrens and Kerber was used to measure the coronavirus titer in cultivated cell lines. Following that, 20×20 mm fabric sample vials were filled with the treated and control fabric samples. 100 µl of viral loads were passed through the treated and control fabrics, and any recoverable viral loads in containers were cleaned with the filter. There was a 10^1 to 10^8 dilution of the coronavirus. All serial dilutions were implanted into Vero-E6 cell lines, where they were then cultured for 3 days at 37 °C with 6% CO_2. The method of Behrens and Karber was employed to determine the Coronavirus titers in the cultivated cell lines.

4.16 Durability of bioactive fabrics

The durability of developed fabric samples was evaluated to check their stability in service. Fabrics were washed with ISO 105-C01. All fabric samples were mixed in a conventional detergent solution with a 50:1 liquor ratio. After that, samples were washed for 35 minutes at 40 °C at a 600-rpm speed. Fabrics were then dried and conditioned for a total of 24 hours under normal atmospheric conditions. Electrical conductivity, antibacterial findings, and SEM observations all supported durability.

5. RESULTS AND DISCUSSIONS

5.1 The Formation of Cu_2O particles (by using three different reducing agents) and Deposition on Cotton Fabric

The study describes the formation of cuprous oxide Cu_2O particles by the Fehling solution method. Three different types of reducing agents were used to make the particles. Subsequently, particles were deposited on cotton fabric. The effect of three different types of reducing agents was analyzed against the bioactive properties.

5.1.1 Morphology of Cu_2O particles coated cotton fabrics

5.1.1.1 SEM microstructure.

Scanning electron microscopy was employed to observe the deposition of cuprous oxide particles which were reduced by different reducing agents. Figure 25 shows nanometres scale images of the surface morphologies of cuprous oxide particles on the surface of cotton fabric. There was an obvious change in the size and surface morphology of cuprous oxide particles reduced by different reducing agents. It was noticed that the cuprous oxide particles which were reduced by glucose, had a big particle size as compared to the cuprous oxide particles reduced by ascorbic acid and Sodium hydrosulphite. While the comparatively smallest and even distribution of particles was observed in case of sodium hydrosulphite. To keep the size of nanoparticles small, the initial concentration of salt and strength of the reducing agent plays an important role. The reason is that sodium hydrosulphite is the strongest and more compatible reducing agent for copper salts as compared to ascorbic acid and glucose [14][16]. The strong reducing agent provides the proper reduction of metal salt and formed the small nanoparticles. While the weak reducing agent (glucose) provided the improper reduction of copper salt and produced the agglomerated structures, which in turn cover the less surface of fiber as shown in Figure 25c. Less salt and a strong reducing agent provide more nucleation of salt and produce finer particles. The theory is further assisted by a study, where different types of reducing agents were used to form metal nanoparticles. The fine nanoparticles were created by the strongest reducing agent 3.8nm ($NaBH_4$) as compared to 4.3nm (N_2H_4), and 15.8nm ($C_6H_8O_6$) [97].

The cuprous oxide particles reduced by sodium hydrosulphite and ascorbic acid covered the complete fiber surface (Figures 3b and 3a). Figure 3a showed the continuous and uniform distribution of particles on the surface of cotton. Furthermore, the deposition was found more uniform and denser with the increase in the concentration of copper salts. This trend was further justified by the particle size images of cuprous oxide particles as shown in Figures 25a, b, and c.

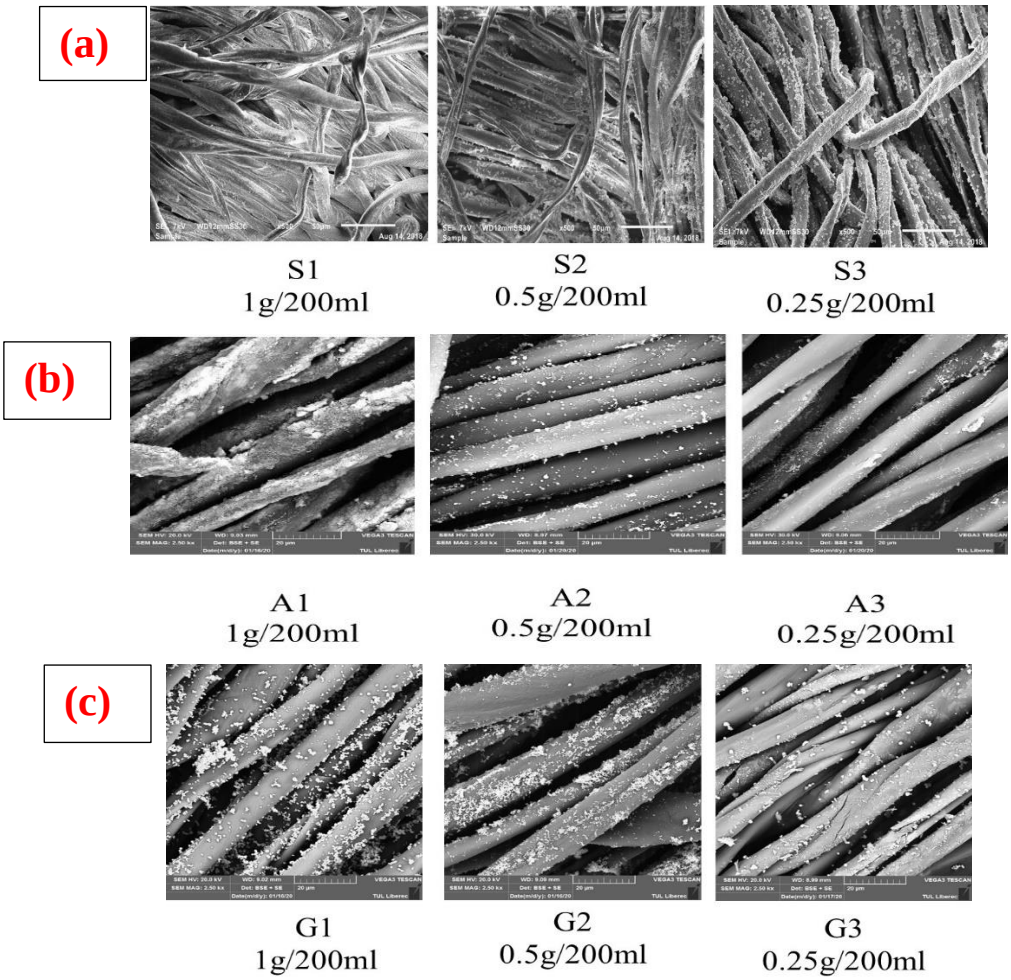
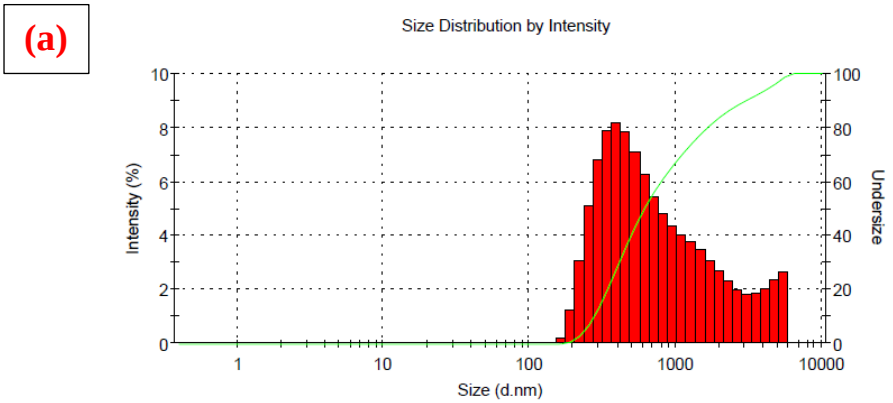


Figure 1: Surface morphology of cotton fabrics coated with cuprous oxide particles reduced by (a) Sodium hydrosulphite, (b) ascorbic acid, (c) glucose.

5.1.1.2 Particle size distribution

The particle size was measured by the dynamic light scattering technique based on Brownian motion. The average particle size distribution of cuprous oxide particles is shown in Figure 26. The cuprous oxide particles were found to have a multi-modal distribution with sizes varying from micrometers to the nanometers range. The average particle size of cuprous oxide particles reduced by glucose was about 900 nanometers, while the average particle size of cuprous oxide particles reduced by ascorbic acid and sodium hydrosulphite was about 500 and 450 nanometers respectively.



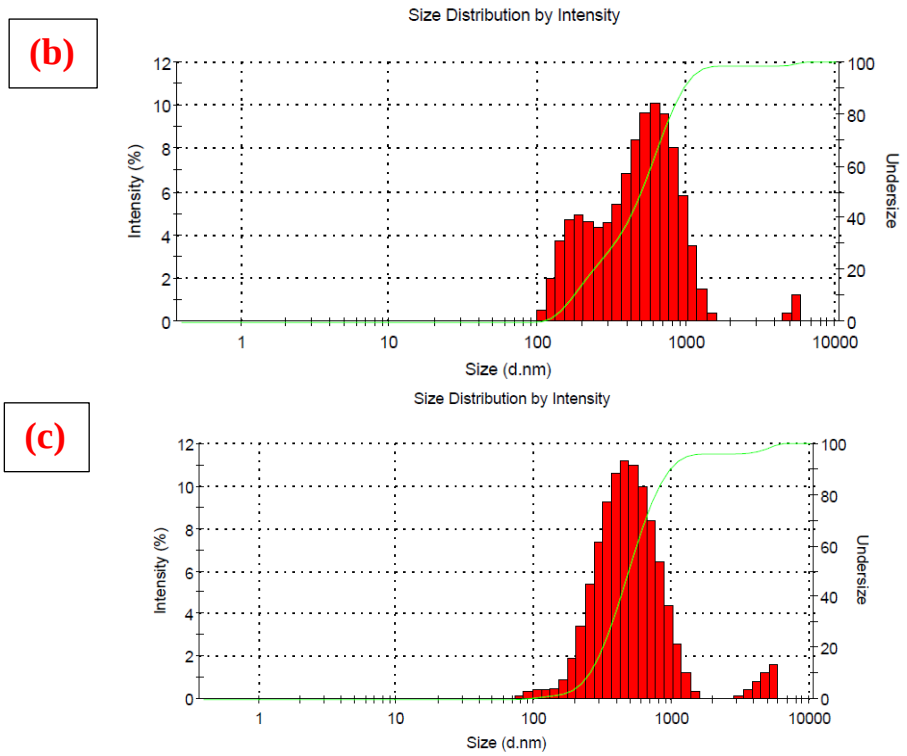


Figure 2: The average particle size distribution of cuprous oxide particles reduced by (a) glucose, (b) ascorbic acid, (c) Sodium hydrosulphite.

5.1.1.3 XRD analysis

The XRD analysis was carried out to know the phase composition of deposited cuprous oxide particles. Figure 27 shows the XRD patterns of samples for the 2θ range of 10 to 80 degrees with a step of 0.02 degrees. The phase purity of the prepared cuprous oxide particles can be seen from the perfect indexing of all the diffraction peaks to the cuprous oxide structure. The diffraction peaks observed at 2θ of 29.6 º, 36.5 º, 42.4 º, 52.1 º, 61.5 º, and 73.7 º represented (1 1 0), (1 1 1), (2 0 0), (2 1 1), (2 2 0) and (3 1 1), reflections respectively [98][99]. The crystalline nature of cuprous oxide particles was confirmed by the sharpness of the peaks, whereas the broadening of the peaks justified the formation of nanoscale cuprous oxide particles. As such no characteristic peaks of impurities were detected, except the peak of copper oxide phase at 2θ of 38º and 78º as shown in Figure 27 [100][101].

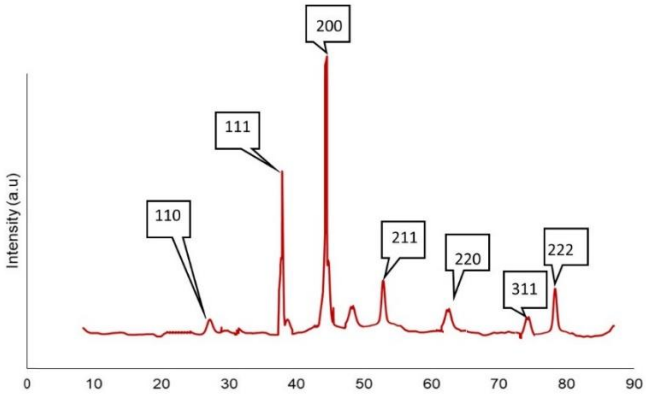


Figure 3:XRD patterns of cuprous oxide particles.

5.1.2 Antibacterial activity of the cuprous oxide nanoparticle-coated fabrics

The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements.

5.1.2.1 Zone of inhibition test (qualitative measurements)

The zone of inhibition test is a type of qualitative measurement. The test was performed against both types of bacteria (Gram-negative *E. coli* and Gram-positive *S. aureus*). Figures 28 and 29 show the clear zones of inhibition around all fabric samples after 24 h of incubation in the dark at 37 °C. The zone of inhibition was less for the cuprous oxide-coated fabrics reduced by glucose. The cuprous oxide-coated fabrics reduced by sodium hydrosulphite showed the most significant antibacterial zone to *E. coli and S. aureus*. The zone of inhibition test was repeated three times for each sample and the average value against each reducing agent is presented in Figures 6, 7, and 8. These results showed that the deposited cuprous oxide particles present strong sterilization to the *E. coli* and *S. aureus* due to the free-standing of the particles. However, *Staphylococcus* aureus depicted the highest sensitivity as compared to *Escherichia coli*. The zone of inhibitions for *Staphylococcus aureus* increased from 5 to 7 mm, while for Escherichia coli it increased from 4 to 6 mm with the increase in sodium hydrosulphite concentration. What is noteworthy is that the annulus of the inhibition zone increases with the increase in the concentration of the reducing agent. In other words, the prepared fibers with more cuprous oxide particles with an increase in the concentration of reducing agents. This is indicating that more cuprous oxide contents will influence the antibacterial activity, which is similar to the previous study [7].

The antibacterial property of coated fabrics can be attributed to the combination of chemical and physical interactions of bacteria with copper particles. The cuprous oxide nanoparticles can incorporate into the cell via endocytic mechanisms. Afterward, the cellular uptake of ions increased as ionic species were subsequently released within the cells by nanoparticle dissolution [102]. This resulted in high intracellular concentration gained within the cell for further massive oxidative stress. The mechanisms associated with the antibacterial behavior of copper nanoparticles can be summarised as shown in Figure 28.

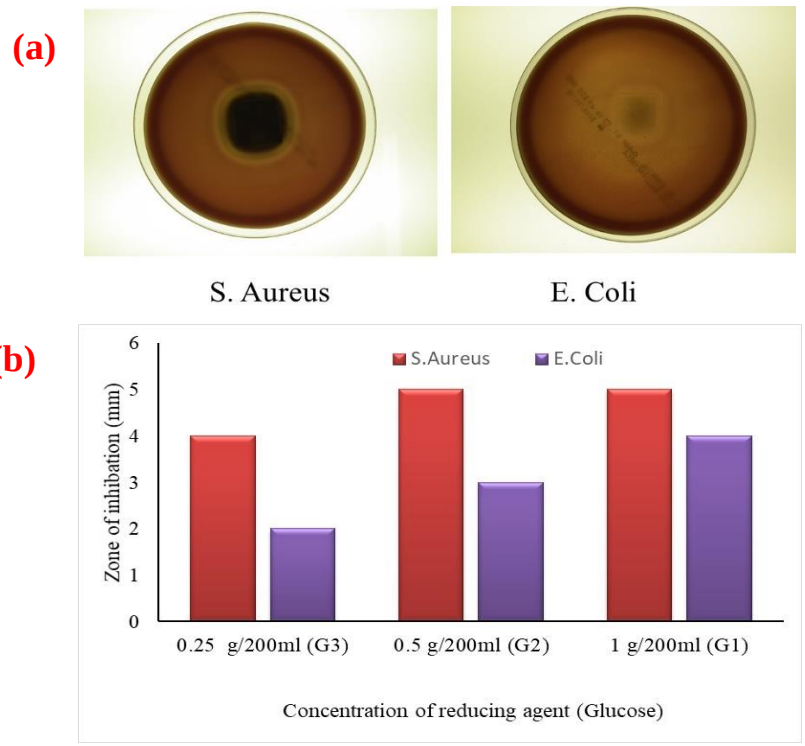


Figure 4: (a) Image of the zone of inhibition (b) the average value of the zone of inhibition against each concentration of the glucose-reducing agent.

(a)

S. Aureus E. Coli

(b)

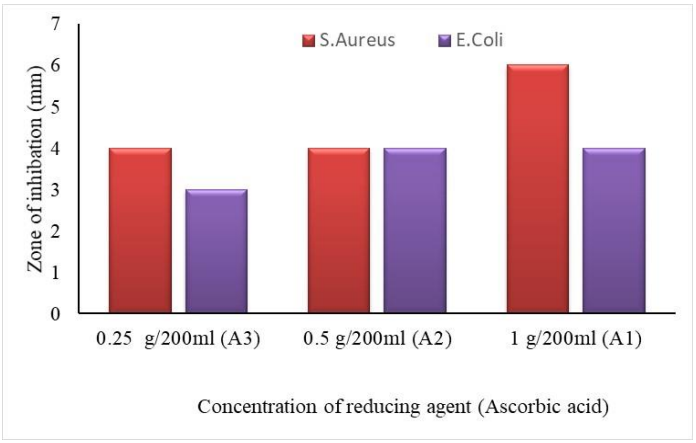


Figure 5:(a) Image of the zone of inhibition (b) the average value of zone of inhibition against each concentration of Ascorbic acid reducing agent.

(a)

S. Aureus E. Coli

(b)

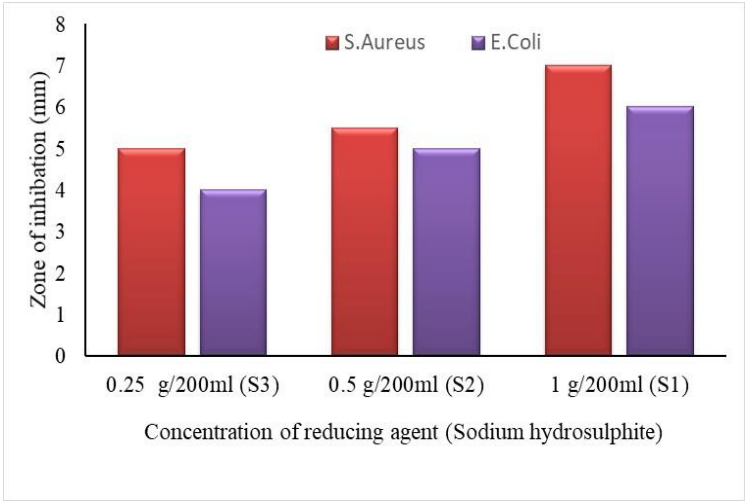


Figure 6:(a) Image of the zone of inhibition (b) the average value of zone of inhibition against each concentration of sodium hydrosulphite reducing agent.

5.1.2.2 Reduction factor (quantitative test)

AATCC test method 100-2004 was adopted for quantitative measurements. This method is based on the reduction (in percent) of the inoculated concentration of the bacteria due to the effect of the sample. The result is many survivor bacteria colonies (CFU) and from this number, there is calculated inhibition degree (in %). The reduction percentage of all samples (untreated, reduced with glucose, ascorbic acid, and sodium hydrosulphite) is given in Table 6. There was no reduction percentage for the untreated sample, while all other samples showed a good reduction percentage against both types of bacteria (gram-positive and negative) [103][104].

Table 3: The reduction (in percent) of the inoculated concentration of the bacteria due to the effect of the cuprous oxide-coated sample.

Sample	**Escherichia coli**	**Staphylococcus aureus**
	The result, % inhibition	**The result, % inhibition**
Untreated standard	0 %	0 %
Glucose G1	99.99%	99.99%
G2	90.9%	99.99%
G3	28.2%	99.99%
Ascorbic Acid A1	99.99%	99.99%
A2	99.99%	99.99%
A3	93.3%	99.99%
Sodium hydrosulphite S1	99.99%	94.7%
S2	99.99%	99.99%
S3	99.99%	99.99%

For a better overview, the log CFU / ml concentration was calculated and the results were plotted. Where the concentration of survival of bacteria was found against both bacteria (S. aureus and E. coli) and their respective graphs are shown in Figures 31 and 32. The untreated sample showed a lot of survival colonies because of no effectiveness against bacteria, while compared to the standard, all other samples (against bacterial strains of E. coli and S. aureus) showed good inhibition and a smaller number of survival colonies. Furthermore, there is a remarkable reduction in the concentration of survival colonies in case of cuprous oxide particles reduced by sodium hydrosulphite.

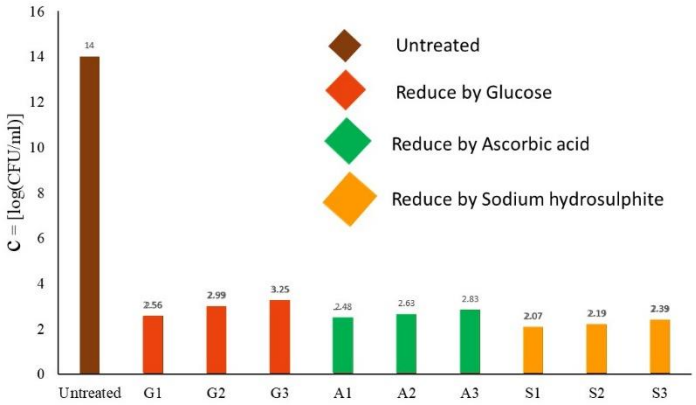


Figure 7: The concentration of survival of bacteria (S. aureus) against cuprous oxide coated fabrics reduced with different reducing agents.

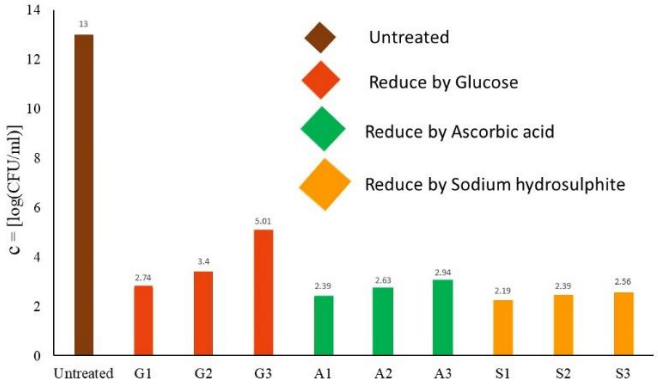
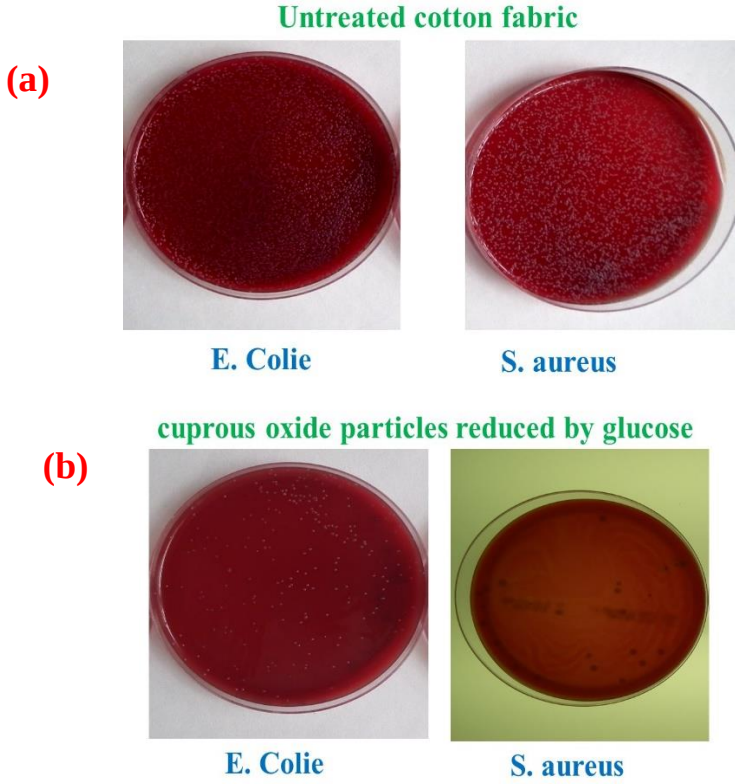


Figure 8: The concentration of survival of bacteria (E. colie) against cuprous oxide coated fabrics reduced with different reducing agents.

The trend was further justified by selected images of the concentration of bacterial growth for the untreated (pristine) cotton fabric and treated (G1, A1, and S1) samples as shown in Figure 33. The untreated sample remained ineffective against bacterial growth, while the treated fabrics coated with cuprous oxide particles showed clear effectiveness against bacterial growth. However, with a further increase in cuprous oxide particles loading at higher concentrations of sodium hydrosulphide (up to 1g/200ml) there was a significant improvement in colony reduction was observed with an efficiency greater than 99% for both types of bacteria.



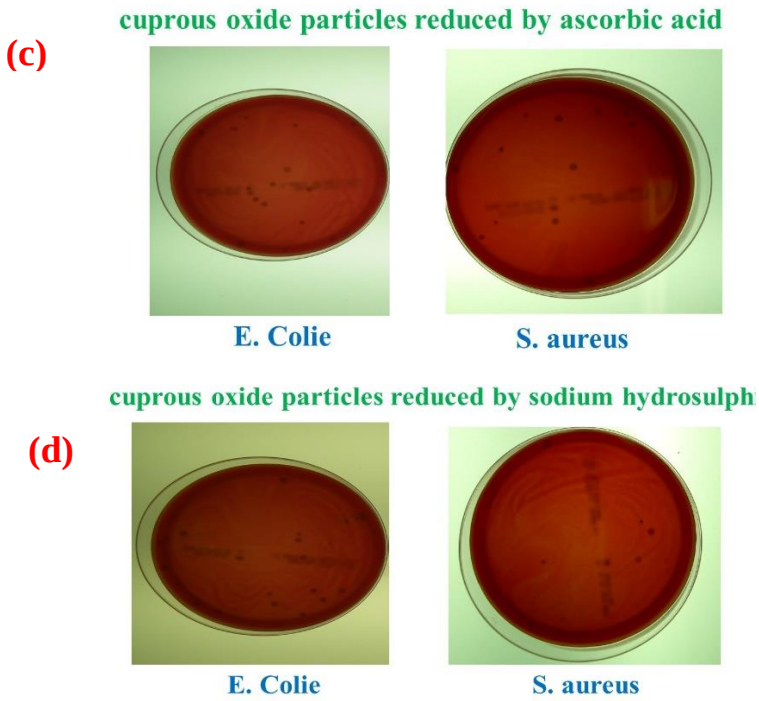
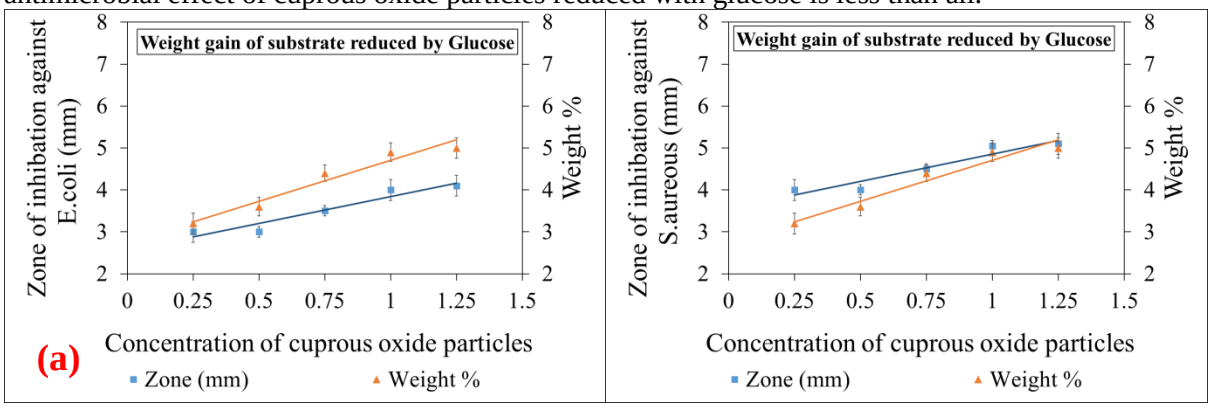


Figure 9: Images of concentration of bacterial growth for the (a) untreated (pristine) cotton fabric, (b) for the G1 fabric sample, (c) for the A1 fabric sample, and (d) S1 fabric sample.

5.1.3 Weight gain percentage and antimicrobial effect

The percentage of fabric weight gain was measured with an increase in the concentration of cuprous oxide particles. The effect of weight gain against antimicrobial properties was measured for all samples and their respective graphs are shown in Figures 34 (a, b, and c). From the trend lines, it is clear that with the increase in the concentration of cuprous oxide particles, the mass gain was going to increase and the antimicrobial effect (zone of inhibition) were significantly increased. The maximum antimicrobial effect (zone of inhibition) and maximum weight gain percentage values were confirmed at 1.25 g/200ml of all reducing agents. The mass gain percentage and antimicrobial effect of cuprous oxide particles reduced with sodium hydrosulfide is higher than all, while the mass gain percentage and antimicrobial effect of cuprous oxide particles reduced with glucose is less than all.



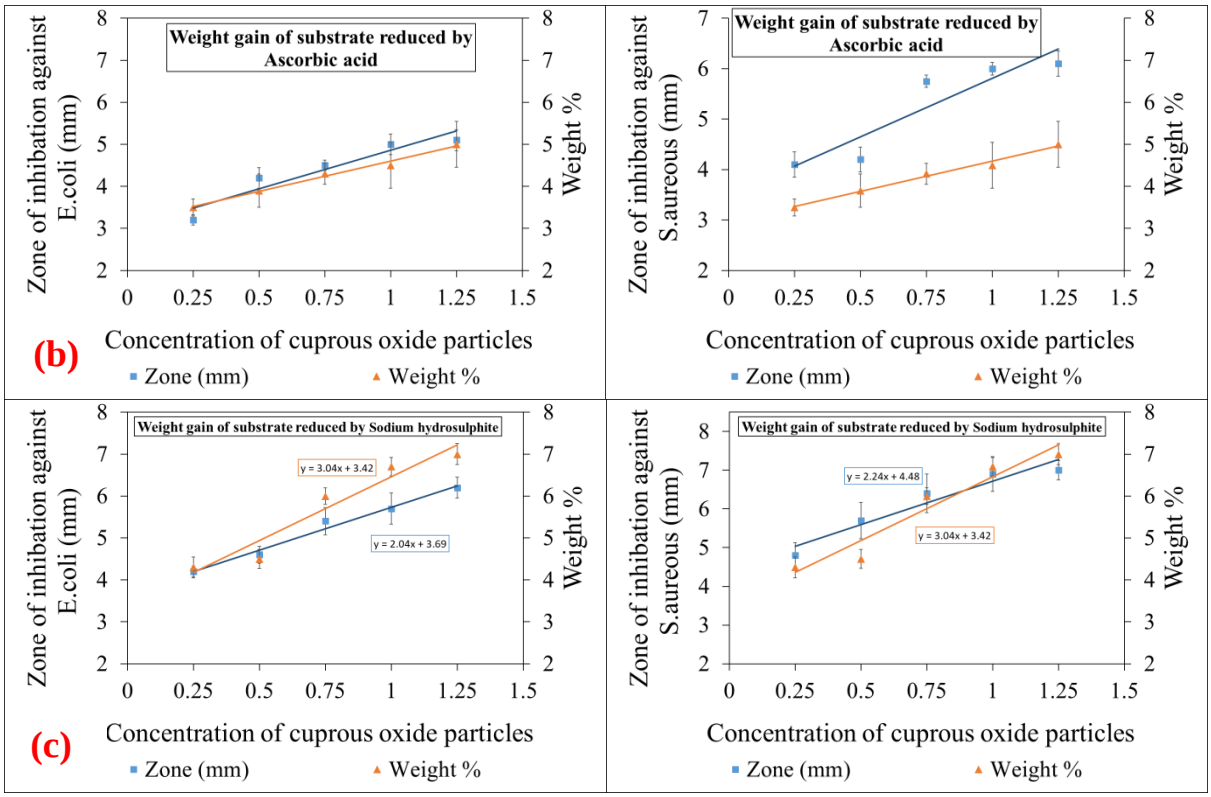


Figure 10: The effect of weight gain against antimicrobial properties for fabric samples (a) reduced by glucose, (b) reduced by ascorbic acid, and (c) reduced by sodium hydrosulphide.

5.1.4 Thermo-physiological comfort properties

The vision behind wearable antimicrobial textiles forecasts future hygienic systems to be an integral part of our everyday outfits. Such hygienic textiles have to meet special requirements regarding wearability. When we talk about wearable antibacterial fabrics, then comfort is the real parameter that we cannot avoid. Air permeability and stiffness are the most important comfort properties for wearable antibacterial textiles.

5.1.4.1 Air permeability

Air permeability is an important parameter for textiles and maintains thermal comfort. It helps to exchange air when heat and perspiration are generated from the body [109]. The results of fabric air permeability are shown in Figure 35. The air permeability results were found for all types of fabrics (treated and untreated). From the results, it is clear that the application of very fine cuprous oxide nanoparticles to the fabrics has very little effect on air permeability. The air permeability of the untreated fabric is about 130 mm/s while the air permeability for all other cuprous oxide nanoparticles coated fabrics is in the range of 123 to 126 mm/s. Showing that there is a minor decrease in air permeability even after depositing the cuprous oxide nanoparticles. There are two factors responsible for this phenomenon. Firstly, there may be a relaxation shrinkage in the fabric structure due to dipping cuprous oxide nanoparticles solution, causing the yarns to come close and hinder the flow of air. Secondly, during the application of nanoparticles, have deposited on the yarn structure and interstices, reducing the fabric air gap spacing (pore size). The fabric pore size has a direct relation with the air permeability of the fabric. Therefore, a reduction in pore size has caused a decrease in the air permeability of the fabric. The spun yarns of cotton have a low uniformity and hairiness, which cause resistance to the flow of air and lead to low air permeability. It is the beauty of nanoparticles that they will cover more surface area over the yarn but will not entrap in the spaces between the fabric structures.

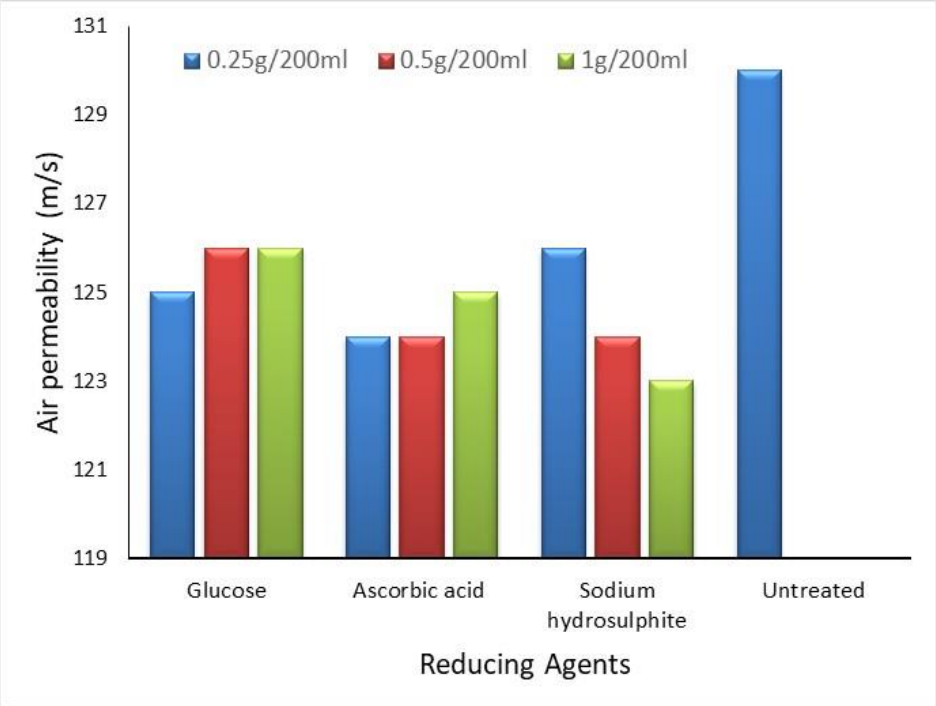


Figure 11: The air permeability results for all types of fabrics.

5.1.5 Stiffness

The term stiffness of fabric describes its ability to resist deformation and keep standing without support. This property is important regarding comfort and desirable draping. Stiffness can be calculated from bending length and flexural rigidity. The stiffness of coated and uncoated fabric samples was found and average values are given in Figure 36. Fabric stiffness was found to increase with the increase in the concentration of reducing agents. It means that an increase in the concentration of reducing agents causes to increase in the deposition of cuprous oxide particles. The reason is that coating increased inter-fiber friction and abrasion at fiber crossover points [110]. However, the effect of the increase in rigidity overall is insignificant.

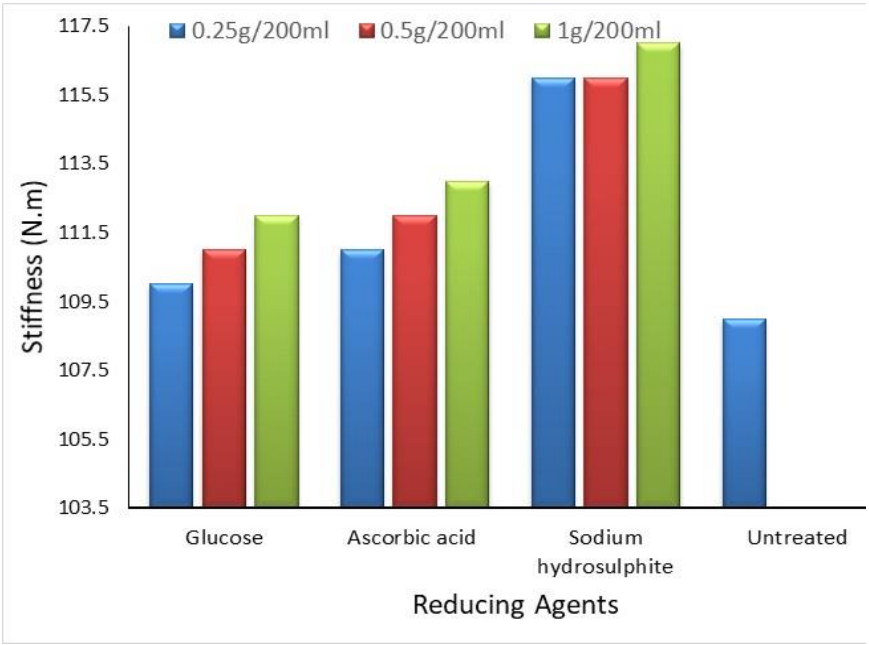


Figure 12: The stiffness of coated and uncoated fabric samples.

5.1.6 Durability of cuprous oxide coated fabrics

As mentioned earlier, the cuprous oxide particles were attached to the surface of fabrics through the combination of various bonding. The additional cuprous oxide particles filled the gaps and interspaces between microfibers and stacked them together to form hygienic antibacterial networks. This behavior of absorbance and adherence was further described by the durability of the antibacterial effect against washing in water. The functionalized fabrics were also soaked, squeezed, and twisted in water. The cuprous oxide-coated samples showed good washing properties without peeling off and precipitating in the water. Later on, an adhesion test was performed with transparent tape. The tape remained transparent i.e. no visible particles were observed on the tape. Hence indicating the robust interactions and reasonable mechanical adhesion properties among hygienic cuprous oxide particles and fabric.

To investigate these properties, the antibacterial properties of all fabrics were investigated after washing. The samples were washed according to the standard washing test method ISO 105-C01. The antimicrobial values of all developed samples before and after washing are given in Table 7.
It is clear from the given values that there is almost a 50% decrease in the zone of inhibition after washing. This shows that prepared samples are effective against pathogens even after severe washing. Furthermore, the trend was also justified by the SEM analysis after washing (for the samples G1, A1, and S1) as shown in Figure 37. The retention of particles over the surface of the fabric reinforced the fact that particles are firmly attached to the fibers and interspaces.

Table 4: Antimicrobial properties of Cu_2O coated fabrics after washing

No of samples	**Code**	**ZOI (mm) E. Colie**	**ZOI (mm) S. Aureus**	**ZOI (mm) E. Colie**	**ZOI (mm) S. Aureus**
		Before washing		**After washing**	
1	G1	4	5	2	3
2	G2	3	5	1	2
3	G3	2	4	0	3
4	A1	4	6	3	4
5	A2	4	4	3	2
6	A3	3	4	2	2
7	S1	6	7	4	4
8	S2	5	5	4	3
9	S3	4	5	2	3

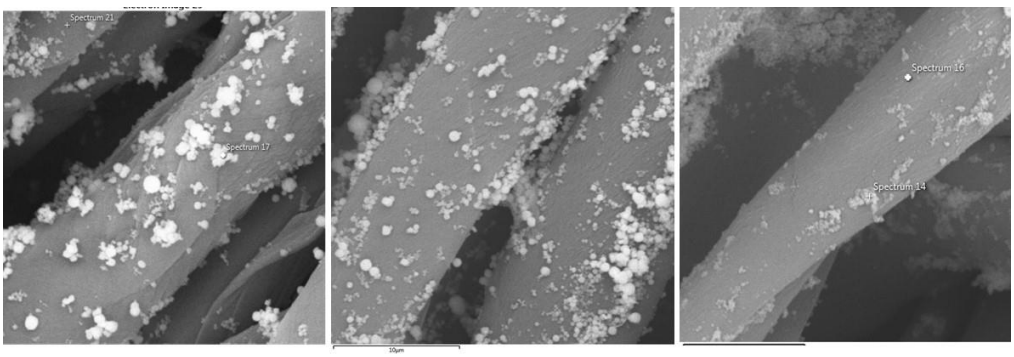


Figure 13: SEM analysis after washing (for the samples G1, A1, and S1)

5.2. Copper-treated environmentally friendly antipathogenic cotton fabric with modified reactive blue 4 dye to improve antibacterial and aesthetic properties

The objectives of this part were to develop an environmentally friendly, low-price, easy, and fast method for developing antipathogenic (antibacterial, antifungal, and antiviral) cuprous oxide-coated multifunctional fabrics. At first, fabrics were sensitized with citric acid then the formation of Cu_2O particles was done by the Fehling solution method. Then, the cuprous oxide particles were deposited on cotton fabrics. Three different types of reducing agents with different concentrations were selected to make the Cu_2O particles. Surface morphology and presence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. In the second step, a reactive antibacterial dye was made (by reacting Reactive Blue 4 with triclosan). The molecular structure of the modified dye was confirmed through FTIR and ^{13}C-NMR. The details about structure of dyestuff, triclosan modification, dyeing procedure and reactivity of modified dye is already described in previous sections 4.4 and 4.5. The resultant antibacterial dye was applied on copper-treated cotton fabrics through exhaust dyeing protocol. The dyed fabric was characterized through colorimetric data (L*, a*, b*, C, H, and K/S), levelness of dye, fastness properties as well as exhaustion and fixation rates. The antipathogenic activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antipathogenic effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite at 1g/L. Furthermore, the utility of hygienic antimicrobial-developed fabrics was analyzed for comfort properties regarding air permeability and stiffness. In the end, the durability of the coating was confirmed by measuring the antibacterial properties and SEM analysis after washing.

5.2.1 FTIR Analysis

FTIR Peaks (KBr): 3323 cm^{-1} (amine -NH_2, -NH stretch), 3378 cm^{-1} (amine -NH_2, N-H stretching), 1046 cm^{-1} (C-O-C ether linkage stretch), 638 cm^{-1} (C-Cl stretch). The FTIR spectra of the unmodified dye (Reactive Blue 4) and modified dye (modified with an antibacterial agent) has shown in Figure 38. The spectra successfully confirmed that an antibacterial agent (triclosan) has been successfully incorporated into the structure of the dye through a covalent bond. It was confirmed by the appearance of a new sharp peak of strong intensity at 1046 cm^{-1}. This sharp peak is a characteristic peak of ether linkage (C-O-C) [111]. The formation of the covalent bond between the hydroxyl group of triclosan and chlorine of the triazine ring has resulted in the development of ether linkage (C-O-C). Therefore, the absence of a sharp and strong peak at 1046 cm^{-1} in unmodified dye and the appearance of this peak in the spectra of modified dye confirmed that modification of dye with the antibacterial agent has been successfully achieved. The modification of the dye was further supported by the increase in the intensity of the peak that occurred at 638 cm^{-1}. This peak is also present in the spectrum of unmodified dye and is attributed to the C-Cl stretching vibrations [112]. However, the intensity of the same peak is significantly increased in the spectrum of modified dye. The increase in peak intensity could be due to the increase in the C-Cl linkages in the structure of the modified dye because three C-Cl linkages are present in the structure of triclosan which further confirmed that triclosan is embedded in the structure of the dye molecule.

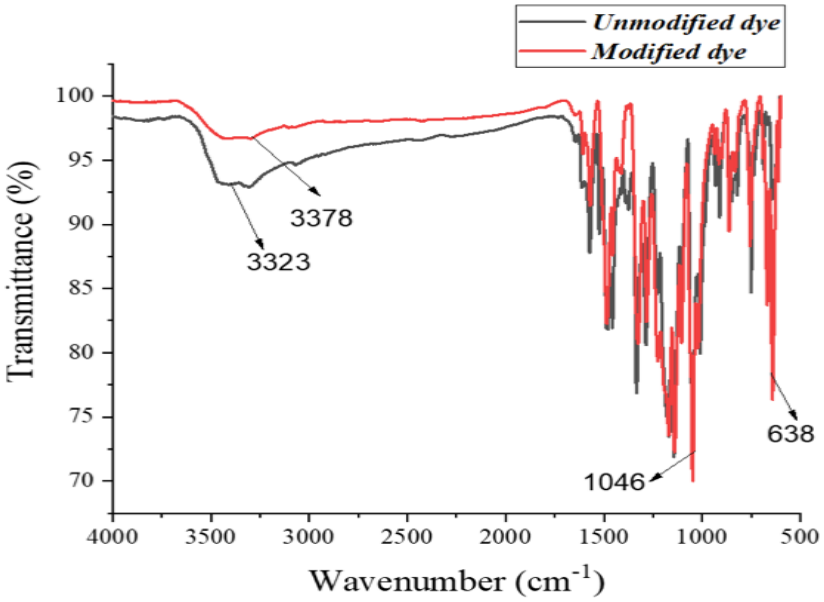


Figure 14: IR spectra of modified and unmodified dye.

5.2.2 NMR Analysis of modified functional reactive dye

^{13}C-NMR (600 MHz, D_2O): δ 32.9. ppm (s), 38.1 ppm (s), 121.4 ppm (s), 128.3 ppm (s), 150.3 ppm (s), 153.6 ppm (s), 161.3 ppm (s), 163.1 ppm (s). Figure 39 shows the ^{13}C -NMR spectrum for the modified Reactive Blue 4 dye. Two sharp up-field singlets occurred at 32.9 and 38.1 ppm could be attributed to the presence of 2 saturated carbon atoms present in the central cycloalkane ring in the anthracene moiety of the dye [113]. Meanwhile, the existence of two sharp peaks that occurred at 121.4 and 128.3 ppm could be due to the presence of a benzene ring in an unmodified dye structure [114]. Whereas, the occurrence of two other sharp singlets at 150.3 and 153.6 pm could be ascribed to the two benzene rings of triclosan attached through oxygen atom [115]. The presence of these two peaks confirmed that an antibacterial agent (triclosan) has successfully incorporated into the structure of reactive blue 4 dye through covalent linkage. Furthermore, the presence of two sharp downfield singlet at 161.3 and 163.1 ppm could be a result of the triazine ring present in the structure of unmodified dye [116].

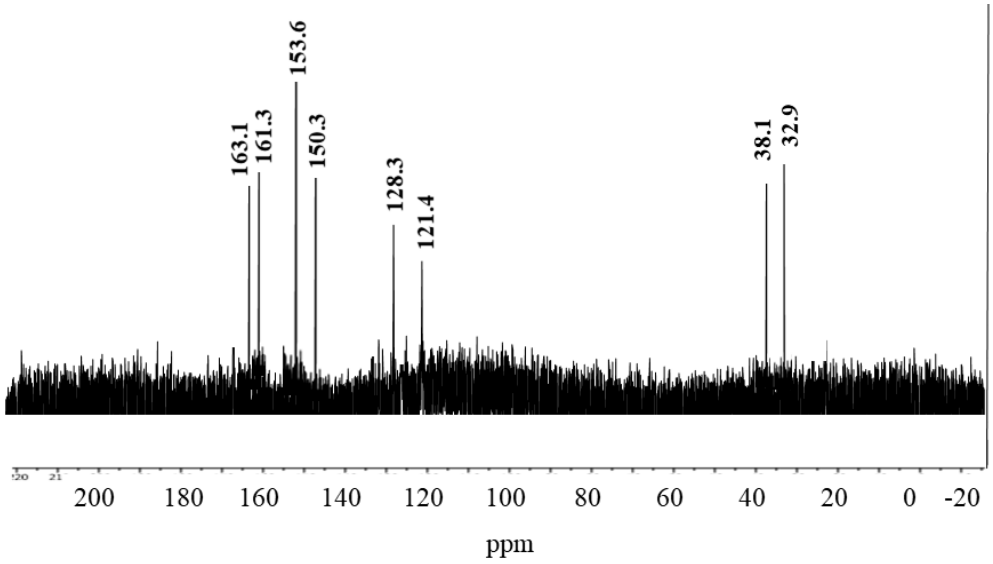


Figure 15: 13C-NMR spectra for modified Reactive Blue 4 dye.

5.2.3 Colorimetric data measurement

The colorimetric data for the copper-treated undyed and dyed fabric samples were evaluated and obtained results are given in Table 8. There was a significant difference between the colorimetric data of copper-treated undyed and dyed fabrics. The K/S value for the dyed cotton fabric was higher (12.13) as compared to undyed fabric (7.08) which showed that the application of dye has changed the light-colored fabric to comparatively dark-colored fabric. The dark shade for the dyed fabric samples was further confirmed by the difference in L* values of both fabrics. The L* value for the dyed fabric sample was lower (39.14) than the L* value of the undyed fabric sample (52.43) which showed that dyed fabric has more dark shade when compared to the undyed fabric. The chroma (C*) value for the dyed sample was also lower (19.14) than for undyed fabric (32.87) which suggested a brighter shade for undyed fabric samples and a darker and duller shade for dyed fabric samples. The a* and b* values for the undyed fabric samples were positive which indicated a reddish and yellowish shade for the undyed fabric whereas both a* and b* values were negative for dyed fabric samples indicating greenish and bluish shade for the dyed fabric samples.

Table 5: Colorimetric data for copper treated undyed and dyed fabric samples.

Sr.#	Properties	Copper-coated fabrics	Copper-coated dyed fabrics
1.	Fabric color		
2.	K/S	7.08	12.13
3.	L*	52.43	39.14
4.	a*	5.15	-9.34
5.	b*	31.56	-16.13
6.	C*	32.87	19.14
7.	H*	80.15	241.13

5.2.4 Levelness of Copper treated undyed and dyed fabric

The color levelness effect was assessed to determine the uniformity of appearance in the dyed fabric. To achieve this, a reflectance spectrophotometer was employed to scan both the undyed copper-coated and dyed copper-coated cotton fabric, and K/S values were collected from 12 distinct points. The obtained K/S values and their corresponding standard deviations are presented in Table 9.

For the dyed copper-coated fabric, the calculated standard deviation was 0.24, indicating excellent levelness properties. This result suggests that the dye is uniformly distributed across the surface of the fabric, leading to an even and consistent appearance. In contrast, the undyed copper-coated fabric exhibited a significantly higher standard deviation of 0.81, highlighting the uneven and inconsistent appearance of the fabric. The outcomes of the dye levelness assessment affirm that the dyed copper-coated fabric achieves a smooth and uniform appearance compared to the undyed copper-coated fabric, aligning with one of the objectives of the study. In the visual evaluation, the dyed copper-coated fabric was awarded a grade of 5 (excellent levelness), while the undyed copper-coated fabric received a grade of 3 (moderate levelness). This visual evaluation further supports the aforementioned findings, emphasizing that the application of dye on the copper-coated fabric results in an even and harmonious appearance of the fabric.

Table 6: Reflectance measurement data for the undyed and dyed copper-coated fabrics.

Number of Scans	K/S Values Undyed sample	Standard deviation (S.D)	K/S Values Dyed sample	Standard deviation (S.D)
Scan 1	7.08		12.35	
Scan 2	8.65		13.17	
Scan 3	9.01		12.55	
Scan 4	7.34		12.73	
Scan 5	8.47	0.81	13.11	0.24

Scan 6	9.34		12.78	
Scan 7	7.56		12.78	
Scan 8	7.38		12.77	
Scan 9	8.39		12.65	
Scan 10	9.01		12.63	

5.2.5 Fastness properties of copper-coated dyed fabric

The exhaustion, fixation, washing, rubbing, and light fastness properties of the three samples—DG, DA, and DS—were evaluated according to standard testing methods, and the obtained results are presented in Table 5. All dyed samples demonstrated favorable washing fastness ratings ranging from 3 to 5, excellent light fastness ratings of 4 to 5, and good rubbing fastness rating of 4.

The exhaustion and fixation rates of the dye for all samples were notably high, with a dye exhaustion rate of 96% and a fixation rate exceeding 89%. These remarkable fastness properties and high exhaustion and fixation rates can be attributed to the fiber-reactive nature of the employed reactive dye. Reactive dyes have the capability to establish strong covalent bonds with the hydroxyl groups present in cotton fabric. This inherent property contributes to achieving excellent dye fixation and fastness properties. Overall, the dyed samples, namely DG, DA, and DS, exhibit impressive performance in terms of fastness properties and dye exhaustion/fixation rates, underscoring the effectiveness of the chosen reactive dye and dyeing procedure.

Table 7: Exhaustion, fixation, and fastness (washing, rubbing, and light) of dyed fabric.

Sr. #	Sample Code	Exhaustion %	Fixation %	Washing fastness	Rubbing fastness	Light fastness
1.	DG	91	84	3-4	3-4	4-5
2.	DA AG	93	87	4-5	4	4-5
3.	DS SG	96	91	5	4-5	5

5.2.6 Morphology of copper coated dyed cotton fabrics

Scanning electron microscopy (SEM) was utilized to analyze the morphological changes of cuprous oxide-coated dyed fabrics resulting from the application of different reducing agents. The nanoscale images of cuprous oxide particles on the surface of dyed cotton fabric are depicted in Figure 40. Notable variations in size and surface morphology were observed among cuprous oxide particles reduced by different reducing agents. Specifically, it was observed that dyed cuprous oxide particle-coated fabrics reduced by glucose exhibited larger particle sizes compared to those reduced by ascorbic acid and sodium hydrosulphite. While the comparatively smallest and even distribution of particles was observed in case of sodium hydrosulphite. The reason is that sodium hydrosulphite is the strongest and more compatible reducing agent for copper salts as compared to ascorbic acid and glucose [30][[31]. The weak reducing agent (glucose) provided the improper reduction of copper salt and produced the agglomerated structures, which in turn cover the less surface of fiber as shown in Figure 40c. The cuprous oxide particles reduced by sodium hydrosulphite and ascorbic acid covered the complete fiber surface (Figures 40b and 40a). Figure 8a showed the continuous and uniform distribution of particles on the surface of cotton. Furthermore, the deposition was found more uniform and denser with the increase in the concentration of copper salts.

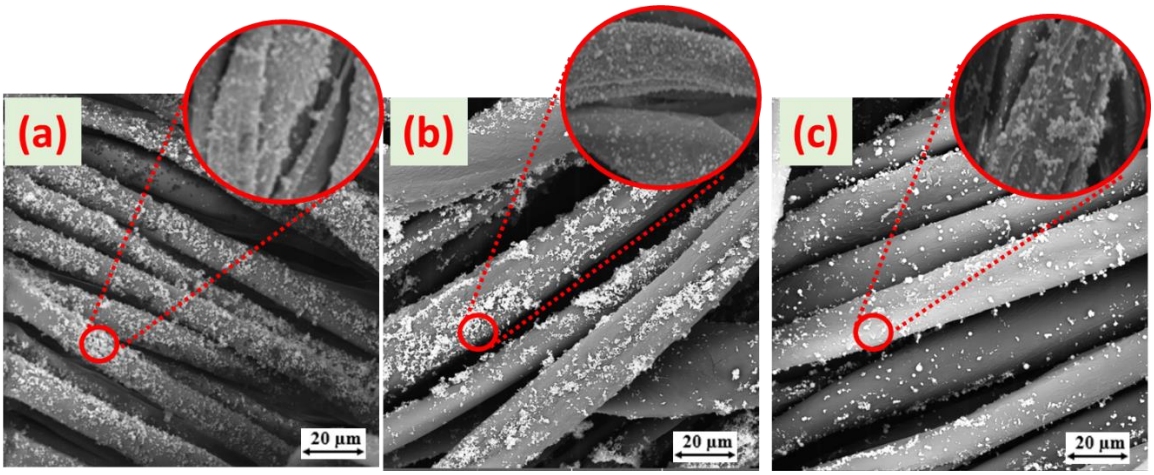


Figure 16: Surface morphology of dyed cotton fabrics coated with cuprous oxide particles (a) DS, (b) DA, (c) DG at 100x magnification and their close view at a magnification of 5kx.

5.2.*7* XRD analysis

The XRD analysis was conducted to determine the phase composition of the deposition of cuprous oxide particles. Figure 41 illustrates the XRD spectrum of the fabric sample in 2θ range of 10-80 degrees with a 0.02-degree shift. The precise identification of all the diffraction signals to the cuprous oxide structure reveals the phase purity of the produced cuprous oxide nanoparticles. The reflections that were represented by the diffraction peaks at 2θ of 29.6 o, 36.5 º, 42.4 º, 52.1 º, 61.5 º, and 73.7 º, respectively, were (1 1 0), (1 1 1), (2 0 0), (2 1 1), (2 2 0), and (3 1 1) [119] [50]. The sharpness of the signals validated the crystalline character of the cuprous oxide nanoparticles; however, the broadening of the signals supported the production of nanosized cuprous oxide particles. As such no characteristic peaks of impurities were detected, except the peak of the copper oxide phase at 2θ of 38º [120][121].

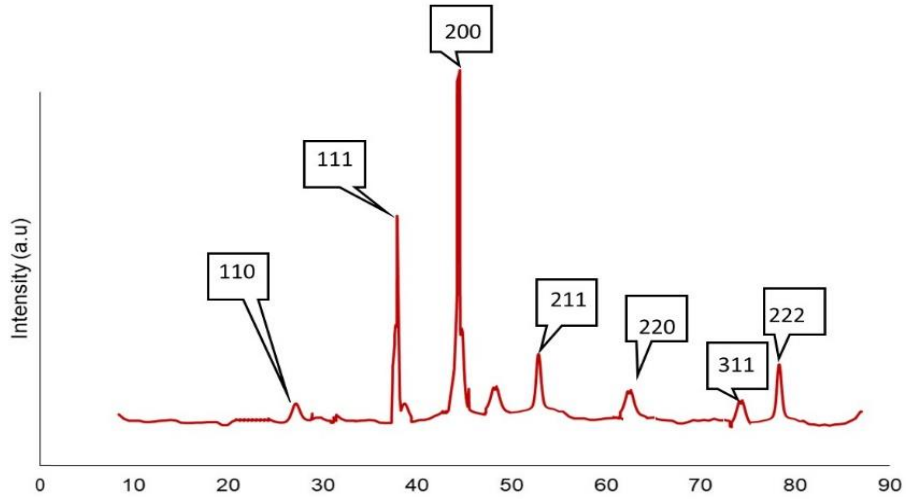


Figure 17: XRD patterns of cuprous oxide particles.

5.2.8 Antibacterial activity of the cuprous oxide nanoparticle coated fabrics

The antibacterial activity of copper-treated undyed and dyed fabric samples was tested both qualitatively and quantitatively using standard testing protocols.

5.2.8.1 Zone of inhibition test (qualitative measurements)

The AATCC-147 (disc-diffusion method) standard testing protocol was followed for the qualitative assessment of all treated samples. The antibacterial efficacy of all samples was evaluated against both Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. The test was carried out three times and the mean value calculated for each sample is presented in Table 12. The obvious zones of inhibition around each fabric sample after 24 hours of incubation at 37 °C in the dark are depicted in

Figures 42 and 43. All samples (undyed and dyed copper-coated fabrics) have shown significant zone of inhibition (ZOI) against both test microbes. However, it was observed that ZOI for dyed copper-coated samples is higher than for undyed copper-coated fabrics. The higher values of ZOI for dyed copper-coated fabrics revealed that the application of antibacterial dye on copper-coated fabric has not masked the antibacterial effect of copper particles, rather antibacterial effectiveness has increased after the dyeing of copper-treated fabrics.

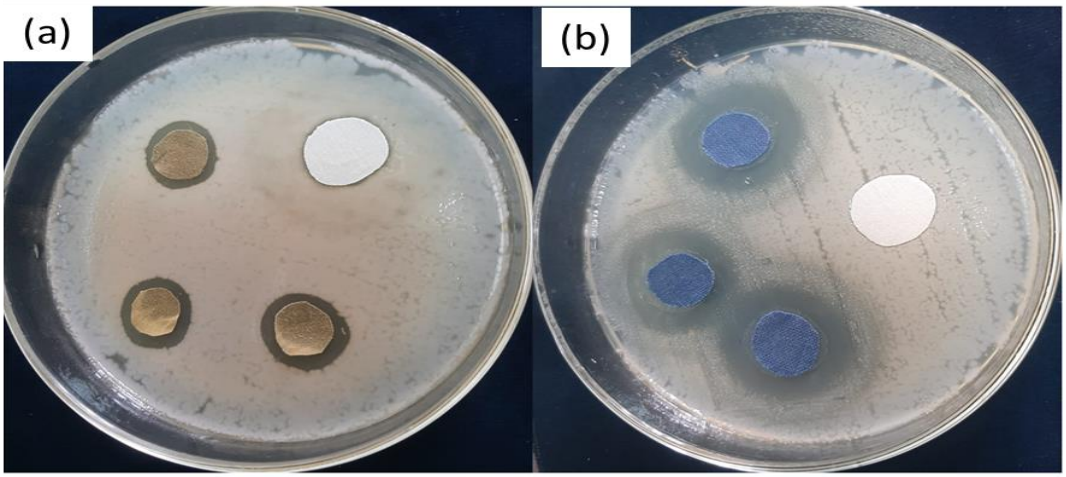


Figure 18: ZOI around (a) Copper-coated fabrics and (b) copper-coated dyed fabrics against *E-coli.*

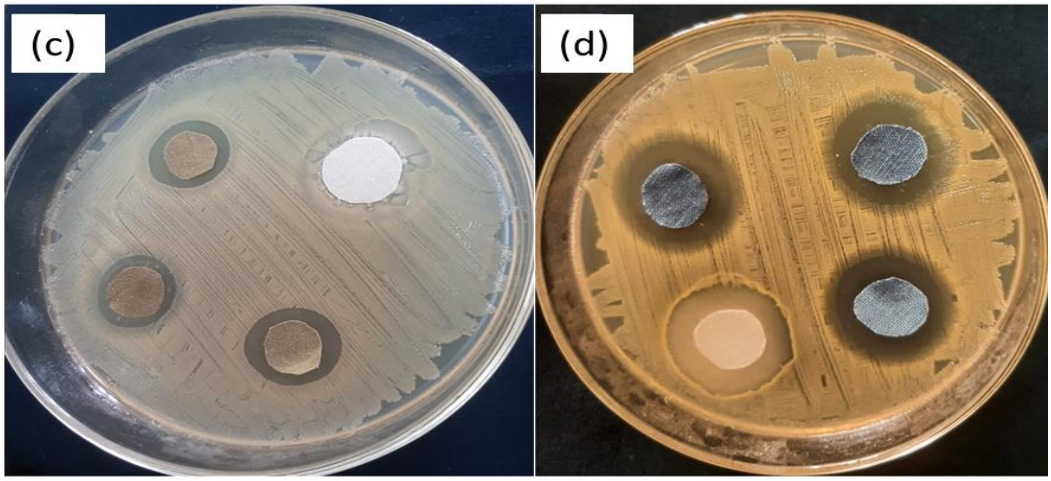


Figure 19: ZOI around (c) Copper-coated fabrics and (d) copper-coated dyed fabrics against *S-aureus.*

Table 8: The values of the zone of inhibitions against *S. aureus* and *E. coli.*

Sr.#	Sample Code	ZOI (mm)	
		S. aureus	*E. coli*
1.	UT	0	0
2.	G	5	2
3.	DG	7	3
4.	A	4	3
5.	DA	7	3
6.	S	5	3
7.	DS	8	4

5.2.8.2 Reduction factor (quantitative test)

ISO-20743 standard testing protocol was followed for the quantitative evaluation of the antibacterial efficacy of all developed samples against Gram-positive *(E. coli*) and Gram-negative (*S. aureus*) bacterial strains. The number of inoculated and survived bacterial colonies was taken and percentage reduction was calculated (Table 12). It was observed that all tested samples exhibited excellent antibacterial potential towards both test microbes i.e., >85% inhibition in bacterial growth. In case of samples G, A, and S, maximum antibacterial action was observed for sample S. It was also observed that the antibacterial activity of all samples increased significantly after the application of modified antibacterial dye on treated fabrics. The activity was increased from 87%, 90%, and 98%, to 99% for samples DG, DA, and DS, respectively against *E. coli*. The same increasing trend was observed for all 3 samples against *S. aureus*. The increase in antibacterial activity after the application of dye could be ascribed to the presence of a strong antibacterial agent i.e., triclosan which is reported to have an excellent antibacterial effect on a broad range of bacterial species. No antibacterial activity was observed for untreated cotton fabric (UT) which further confirmed that antibacterial activity in treated samples was due to the application of nanoparticles and antibacterial dye.

Table 9: The percentage reduction of developed samples against *S. aureus* and *E. coli.*

Sr.#	**Reducing agent**	**Application of dye**	**Sample code**	**E. coli**	**S. aureus**
1.	Untreated cotton	No	UT	0 %	0 %
2.	Glucose	No	G	87 %	91 %
3.	Glucose	Yes	DG	99.99 %	97.99 %
4.	Ascorbic acid	No	A	90.99%	95.99%
5.	Ascorbic acid	Yes	DA	99.9%	99.99%
6.	Sodium hydrosulphite	No	S	98.99%	99.99%
7.	Sodium hydrosulphite	Yes	DS	99.99%	99.99%

For more clarification, the reduction in colony-forming units (CFU/ml) of survived bacterial colonies was calculated, and obtained results are presented in Figure 44. The untreated cotton fabric exhibited a substantial number of survived bacterial colonies and higher values CFUs/ml were obtained (7.34 for E. coli and 6.44 for the S. aureus bacterial strain). The results revealed that CFUs concentration was remarkably reduced for all treated samples. The samples S and DS exhibited the highest reduction in survived bacterial colonies and the CFUs values reached 0 from 7.34 and 6.44 for E. coli and S. aureus, respectively.

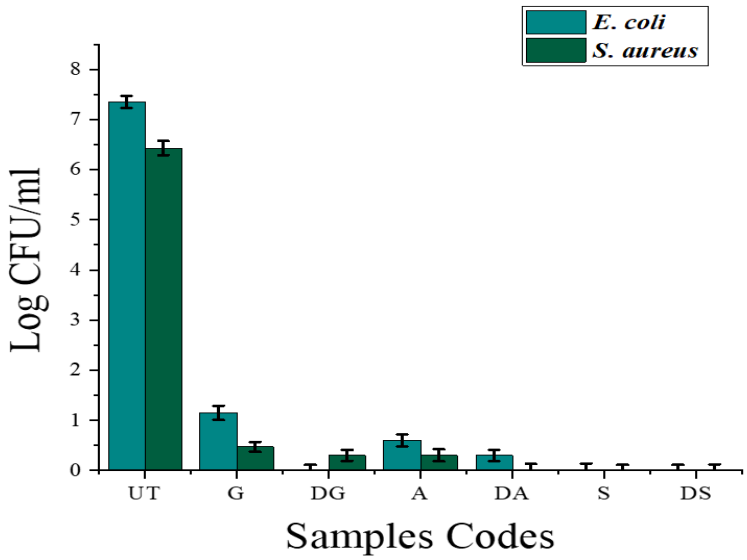


Figure 20: The reduction in CFUs of survived bacterial colonies for all developed samples

The validity of the obtained results was reaffirmed through the visual evidence depicted in Figures 45a and 46b, showcasing a clear comparison between untreated and treated samples against S. aureus and E. coli. The stark contrast between these images provides compelling insight into the efficacy of the antibacterial treatment.

In the case of untreated fabric samples, a discernible increase in the number of bacterial colonies is apparent, which strongly indicates the absence of any antibacterial action in the untreated (UT) sample. Conversely, for all treated samples, a remarkable reduction in the number of bacterial colonies is evident. This striking visual representation reinforces the fact that the treated samples exhibited a remarkable reduction of over 99% in bacterial growth, underlining the potent antibacterial effectiveness of the treatments.

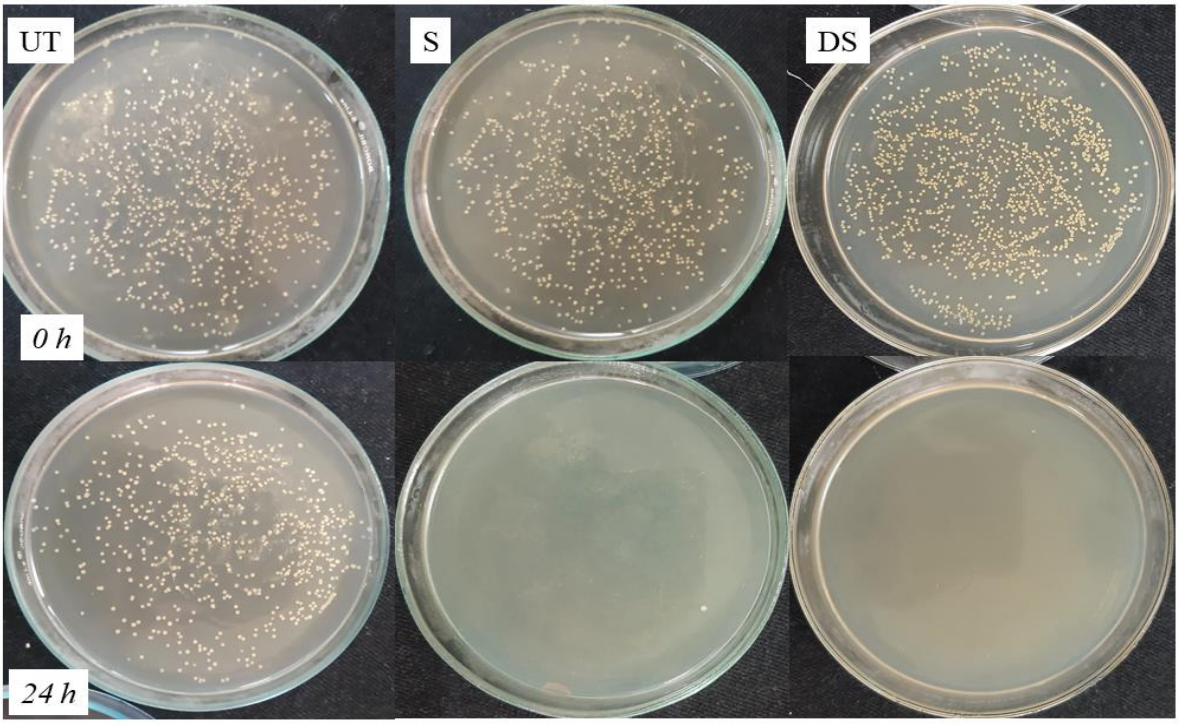


Figure 21:a: Images for the number of bacterial colonies inoculated (0 h) and survived (24 h) for sample UT, S, and DS against *S. aureus.*

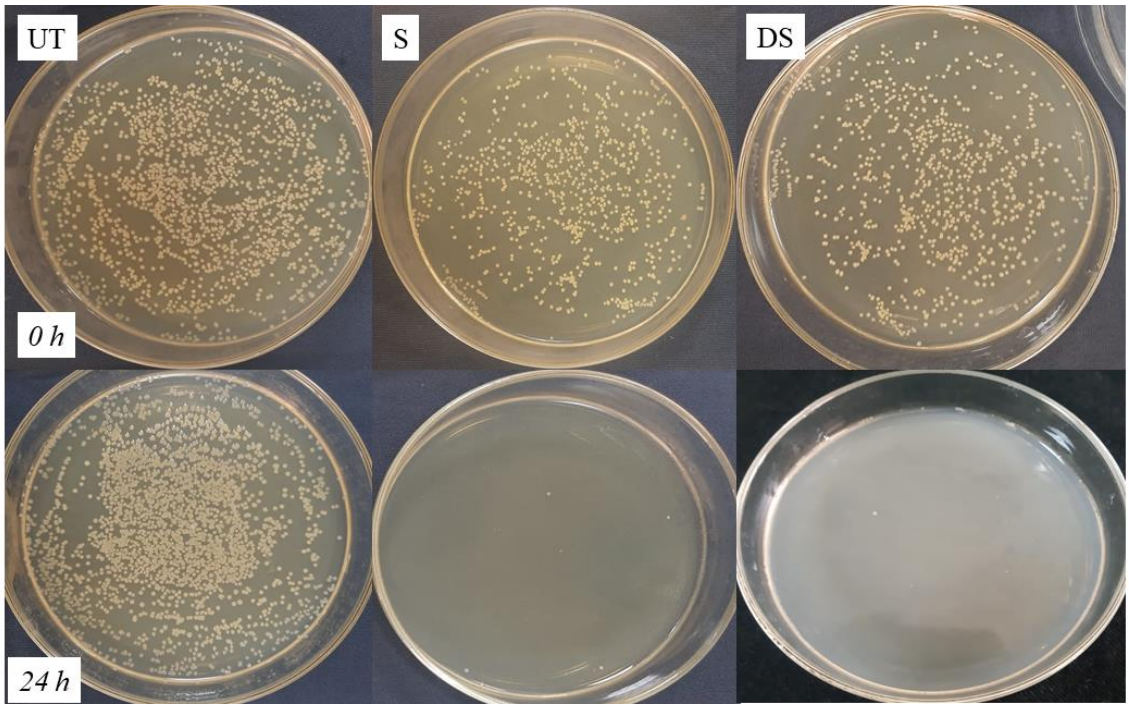


Figure 22:b: Images for the number of bacterial colonies inoculated (0 h) and survived (24 h) for sample UT, S, and DS against *E. coli.*

5.2.9 Antifungal Activity of Treated Samples

The quantitative assessment of antifungal activity against the A. Niger fungal species was conducted following the AATCC-100 standard method. The results, summarized in Table 13, showcase the percentage reduction in fungal growth achieved by all fabric samples, both treated undyed and treated dyed. Impressively, all treated fabric samples, whether dyed or undyed, exhibited effective antifungal activity against the tested microbe.

A noteworthy trend is observed, with the dyed samples demonstrating an increase in antifungal activity compared to their undyed counterparts. This enhancement could be attributed to the effective antifungal action of the incorporated dye itself. However, it's important to acknowledge that the antibacterial activity of the colored samples remains more pronounced than their antifungal counterpart. This disparity could be attributed to the nature of the incorporated triclosan, which has a higher efficacy against bacteria compared to fungi.

Among the undyed samples, sample S demonstrated the highest reduction in fungal spore growth, displaying a substantial antifungal activity of 89%. Similarly, among the dyed samples, sample DS exhibited exceptional antifungal action, attaining a maximum antifungal activity of 91%. The comparison against untreated fabric once again reiterates the ineffectiveness of untreated samples against the test microbe, thereby confirming that the observed antifungal activity in treated samples stems from the integration of nanoparticles and the modified antimicrobial dye.

Table 10: Reduction percentage of antifungal activity.

Sr.#	Reducing agent	Application of dye	Sample code	*A. Niger*
1.	Untreated cotton	No	UT	0 %
2.	Glucose	No	G	75%
3.	Glucose	Yes	DG	79 %
4.	Ascorbic acid	No	A	83%
5.	Ascorbic acid	Yes	DA	85%
6.	Sodium hydrosulphite	No	S	89%
7.	Sodium hydrosulphite	Yes	DS	91%

5.2.10 Antiviral effectiveness

Behrens and Karber's method were used for the evaluation of virus titers reduction from the initial viral titer of infectivity (10^8) against Corona Virus. Figure 47 showed the virus infectivity titer log at contact time (0 h and 60 mins). Figure 47 (a) showed the infectivity titer change of coronavirus for all tested samples. It was noticed that antiviral activity was increased for all dyed samples which indicated that dye has significant antiviral activity. However, the antibacterial action of dyed samples was more pronounced as compared to antiviral activity. This could be explained by the fact that the antibacterial action of triclosan is stronger than antiviral and antifungal action. The observed trend supported the obtained results of the antibacterial activities of these samples. The viral infectivity titer was decreased significantly for all treated samples. However, the maximum reduction was exhibited by the sample S (among all undyed samples) and DS (among all dyed samples) which showed 79% and 83% antiviral action, respectively. The untreated fabric remained ineffective against the virus which further confirmed that antiviral action in all treated samples was due to the application of nanoparticles and modified antimicrobial dye. The antiviral action shown by the fabrics treated with copper nanoparticles and dyed fabric samples could be due to the binding of metallic NPs and the non-polar part (benzene ring) of the triclosan with glycoproteins at the viral surface working as an inhibitory action for viruses. The reduction percentages of all tested samples are given in Table 14.

Table 11: Reduction percentage of antifungal activity.

Sr.#	Reducing agent	Application of dye	Sample code	Corona Virus
1.	Untreated cotton	No	UT	0 %
2.	Glucose	No	G	70 %
3.	Glucose	Yes	DG	72 %
4.	Ascorbic acid	No	A	75 %
5.	Ascorbic acid	Yes	DA	75 %
6.	Sodium hydrosulphite	No	S	79 %
7.	Sodium hydrosulphite	Yes	DS	83 %

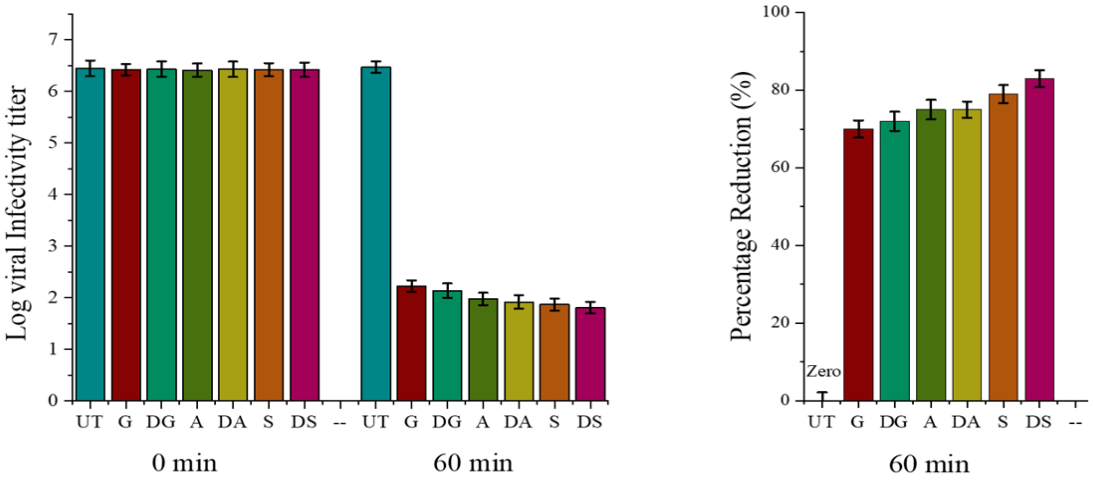


Figure 23: Reduction in viral infectivity titer (a) and percentage reduction (b) calculated from viral infectivity at a contact time of 0 and 60 min.

5.2.11 Durability of cuprous oxide coated fabrics

Copper nanoparticles (Cu-NPs) were previously noted to be attached to the fabric surface through a combination of physical and chemical linkages. The introduced Cu-NPs further aggregated the microfibers, effectively forming hygienic antibacterial networks by filling the gaps and voids between them. This property of absorption and adhesion was further underscored by the durability of antibacterial activity, even after repeated washing.

To validate this behavior of absorption and adhesion, fabric samples coated with Cu-NPs were subjected to various tests. These included soaking, squeezing, and twisting the samples in water. Notably, the Cu-NP-coated samples exhibited favorable washing characteristics, with no observable peeling from the fabric surface or precipitation in the water. Subsequently, an adhesion test was performed using transparent tape. The tape retained its transparency, indicating the absence of detectable particles on its surface. This outcome serves to confirm the robust interactions and excellent mechanical adherence between Cu-NPs, dye molecules, and the fabric itself.

The durability of the antibacterial activity was assessed by evaluating the antibacterial properties of all treated fabric samples after undergoing washing cycles. Compliance with the ISO 105-C01 standard washing test protocol was followed. Table 15 provides a comprehensive overview of the antibacterial values for all tested samples, both before and after washing. The results clearly indicate that the inhibition zone values underwent negligible changes even after multiple washing cycles.

Furthermore, this pattern was substantiated by SEM analysis carried out on the washed samples A1, S1, and G1, as depicted in Figure 20. The retention of nanoparticles on the fabric surface post-washing reaffirmed their strong adherence to the fibers and interstices. This evidence serves to emphasize the enduring nature of the antibacterial functionality of the treated fabrics, even after undergoing washing procedures.

Table 12: Antimicrobial properties of Cu_2O coated fabrics after washing.

Sr.#	**Sample Code**	**ZOI (mm)**		**ZOI (mm)**	
		Unwashed samples		Washed samples	
		S. aureus	*E. coli*	*S. aureus*	*E. coli*
1.	G	5	2	5	2
2.	DG	7	3	6	3
3.	A	4	3	4	2
4.	DA	7	3	7	3
5.	S	5	3	5	2
6.	DS	8	4	7	4

6. CONCLUSIONS

The cuprous nanoparticles have already been studied extensively due to their potential technological applications in medical fields. The main aim of the thesis was to investigate preparation properties and selected applications of bioactive textiles having antimicrobial ability, antiviral, antifungal, and durability. The first part of thesis was the formation of Cu_2O particles by the Fehling solution method and the effect of three different reducing agents was analyzed to prepare the Cu_2O particles. A total of nine hygienic multifunctional textile samples were developed using nanoparticle of cuprous oxide particles.

In the second step, the deposition of cuprous oxide particles was done on woven cotton fabric, and studied their antipathogenic (antibacterial, antifungal, and antiviral) properties. Surface morphology and the existence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. The antibacterial activity of cuprous oxide-coated fabrics was tested against qualitative and quantitative measurements. The strongest antibacterial effect was found for the fabrics coated with cuprous oxide particles reduced with sodium hydrosulphite at 1g/L.

Furthermore, the developed fabrics were analyzed for comfort properties regarding air permeability and stiffness. The particles were so fine, ensuring that they did not obstruct the pores of the fabric structure. Hence, ensured improvement in stiffness and air permeability. In the end, the durability of deposition was confirmed by measuring the antibacterial properties and SEM analysis after washing. The retention of particles over the surface of the fabric reinforced the fact that particles are firmly attached to the fibers and interspaces. Developed process very easy, less in cost, and provide odorless work wear. The third study was to dye the already cuprous oxide-coated fabrics with antibacterial dye. The dyeing of the coated textiles has been performed to overcome discoloration and

staining but their antibacterial effectiveness was compromised. Thus, the development of highly effective antimicrobial textiles with improved aesthetics was challenging.

In the second part, a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with an excellent aesthetic appearance was proposed. At first, fabrics were sensitized with citric acid. Then, the Fehling solution method was followed for the synthesis of Cu_2O nanoparticles. The synthesized nanoparticles were then applied to cotton fabric. Then, a reactive dye was selected and functionalized with an antibacterial agent. Subsequently, the cuprous oxide particles coated fabric were subjected to exhaust dyeing through the solution of functional bioactive dye. A total of 6 hygienic multifunctional textile samples (3 dyed and 3 undyed) were developed using nanoparticles of cuprous oxide particles along with varying the concentrations of three different reducing agents. The surface morphology and existence of metals were analyzed by scanning electron microscopy, dynamic light scattering, FTIR, EDS, and XRD. After that, the Reactive blue 4 dye was functionalized with triclosan to impart antibacterial activity to the dye. FTIR and 13C-NMR results confirmed the successful modification of dye with an antibacterial agent. The modified dye was applied to copper-treated cotton fabrics through the exhaust dyeing method. The modified dye exhibited excellent fixation, exhaustion, and dye levelness on copper-treated fabric. The antibacterial activity of copper-treated fabrics was also increased after the application of dye. The antibacterial activity of cuprous oxide-coated dyed and undyed fabrics was tested against qualitative and quantitative measurements. It was observed that ZOI for dyed copper-coated samples is higher than for undyed copper-coated fabrics. The strongest antibacterial effect was found for the dyed fabric sample DS (sodium hydrosulphite). In case of quantitative analysis, the samples S and DS exhibited the highest reduction in survived bacterial colonies and the CFUs values reached 0 from 7.34 and 6.44 for E. coli and S. aureus, respectively. However, the antibacterial action of colored samples was more prominent in comparison to antifungal activity. This might be accounted to the fact that the antibacterial action of triclosan is higher than its antifungal and antiviral action. Furthermore, the durability of deposition was confirmed by measuring the antibacterial properties and SEM analysis after washing. The retention of particles over the surface of the fabric (SEM images) reinforced the fact that particles are firmly attached to the fibers and interspaces. Moreover, the change in antimicrobial activities of all treated fabric samples after repeated laundry cycles was insignificant which further confirmed the durability of particles on fabric. The developed process is very easy, less in cost, and provides odorless work wear. The successful application of cuprous oxide-coated fabrics explained their potential applications in the field of medical textiles to develop antibacterial surgical drapes, pants, socks, panels, bed sheets, surgical gowns, curtains, panel covers, wallpapers/sheets coverage, shoe mats, outlet covers, seat chair covers, Table covers, patient and doctors' socks, etc.

7. REFERENCES

[1] A. Ali, V. Baheti, M. Vik, and J. Militky, "Copper electroless plating of cotton fabrics after surface activation with deposition of silver and copper nanoparticles," *Journal of Physics and Chemistry of Solids*, vol. 137, no. October 2018, p. 109181, 2020, doi: 10.1016/j.jpcs.2019.109181.

[2] F. Ahmed, I. A. Shaikh, T. Hussain, I. Ahmad, S. Munir, and M. Zameer, "Developments in health care and medical textiles - A mini review-1," *Pakistan Journal of Nutrition*, vol. 13, no. 12, pp. 780–783, 2014, doi: 10.3923/pjn.2014.780.783.

[3] D. SJ, "How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals," *J Hosp Infect*, vol. 56, pp. 10–15, 2004.

[4] Pe. G. S. Hubert H.AttawayIIIMSaSarahFaireyBSaLisa L.SteedPhDbCassandra D.SalgadoMDcHarold T.MichelsPhD, "Intrinsic bacterial burden associated with intensive care unit hospital beds: Effects of disinfection on population recovery and mitigation of potential infection risk," *Am J Infect Control*, vol. 40, pp. 907–912, 2012.

[5] D. M. G. Usha Stiefel (a1) (a2), Jennifer L. Cadnum (a1), Brittany C. Eckstein (a1), "Contamination of Hands with Methicillin-Resistant Staphylococcus aureus after Contact with

Environmental Surfaces and after Contact with the Skin of Colonized Patients," *Infect Control Hosp Epidemiol*, vol. 32, pp. 185–187, 2011.
[6] L. Van Der Hoek *et al.*, "Identification of a new human coronavirus," *Nat Med*, vol. 10, no. 4, pp. 368–373, 2004, doi: 10.1038/nm1024.
[7] A. Ali, V. Baheti, J. Militky, Z. Khan, V. Tunakova, and S. Naeem, "Copper-coated multifunctional cotton fabrics," *Journal of Industrial Textiles*, vol. 48, pp. 448–464, Sep. 2017, doi: 10.1177/1528083717732076.
[8] K. C. Weaver L, Michels HT, "Potential for preventing spread of fungi in air-conditioning systems constructed using copper instead of aluminium," *Lett Appl Microbiol*, vol. 50, pp. 18 – 23, 2010.
[9] F. G. Otter JA, Yezli S, Salkeld JAG, "Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings," *Am J Infect Control*, vol. 41, pp. S6–11, 2013.
[10] W. D. Rutala WA, "Are room decontamination units needed to prevent transmission of environmental pathogens?," *Infect Control Hosp Epidemiol*, vol. 32, pp. 743–747, 2011.
[11] B. H. Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, "Survival of influenza viruses on environmental surfaces," *J Infect Dis*, vol. 146, pp. 47–51, 1982.
[12] S. AnitaT. R. R. all 5 authorsM. Mahalakshmi, "A study of the antimicrobial property of encapsulated copper oxide nanoparticles on cotton fabric," *Textile Research Journal*, vol. 81, pp. 1081–1088, 2011.
[13] J. M. Azam Ali, Vijay Baheti, Muhammad Usman Javaid, "Enhancement in ageing and functional properties of copper-coated fabrics by subsequent electroplating," *Applied Physics A*, vol. 124, no. 9, p. 651, 2018.
[14] A. Ali, V. Baheti, J. Militky, Z. Khan, and S. Q. Z. Gilani, "Comparative Performance of Copper and Silver Coated Stretchable Fabrics," *Fibers and Polymers*, vol. 19, no. 3, 2018, doi: 10.1007/s12221-018-7917-5.
[15] M. Shahid, A. Ali, H. Khaleeq, M. Farrukh Tahir, J. Militky, and J. Wiener, "Development of antimicrobial multifunctional textiles to avoid from hospital-acquired infections," *Fibers and Polymers*, vol. 22, no. 11, pp. 3055–3067, 2021.
[16] M. Shahid, Shahid-ul-Islam, L. J. Rather, N. Manzoor, and F. Mohammad, "Simultaneous shade development, antibacterial, and antifungal functionalization of wool using Punica granatum L. Peel extract as a source of textile dye," *Journal of Natural Fibers*, vol. 16, no. 4, pp. 555–566, 2019.
[17] A. Keller, T. and Kuhn, "Electrodes for transcutaneous (surface) electrical stimulation," *Journal of Automatic Control*, vol. 18, no. 2, pp. 35–45, 2008.
[18] F. Ahmed, A. Shaikh, T. Hussain, I. Ahmad, S. Munir, and M. Zameer, "Developments in Health Care and Medical Textiles-A Mini Review-1," *Pakistan Journal of Nutrition*, vol. 13, no. 12, pp. 780–783, 2014.
[19] M. Shahid, Shahid-ul-Islam, L. J. Rather, N. Manzoor, and F. Mohammad, "Simultaneous shade development, antibacterial, and antifungal functionalization of wool using *Punica granatum* L. Peel extract as a source of textile dye," *Journal of Natural Fibers*, vol. 16, no. 4, pp. 555–566, May 2019, doi: 10.1080/15440478.2018.1428846.
[20] K. E. Jones *et al.*, "Global trends in emerging infectious diseases," *Nature*, vol. 451, no. 7181, pp. 990–993, Feb. 2008, doi: 10.1038/nature06536.
[21] J. D. Cherry and P. Krogstad, "SARS: The First Pandemic of the 21st Century," *Pediatr Res*, vol. 56, no. 1, pp. 1–5, Jul. 2004, doi: 10.1203/01.PDR.0000129184.87042.FC.
[22] M. T. Mascellino, F. di Timoteo, M. de Angelis, and A. Oliva, "Overview of the Main Anti-SARS-CoV-2 Vaccines: Mechanism of Action, Efficacy and Safety," *Infect Drug Resist*, vol. Volume 14, pp. 3459–3476, Aug. 2021, doi: 10.2147/IDR.S315727.
[23] E. Janik, M. Ceremuga, M. Niemcewicz, and M. Bijak, "Dangerous Pathogens as a Potential Problem for Public Health," *Medicina (B Aires)*, vol. 56, no. 11, p. 591, Nov. 2020, doi: 10.3390/medicina56110591.

[24] D. Montegut, N. Indictor, and R. J. Koestler, “Fungal deterioration of cellulosic textiles: a review,” *International Biodeterioration*, vol. 28, no. 1–4, pp. 209–226, Jan. 1991, doi: 10.1016/0265-3036(91)90043-Q.
[25] S. A. Sattar *et al.*, “Transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using *Staphylococcus aureus* as a model,” *J Appl Microbiol*, vol. 90, no. 6, pp. 962–970, Jun. 2001, doi: 10.1046/j.1365-2672.2001.01347.x.
[26] R. W. Sidwell, G. J. Dixon, and E. Mcneil, “Quantitative Studies on Fabrics as Disseminators of Viruses,” *Appl Microbiol*, vol. 14, no. 1, pp. 55–59, Jan. 1966, doi: 10.1128/am.14.1.55-59.1966.
[27] Z. U. Iyigundogdu, O. Demir, A. B. Asutay, and F. Sahin, “Developing Novel Antimicrobial and Antiviral Textile Products,” *Appl Biochem Biotechnol*, vol. 181, no. 3, pp. 1155–1166, Mar. 2017, doi: 10.1007/s12010-016-2275-5.
[28] M. Egbuta, M. Mwanza, and O. Babalola, “Health Risks Associated with Exposure to Filamentous Fungi,” *Int J Environ Res Public Health*, vol. 14, no. 7, p. 719, Jul. 2017, doi: 10.3390/ijerph14070719.
[29] V. Dixit, J. Tewari, and S. K. Obendorf, “Fungal Growth Inhibition of Regenerated Cellulose Nanofibrous Membranes Containing Quillaja Saponin,” *Arch Environ Contam Toxicol*, vol. 59, no. 3, pp. 417–423, Oct. 2010, doi: 10.1007/s00244-010-9493-6.
[30] R. Purwar, “Antimicrobial textiles,” in *The Impact and Prospects of Green Chemistry for Textile Technology*, Elsevier, 2019, pp. 281–306. doi: 10.1016/B978-0-08-102491-1.00010-1.
[31] A. Ali *et al.*, “Development of silver plated electrically conductive elastomers embedded with carbon black particles obtained from Kevlar waste source,” *Polym Test*, vol. 116, p. 107793, Dec. 2022, doi: 10.1016/j.polymertesting.2022.107793.
[32] M. Piętka-Ottlik, H. Wójtowicz-Młochowska, K. Kołodziejczyk, E. Piasecki, and J. Młochowski, “New Organoselenium Compounds Active against Pathogenic Bacteria, Fungi and Viruses,” *Chem Pharm Bull (Tokyo)*, vol. 56, no. 10, pp. 1423–1427, 2008, doi: 10.1248/cpb.56.1423.
[33] D. Gerrity, H. Ryu, J. Crittenden, and M. Abbaszadegan, “Photocatalytic inactivation of viruses using titanium dioxide nanoparticles and low-pressure UV light,” *Journal of Environmental Science and Health, Part A*, vol. 43, no. 11, pp. 1261–1270, Jul. 2008, doi: 10.1080/10934520802177813.
[34] J. Yu, C. Kuwentrai, J.-D. Huang, and C. Xu, “Carbon-based nanomaterials for viral infection management,” *Biomicrofluidics*, vol. 15, no. 1, p. 011501, Jan. 2021, doi: 10.1063/5.0032427.
[35] A. Hassabo and mariam kamel, “Anti-microbial finishing for natural textile fabrics,” *Journal of Textiles, Coloration and Polymer Science*, vol. 0, no. 0, pp. 0–0, May 2021, doi: 10.21608/jtcps.2021.72333.1054.
[36] L. B. Williams and S. E. Haydel, “Evaluation of the medicinal use of clay minerals as antibacterial agents,” *Int Geol Rev*, vol. 52, no. 7–8, pp. 745–770, May 2010, doi: 10.1080/00206811003679737.
[37] C. Hobson, A. N. Chan, and G. D. Wright, “The Antibiotic Resistome: A Guide for the Discovery of Natural Products as Antimicrobial Agents,” *Chem Rev*, vol. 121, no. 6, pp. 3464–3494, Mar. 2021, doi: 10.1021/acs.chemrev.0c01214.
[38] T. Arif *et al.*, “Natural products – antifungal agents derived from plants,” *J Asian Nat Prod Res*, vol. 11, no. 7, pp. 621–638, Jul. 2009, doi: 10.1080/10286020902942350.
[39] Shahid-ul-Islam, M. Shahid, and F. Mohammad, “Perspectives for natural product based agents derived from industrial plants in textile applications – a review,” *J Clean Prod*, vol. 57, pp. 2–18, Oct. 2013, doi: 10.1016/j.jclepro.2013.06.004.
[40] R. A. Matula, “Electrical resistivity of copper, gold, palladium, and silver,” *J Phys Chem Ref Data*, vol. 8, no. 4, pp. 1147–1298, 1979.
[41] P. T. Coleridge and I. M. Templeton, “Fermi-surface radii in copper, silver, and gold,” *Phys Rev B*, vol. 25, no. 12, p. 7818, 1982.
[42] A. Ali, V. Baheti, J. Militky, Z. Khan, V. Tunakova, and S. Naeem, “Copper-coated multifunctional cotton fabrics,” *Journal of Industrial Textiles*, vol. 48, no. 2, pp. 448–464, 2018.

[43] K. Bertuleit, “Silver coated polyamide: a conductive fabric,” *Journal of Coated Fabrics*, vol. 20, no. 3, pp. 211–215, 1991.
[44] W. C. Smith, *Smart Textile Coatings and Laminates*. Woodhead Publishing, UK and CRC Press, Boca Raton, FL, 2010.
[45] L. I. Murthy and W. E. Halperin, “Medical screening and biological monitoring: A guide to the literature for physicians,” *J Occup Environ Med*, pp. 170–184, 1995.
[46] A. Ali, V. Baheti, J. Militky, and Z. Khan, “Utility of silver-coated fabrics as electrodes in electrotherapy applications,” *J Appl Polym Sci*, vol. 135, no. 23, 2018, doi: 10.1002/app.46357.
[47] J. Molina, A. I. Del Río, J. Bonastre, and F. Cases, “Electrochemical polymerisation of aniline on conducting textiles of polyester covered with polypyrrole/AQSA,” *Eur Polym J*, vol. 45, no. 4, pp. 1302–1315, 2009.
[48] P. Rytlewski, B. Jagodziński, and K. Moraczewski, “Laser-assisted electroless metallization of polymer materials: a critical review,” *Reviews of Adhesion and Adhesives*, vol. 4, no. 3, pp. 334–366, 2016.
[49] S. Q. Jiang, E. Newton, C. W. M. Yuen, and C. W. Kan, “Chemical silver plating on cotton and polyester fabrics and its application on fabric design,” *Textile research journal*, vol. 76, no. 1, pp. 57–65, 2006.
[50] X. Gan, Y. Wu, L. Liu, B. Shen, and W. Hu, “Electroless plating of Cu–Ni–P alloy on PET fabrics and effect of plating parameters on the properties of conductive fabrics,” *J Alloys Compd*, vol. 455, no. 1–2, pp. 308–313, 2008.
[51] A. A. Tracton, *Coatings technology handbook*. CRC press, 2005.
[52] B. Alemour, M. H. Yaacob, H. N. Lim, and M. R. Hassan, “Review of Electrical Properties of Graphene Conductive Composites.,” *International Journal of Nanoelectronics & Materials*, vol. 11, no. 4, 2018.
[53] R. A. Serway, *Principles of Physics*, Second. London: Saunders College Pub, 1998.
[54] C. Douglas, “Giancoli, physics for scientists and engineers.” Prentice Hall, New Jersey, 1989.
[55] R. M. Pashley, M. Rzechowicz, L. R. Pashley, and M. J. Francis, “De-gassed water is a better cleaning agent,” *J Phys Chem B*, vol. 109, no. 3, pp. 1231–1238, 2005.
[56] S. D. Pawar, P. Murugavel, and D. M. Lal, “Effect of relative humidity and sea level pressure on electrical conductivity of air over Indian Ocean,” *Journal of Geophysical Research: Atmospheres*, vol. 114, no. D2, 2009.
[57] N. Muthukumar and G. Thilagavathi, “Development and characterization of electrically conductive polyaniline coated fabrics,” *Indian journal of chemical technology*, vol. 19, no. November, pp. 434–441, 2012.
[58] G. Wu, X. Huang, Z. Dou, S. Chen, and L. Jiang, “Electromagnetic interfering shielding of aluminum alloy–cenospheres composite,” *J Mater Sci*, vol. 42, no. 8, pp. 2633–2636, 2007.
[59] Z. An, X. Zhang, and H. Li, “A preliminary study of the preparation and characterization of shielding fabric coated by electrical deposition of amorphous Ni-Fe-P alloy,” *J Alloys Compd*, vol. 621, pp. 99–103, 2015, doi: 10.1016/j.jallcom.2014.09.163.
[60] T. Keller and A. Kuhn, “Electrodes for transcutaneous (surface) electrical stimulation,” *Journal of automatic control*, vol. 18, no. 2, pp. 35–45, 2008.
[61] K. W. Oh, H. J. Park, and S. H. Kim, “Stretchable conductive fabric for electrotherapy,” *J Appl Polym Sci*, vol. 88, no. 5, pp. 1225–1229, 2003.
[62] M. Montazer and M. Maali Amiri, “ZnO nano reactor on textiles and polymers: ex situ and in situ synthesis, application, and characterization,” *J Phys Chem B*, vol. 118, no. 6, pp. 1453–1470, 2014.
[63] L. Tamayo, M. Azócar, M. Kogan, A. Riveros, and M. Páez, “Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces,” *Materials Science and Engineering: C*, vol. 69, pp. 1391–1409, 2016.
[64] I. Perelshtein *et al.*, “CuO–cotton nanocomposite: Formation, morphology, and antibacterial activity,” *Surf Coat Technol*, vol. 204, no. 1–2, pp. 54–57, 2009.

[65] D. Marković *et al.*, “Broad Spectrum of Antimicrobial Activity of Cotton Fabric Modified with Oxalic Acid and CuO/Cu2O Nanoparticles,” *Fibers and Polymers*, vol. 20, no. 11, pp. 2317–2325, 2019, doi: 10.1007/s12221-019-9131-5.
[66] A. B. Rezaie, M. Montazer, and M. M. Rad, “A cleaner route for nanocolouration of wool fabric via green assembling of cupric oxide nanoparticles along with antibacterial and UV protection properties,” *J Clean Prod*, vol. 166, pp. 221–231, 2017.
[67] D. Marković *et al.*, “Broad Spectrum of Antimicrobial Activity of Cotton Fabric Modified with Oxalic Acid and CuO/Cu2O Nanoparticles,” *Fibers and Polymers*, vol. 20, no. 11, pp. 2317–2325, Nov. 2019, doi: 10.1007/s12221-019-9131-5.
[68] L. E. Román *et al.*, “In situ growth of CuO nanoparticles onto cotton textiles,” *Advances in Natural Sciences: Nanoscience and Nanotechnology*, vol. 11, no. 2, p. 25009, 2020.
[69] I. Perelshtein *et al.*, “Making the hospital a safer place by sonochemical coating of all its textiles with antibacterial nanoparticles,” *Ultrason Sonochem*, vol. 25, pp. 82–88, 2015.
[70] P. Mantecca *et al.*, “Airborne nanoparticle release and toxicological risk from metal-oxide-coated textiles: toward a multiscale safe-by-design approach,” *Environ Sci Technol*, vol. 51, no. 16, pp. 9305–9317, 2017.
[71] I. Perelshtein *et al.*, “The sonochemical coating of cotton withstands 65 washing cycles at hospital washing standards and retains its antibacterial properties,” *Cellulose*, vol. 20, no. 3, pp. 1215–1221, 2013.
[72] D. Zarbaf, M. Montazer, and A. Sadeghian Maryan, “In-situ synthesis of nano-copper on denim garment along with nano-clay for antibacterial and decoloration purposes,” *Cellulose*, vol. 24, no. 9, pp. 4083–4095, 2017.
[73] A. Khani and N. Talebian, “In vitro bactericidal effect of ultrasonically sol–gel-coated novel CuO/TiO2/PEG/cotton nanocomposite for wound care,” *J Coat Technol Res*, vol. 14, no. 3, pp. 651–663, 2017.
[74] M. Gouda, A. Aljaafari, Y. Al-Fayz, and W. E. Boraie, “Preparation and characterization of some nanometal oxides using microwave technique and their application to cotton fabrics,” *J Nanomater*, vol. 2015, 2015.
[75] A. Ali, V. Baheti, M. Vik, and J. Militky, “Copper electroless plating of cotton fabrics after surface activation with deposition of silver and copper nanoparticles,” *Journal of Physics and Chemistry of Solids*, vol. 137, no. October 2018, p. 109181, 2020, doi: 10.1016/j.jpcs.2019.109181.
[76] V. V Thampi, S. Thanka Rajan, K. Anupriya, and B. Subramanian, “Functionalization of fabrics with PANI/CuO nanoparticles by precipitation route for anti-bacterial applications,” *Journal of Nanoparticle Research*, vol. 17, no. 1, pp. 1–12, 2015.
[77] S. Vasantharaj *et al.*, “Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: characterization of antibacterial activity and dye degradation potential,” *J Photochem Photobiol B*, vol. 191, pp. 143–149, 2019.
[78] A. Ali, V. Baheti, M. Vik, and J. Militky, “Copper electroless plating of cotton fabrics after surface activation with deposition of silver and copper nanoparticles,” *Journal of Physics and Chemistry of Solids*, vol. 137, p. 109181, 2020, doi: 10.1016/j.jpcs.2019.109181.
[79] A. Ali, V. Baheti, J. Militky, Z. Khan, and S. Q. Z. Gilani, “Comparative Performance of Copper and Silver Coated Stretchable Fabrics,” *Fibers and Polymers*, vol. 19, no. 3, pp. 607–619, 2018, doi: 10.1007/s12221-018-7917-5.
[80] S. Iravani, “Green synthesis of metal nanoparticles using plants,” *Green Chemistry*, vol. 13, no. 10, pp. 2638–2650, 2011.
[81] P. Mukherjee *et al.*, “Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis,” *Nano Lett*, vol. 1, no. 10, pp. 515–519, 2001.
[82] B. Turakhia, M. B. Divakara, M. S. Santosh, and S. Shah, “Green synthesis of copper oxide nanoparticles: a promising approach in the development of antibacterial textiles,” *J Coat Technol Res*, vol. 17, no. 2, pp. 531–540, 2020, doi: 10.1007/s11998-019-00303-5.
[83] P. Sharma, S. Pant, V. Dave, K. Tak, V. Sadhu, and K. R. Reddy, “Green synthesis and characterization of copper nanoparticles by Tinospora cardifolia to produce nature-friendly

copper nano-coated fabric and their antimicrobial evaluation," *J Microbiol Methods*, vol. 160, pp. 107–116, 2019, doi: https://doi.org/10.1016/j.mimet.2019.03.007.
[84] N. Sarwar *et al.*, "Citric acid mediated green synthesis of copper nanoparticles using cinnamon bark extract and its multifaceted applications," *J Clean Prod*, vol. 292, p. 125974, 2021, doi: 10.1016/j.jclepro.2021.125974.
[85] S. Vasantharaj *et al.*, "Synthesis of ecofriendly copper oxide nanoparticles for fabrication over textile fabrics: Characterization of antibacterial activity and dye degradation potential," *J Photochem Photobiol B*, vol. 191, pp. 143–149, 2019, doi: 10.1016/j.jphotobiol.2018.12.026.
[86] A. Vinothkanna, K. Mathivanan, S. Ananth, Y. Ma, and S. Sekar, "Biosynthesis of copper oxide nanoparticles using Rubia cordifolia bark extract: characterization, antibacterial, antioxidant, larvicidal and photocatalytic activities," *Environmental Science and Pollution Research*, no. 0123456789, 2022, doi: 10.1007/s11356-022-18996-4.
[87] Massella *et al.*, "Bio-Functional Textiles: Combining Pharmaceutical Nanocarriers with Fibrous Materials for Innovative Dermatological Therapies," *Pharmaceutics*, vol. 11, no. 8, p. 403, Aug. 2019, doi: 10.3390/pharmaceutics11080403.
[88] D. Atanasova, D. Staneva, and I. Grabchev, "Textile Materials Modified with Stimuli-Responsive Drug Carrier for Skin Topical and Transdermal Delivery," *Materials*, vol. 14, no. 4, p. 930, Feb. 2021, doi: 10.3390/ma14040930.
[89] C. Fenton and Z. T. Al-Salama, "Fabrics can greatly improve or exacerbate atopic dermatitis," *Drugs & Therapy Perspectives*, vol. 37, no. 4, pp. 157–161, Apr. 2021, doi: 10.1007/s40267-021-00822-5.
[90] M. Elnaggar, H. Emam, M. Fathalla, M. Abdel-Aziz, and M. Zahran, "Chemical synthesis of silver nanoparticles in its solid state: highly efficient antimicrobial cotton fabrics for wound healing properties," *Egypt J Chem*, vol. 0, no. 0, pp. 0–0, Feb. 2021, doi: 10.21608/ejchem.2021.57667.3236.
[91] N. Hedayati, M. Montazer, M. Mahmoudirad, and T. Toliyat, "Ketoconazole and Ketoconazole/β-cyclodextrin performance on cotton wound dressing as fungal skin treatment," *Carbohydr Polym*, vol. 240, p. 116267, Jul. 2020, doi: 10.1016/j.carbpol.2020.116267.
[92] K. Jagadeesan, "Antimicrobial Textiles for Long Duration Space Flight." [Online]. Available: https://digitalcommons.uri.edu/tmd_major_papers
[93] I. G. Klepp, M. Buck, K. Laitala, and M. Kjeldsberg, "What's the Problem? Odor-control and the Smell of Sweat in Sportswear," *Fashion Practice*, vol. 8, no. 2, pp. 296–317, Jul. 2016, doi: 10.1080/17569370.2016.1215117.
[94] R. M. El-Shishtawy, Y. A. Youssef, N. S. E. Ahmed, and A. A. Mousa, "The use of sodium edate in dyeing: II. Union dyeing of cotton/wool blend with hetero bi-functional reactive dyes," *Dyes and Pigments*, vol. 72, no. 1, pp. 57–65, 2007, doi: 10.1016/j.dyepig.2005.07.017.
[95] A. VinodVelloraThekkaePadil, NhungH.A.Nguyen, AlenaŠevcR, "Fabrication, Characterization, and Antibacterial Properties of Electrospun Membrane Composed of Gum Karaya, Polyvinyl Alcohol, and Silver Nanoparticles," *J Nanomater*, vol. 2015, pp. 1–10, 2014, doi: 10.1155/2015/750726.
[96] V. V. T. Padil, N. H. A. Nguyen, Z. Rozek, A. Ševců, and M. Černík, "Synthesis, fabrication and antibacterial properties of a plasma modified electrospun membrane consisting of gum Kondagogu, dodecenyl succinic anhydride and poly (vinyl alcohol)," *Surf Coat Technol*, vol. 271, pp. 32–38, 2015, doi: 10.1016/j.surfcoat.2015.01.035.
[97] R. I. Palomares, R. E. Navarro, R. H. Urbina, C. I. Palomares, and A. Maldonado, "León, E. R., Tánori, J., and Synthesis of silver nanoparticles using reducing agents obtained from natural sources (Rumex hymenosepalus extracts). Nanoscale. Res. Lett. .," *Nanoscale Res Lett*, vol. 8, pp. 318–326, 2013.
[98] Y. Yang, D. Xu, Q. Wu, and P. Diao, "Cu2O/CuO bilayered composite as a high-efficiency photocathode for photoelectrochemical hydrogen evolution reaction," *Sci Rep*, vol. 6, no. November, pp. 7–14, 2016, doi: 10.1038/srep35158.
[99] X. Gan, Y. Wu, L. Liu, B. Shen, and W. Hu, "Electroless plating of Cu-Ni-P alloy on PET fabrics and effect of plating parameters on the properties of conductive fabrics," *J Alloys Compd*, vol. 455, no. 1–2, pp. 308–313, 2008, doi: 10.1016/j.jallcom.2007.01.054.

[100] Q. He *et al.*, "Electrochemical sensor for rapid and sensitive detection of tryptophan by a cu2o nanoparticles- coated reduced graphene oxide nanocomposite," *Biomolecules*, vol. 9, no. 5, pp. 1–15, 2019, doi: 10.3390/biom9050176.
[101] Y. Zhao, Z. Cai, X. Fu, B. Song, and H. Zhu, "Electrochemical deposition and characterization of copper crystals on polyaniline/poly(ethylene terephthalate) conductive textiles," *Synth Met*, vol. 175, pp. 1–8, 2013, doi: 10.1016/j.synthmet.2013.04.018.
[102] W. J. Studer, A.M.; Limbach, L.K.; van Duc, L.; Krumeich, F.; Athanassiou, E.K.; Gerber, L.C.; Moch, H.; Stark, "Nanoparticle cytotoxicity depends on intracellular solubility: Comparison of stabilized copper metal and degradable copper oxide nanoparticles," *Toxicol. Lett*, vol. 197, pp. 169–174, 2010.
[103] "AATCC Test Method 100 - 2012, Antibacterial Finishes on Textile Materials: Assessment of.".
[104] "AATCC Test Method 147 - 2016, Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method.".
[105] S. ; Karlsson, H.L.; Cronholm, P.; Hedberg, Y.; Tornberg, M.; de Battice, L.; Svedhem and I. O. Wallinder, "Cell membrane damage and protein interaction induced by copper containing nanoparticles—Importance of the metal release process," *Toxicology*, vol. 313, pp. 59–69, 2013.
[106] D. F. and H. X. Zheng M, "Ethylene glycol monolayer protected nanoparticles for eliminating nonspecific binding with biological molecules," *J Am Chem Soc*, vol. 125, pp. 7790–7791, 2003.
[107] R. J. Lemire, J.A.; Harrison, J.J.; Turner, "Antimicrobial activity of metals: Mechanisms, molecular targets and applications," *Nat. Rev. Microbiol*, vol. 11, pp. 371–384, 2013.
[108] M. Solioz, *Copper and Bacteria : Evolution, Homeostasis and Toxicity*. 2018.
[109] J. B. Ballou, "Static electricity in textiles," *Textile Research Journal*, vol. 24, pp. 146–155, 1954.
[110] S. Perera, B. Bhushan, R. Bandara, and G. Rajapakse, "Colloids and Surfaces A : Physicochemical and Engineering Aspects Morphological , antimicrobial , durability , and physical properties of untreated and treated textiles using silver-nanoparticles," *Colloids Surf A Physicochem Eng Asp*, vol. 436, pp. 975–989, 2013, doi: 10.1016/j.colsurfa.2013.08.038.
[111] G. Nikolic, S. Zlatkovic, M. Cakic, S. Cakic, C. Lacnjevac, and Z. Rajic, "Fast fourier transform IR characterization of epoxy GY systems crosslinked with aliphatic and cycloaliphatic EH polyamine adducts," *Sensors*, vol. 10, no. 1, pp. 684–696, 2010.
[112] T. Mondal, A. K. Bhowmick, and R. Krishnamoorti, "Chlorophenyl pendant decorated graphene sheet as a potential antimicrobial agent: synthesis and characterization," *J Mater Chem*, vol. 22, no. 42, pp. 22481–22487, 2012.
[113] C. L. Hoop and R. J. Iuliucci, "C chemical-shift anisotropy of alkyl-substituted aromatic carbon in anthracene derivatives," *Solid State Nucl Magn Reson*, vol. 53, pp. 1–12, 2013, doi: 10.1016/j.ssnmr.2013.01.004.
[114] W. J. Horsley, H. Sternlicht, and J. S. Cohen, "Carbon-13 magnetic resonance studies of amino acids and peptides. II," *J Am Chem Soc*, vol. 92, no. 3, pp. 680–686, 1970.
[115] S. K. Dewan, "Organic Spectroscopy (Nmr , Ir , Mass And Uv)," vol. 22, p. 565, 2011.
[116] Y. Fan, Y. Zhang, K. Yan, and J. Long, "Synthesis of a novel disperse reactive dye involving a versatile bridge group for the sustainable coloration of natural fibers in supercritical carbon dioxide," *Advanced Science*, vol. 6, no. 1, p. 1801368, 2019.
[117] A. Ali, V. Baheti, J. Militky, Z. Khan, and S. Q. Z. Gilani, "Comparative Performance of Copper and Silver Coated Stretchable Fabrics," *Fibers and Polymers*, vol. 19, no. 3, 2018, doi: 10.1007/s12221-018-7917-5.
[118] A. Ali, V. Baheti, J. Militky, "Enhancement in ageing and functional properties of copper-coated fabrics by subsequent electroplating," *Applied Physics A*, vol. 124, no. 9, p. 651, 2018.
[119] Y. Yang, D. Xu, Q. Wu, and P. Diao, "Cu_2O/CuO bilayered composite as a high-efficiency photocathode for photoelectrochemical hydrogen evolution reaction," *Sci Rep*, vol. 6, no. November, pp. 7–14, 2016, doi: 10.1038/srep35158.

[120] Q. He *et al.*, "Electrochemical sensor for rapid and sensitive detection of tryptophan by a Cu_2O nanoparticles- coated reduced graphene oxide nanocomposite," *Biomolecules*, vol. 9, no. 5, pp. 1–15, 2019, doi: 10.3390/biom9050176.
[121] Y. Zhao, Z. Cai, X. Fu, B. Song, and H. Zhu, "Electrochemical deposition and characterization of copper crystals on polyaniline/poly(ethylene terephthalate) conductive textiles," *Synth Met*, vol. 175, pp. 1–8, 2013, doi: 10.1016/j.synthmet.2013.04.018.
[122] P. P. Fu, Q. Xia, H.-M. Hwang, P. C. Ray, and H. Yu, "Mechanisms of nanotoxicity: generation of reactive oxygen species," *J Food Drug Anal*, vol. 22, no. 1, pp. 64–75, 2014.

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List of Publications

1. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, Development of antimicrobial multifunctional Textiles to avoid from Hospital-Acquired Infections' Fibre and polymers, 2021; 22(11): 3055–3067
2. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, Copper-Treated Environmentally Friendly Antipathogenic Cotton Fabric with Modified Reactive Blue 4 Dye to Improve Its Antibacterial and Aesthetic Properties' Coatings, 2022; 13; 33 https://doi.org/10.3390/coatings13010133
3. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, Zuhaib Ahmad, Impact of cuprous based nanoparticles on coronavirus' Science Advance Seditorial Under Review

4. **Muhammad Shahid,** Jakub Wiener and Boris Mahltig, Copper and copper oxide nanoparticles for textile finishing, Nano Con, International Conferences (2014)
5. **Muhammad Shahid**, Azam Ali, Jakub Wiener, Jiri Militky, A comparative performance of phytochemicals, green synthesized silver nanoparticles and green synthesized of copper nanoparticles loaded textiles to avoid from nosocomial infections, Journal of Applied polymer science, under process
6. Azeem, M., Javed, A., Morikawa, H., Noman, M. T., Khan, M. Q., **Muhammad Shahid**., & Wiener, J. (2019). Hydrophilization of Polyester Textiles by Nonthermal Plasma. Autex Res. J, 142-149. 5.
7. Azeem, M., Noman, M. T., **Shahid**, M., Khan, M. Q., & Wiener, J. (2021). Surface Wettability of Vertical Harp for Fog Collection. Polymers.
8. Azam Ali., **Muhammad Shahid**, M. and Jiri Militky., (2020). Copper based viral inhibition. 13th Textile Bioengineering and Informatics Symposium (TBIS). Manchester UK
9. Azeem, M., Wiener, J., Khan, M. Z., Havelka, A., & **Muhammad Shahid**, M. (2017). Hydrophobic Treatment of Nano-Filament Polyester Fabric. 9th Central European Conference, Liberec, Czech Republic.
10. Silver-plated stretchable elastomeric electrodes for electrotherapy applications, Azam Ali, Muhammad Shahid, Nageena Zahid, Muhammad Shahzad Anjam, Tereza Subrova, Jakub Wiener, Jiri Militky, Blanka Tomkova, *journal of industrial textile*, Accepted
11. Micro and Nano-plastics produced from textile finishes: A review, Azam Ali, Muhammad Shahid, Nageena Jakub Wiener, Jiri Militky, Blanka Tomkova, *ACS, Langmuir*, **Minor revision**

Recommendation of Supervisor

Date: 07/05/2024

LETTER OF RECOMMENDATION FOR DEFENSE

I have known Mr. Muhammad Shahid since 2013, when he started his PhD degree under my supervision. Throughout this journey, he showed an outstanding commitment and achievements in his field of work. He efficiently contributed to the theoretical and experimental domains and succeeded in gathering fruitful results on his part of research. He has done a lot of work on his PhD topic **Development of Cuprous Oxide based Antipathogenic Textiles.** He has published about more than 5 research articles and have been participation in some international conferences. Some of his articles related to PhD study are also in process.

He was also attending and actively participating in other educational programs and projects, expressing critical approach, intervening questions and interpretation. He passed his exams with good grades too. I must say he did his research with great responsibility, motivation and honesty.

Mr. Shahid has, in my opinion, prepared the submitted doctoral thesis carefully and in all respects adequately. Only negligible similarities with other texts were detected during the plagiarism check, and even this can be explained, for example, by collaborative publications with other authors. I believe that his work and performance are of significant quality.

I recommend Mr. Muhammad Shahid thesis to be accepted for defense.

Prof. Ing. Jakub Wiener, PhD.

Department of Material Engineering

Technical University of Liberec

Reviews of the Opponents

Opponent's review

Title: Development of Cuprous Oxide based Antipathogenic Textiles

Author: Muhammad Shahid

The presented thesis deals with the study of the antimicrobial properties of cotton fabrics coated with Cuprous oxide, as well as the possibility of using a reactive dye as a carrier of an antimicrobial active component.

Thesis contains 93 pages divided into 6 main chapters and contains 15 tables and 47 pictures and graphs.

In the first part of the thesis, the doctoral student first briefly describes the current issue of antimicrobial textiles esspecially used in medicine. Then author defines the goals of his work. He divided his work into 3 parts, the first is preparing couprous oxide particles using various reducing agents, the second part is applying the thus prepared oxide to cotton fabric, and the third part was binding the reactive dye with triclosan to the treated cotton fabric.

In the literature review, the author devoted himself to the study of current knowledge of the preparation and behavior of antimicrobial, antiviral and antifungal textiles, not only in terms of their properties, but also in terms of usable active substances. In the next part of the research, the author focused on copper compounds, also from the point of view of antimicrobial behavior. The largest part of the conducted literature review is the chapter concerning copper nanoparticles.

The leterature review are written with logical sequence.

In the experimental part, the doctoral student describes the procedures of the preparation of Cuprous oxide from copper sulfate, using three different reducing agents, also the procedure of treatment the oxide to cotton fabric. Next procedure describes the preparation of a functional reactive dye and its fixation on the surface of the cotton substrate. The author did not forget to describe the individual evaluation parameters that he used in his work.

The procedures are described relatively briefly. For some of them, I am missing basic measurement conditions, e.g. for colorimetric measurements, or a diagram of the dyeing procedure, where the exhaustion and fixation part of dyeing would be clearly recognizable. However, the evaluation methods of the prepared antimicrobial fabrics were chosen appropriately.

In the first part of the results, the doctoral student focuses on the evaluation of the preparation of cotton textiles coated with cuprous oxide. The evaluation of the size and distribution of particles using SEM and light scattering technique methods were used. The presence of the oxide was further confirmed by XRD analysis.

The narrowest distribution of particles was achieved by the use of the reducing agent sodium hydrosulfite, which can be agreed with.

The second discussed part of this thesis was the determination of the antimicrobial activity of the prepared cotton fabrics. Not only for standard gram-positive and gram-negative bacteria, but also for viruses and fungi. The author monitored the effect of the amount of reducing agent used on these properties.

The best results of antimicrobial behavior were again achieved by the reducing agent sodium hydrosulfite, although the air premeability decreased with increasing concentration of the agent (in contrast to the other reducing agents, where it remained constant).

The third discussed part of the thesis was the preparation of a functional textile material with a textile reactive dye. The effect of the three reducing agents used on the observed parameters was evaluated.

The use of the antimicrobial product triclosan bound by means of a reactive group to the textile substrate has better results than the use of cuprous oxide according to the presented results. However, the increase is not significant.

Formally, the thesis is written at a good language level. Unfortunately, the work contain typing errors and missing parts, e.g. in figure 34, some regression equations in the graphs are missing. Standard deviations are also graphically displayed for some evaluation properties, but there is no information about the number of measurements.

The thesis presents interesting results of antimicrobial treatments on the possibility of using cuprous oxide as well as the use of a reactive dye as a carrier of the active ingredient.

The doctoral student demonstrated independent scientific work. According to the attached list of publications, he published the results of his thesis in 3 articles (one under review) and 1 conference. He is also the co-author of 3 other articles, 1 of which has already been published.

My questions:

1. How does the author explain the reduction of air permeability with increasing of concentration of sodium hydrosulfite?
2. How many wash cycles were used? What temperature was used?
3. I miss the application of a reactive dye with triclosan directly on cotton fabric not treated with cuprous oxide, for a comparison of the antimicrobial behavior of a fabric prepared in this way with a fabric treated only with cuprous oxide. It would confirm or disprove a synergistic antimicrobial effect. Has this option been studied?

In conclusion, **I recommend** this thesis for the defense.

Ing. Michal Černý, Ph.D. **In Pardubice on May 3, 2024**

Referee´s report
on PhD. thesis of
Muhammad Shahid

"Development of Cuprous Oxide Based Antipathogenic Textiles"

by
Doc. Ing. Stanislav Petrík,CSc.

The presented thesis consists of 109 pages divided into 5 chapters. The main theme of the thesis was to investigate preparation properties and selected applications of bioactive textiles having the antimicrobial ability (antiviral, antifungal) and durability. The thesis main objectives were the development of an environmentally friendly, low-cost, easy and fast method for synthesis of cuprous oxide particles. Subsequently, a novel approach for the development of cuprous oxide-coated antibacterial cotton fabric with excellent aesthetic appearance was investigated in detail. This thesis is focused on the copper deposition on cotton fabric and subsequently dyeing it with antimicrobial dye (modified reactive blue 4 dye). The studied properties and applications of bioactive textiles were bioactive (antibacterial qualitative antibacterial quantitative, antiviral, antifungal), comfort parameters, dye exhaustion, fixation, and levelness.

The thesis has standard parts - Introduction, Thesis objectives, Literature review, Methodology, Results and discussions, Conclusions and Future works. In the end, there are References and a List of author´s publications. The author´s record of publications is very good.

First pages

Before the introduction, there are Contents, List of tables, List of figures, List of abbreviations. These lists contain almost all type of contents complete.

Thesis significance, scope and objectives

The author made here an introduction to his work and explained the objectives of the study. The work was split into these major parts: Formation of Cu_2O particles by the Fehling solution method, Deposition of cuprous oxide particles on woven cotton fabric, Modification of reactive dye as antipathogenic, and dyeing the already cuprous oxide-coated fabrics with antibacterial dye. These all topics are coinciding with each other makes a unification bridge and make a sense.

Literature review

There is a sufficient number of literature sources divided into twelve parts. Even the whole chapter is well written, only some parts and Figures are questionable. The labelling of all figures is very clear. Both Figure 1 and Figure 2 are showing the interaction of copper particles with bacterial cell, only one figure was enough. In literature, author mostly provided the antibacterial effect, while the thesis work contains all antipathogenic (antiviral, antifungal and anti-bacterial) properties. So, it was better if these should refer to more studies related to anti-fungal or antiviral textiles. I think the literature part well explained previous work and the most critical literature findings are related to the topic. But it should not repeat figures published previously (at least in such quantity).

Methodology

The Fehling solution method was applied, with three different reducing agents for the preparation of Cu_2O particles, subsequently coating over fabric. The importance of Cu_2O as compared to other copper ions is described well regarding the antipathogenic properties.

There focussed methodology was adopted for the preparation, characterization and application of the fabrics. Two different types of bioactive textile materials were prepared – coated with Cu_2O particles on textiles, against three different reducing agents; second substrate focused on the copper deposition on cotton fabric and subsequently dyeing it with antimicrobial dye (modified reactive blue 4 dye).

There are other methods of testing involved like surface testing, tests related to dye modification and its properties, antipathogenic testing and durability. I suppose all these methods are essential for the final topic of the thesis.

Results and discussions

The chapter summarizes all results of the thesis. For each bioactive textile, several measurements were performed – antibacterial, antiviral, antifungal, SEM analysis, mechanisms of attachment, and durability of fabrics. Error bars might be effective for Fig. 35 and Fig. 36.

The scale on SEM images in Figure 37 is not readable.

Conclusions

This chapter summarizes the findings of the previous chapters. The author presents interesting results of experimental work in the field of aesthetic bioactive fabric. The doctoral student demonstrated good scientific work and published his results sufficiently. The conclusions from the study are well explained.

Referee remarks, question and conclusions

1. What does the diffraction peak (222) in Fig. 27 represent as it does not belong to Cu_2O.
2. Explain the reason why the cuprous oxide particles reduced by glucose are larger than those reduced by ascorbic acid and sodium hydrosulphite.
3. In deposition of Cu_2O particles on cotton fabric process, the fabric was pre-treated with citric acid. What kind of reaction proceeds in this process? What about transformation ratio of citric acid labeled sugar unit of fabrics?
4. How about the stability of Cu_2O layer? Does it transfer into CuO?

Referee´s conclusion

The presented thesis of Muhammad Shahid has all the formal parts and shows the author is able to carry out scientific work. The author also showed good understanding of chemical processes applied in the thesis. The language is acceptable and entirely understandable.

The thesis meets the criteria, I recommend it to be taken to the defense.

In Liberec on April 9, 2024

doc. Ing. Stanislav Petrík, CSc.