Breathability performance of antiviral cloth masks treated with silver nanoparticles for protection against COVID-19

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Abstract
The global widespread of coronavirus disease 2019 (COVID-19) has caused shortage of medical face masks and led to developing of various types of cloth masks with different levels of protection and comfort to meet the market demands. Breathing comfort is a significant aspect that should be considered during the design of cloth masks along with the filtration efficiency; otherwise, the wearer will feel suffocated. In this work, different types of cotton and polyester knitted fabrics blended with spandex yarns were produced and treated with silver nanoparticles to be used as antiviral cloth masks. Scanning electron microscope, transmission electron microscope, and EDX were used to characterize the silver nanoparticles (AgNPs). Antiviral activity was assessed against SARS-CoV-2 coronavirus as well. The influence of using different fabric materials, number of layers, and hybrid layers on their air permeability and breathability were investigated to evaluate the comfortability of the cloth masks. Physiological impacts of wearing the cloth masks were evaluated by measuring oxygen saturation of hemoglobin and heart rate of the wearers while doing various activities. The results indicated that AgNPs have low cytotoxicity and considerable efficiency in inhibition of SARS-CoV-2. Adding spandex yarns with different count and ratios reduced the porosity and air permeability of the fabrics. Moreover, the combination of three hybrid layers’ mask made of polyester fabric in the outer layer with

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100% cotton fabric in the inner layer showed high comfortability associated with high air permeability and breathability. Also, wearing these masks while doing activities showed no significant effect on blood oxygen saturation and heart rate of the wearers.

Keywords
COVID-19, SARS-CoV-2, cloth face mask, cotton, silver nanoparticles, breathability, blood oxygen saturation

Introduction
The emergence of new pathogenic microbes in the last decades had caused severe negative impacts on human health and economic stability of many countries all over the world. HIV/AIDS, SARS, Hepatitis C, Ebola, and H1N1 influenza are few examples of the emerging infectious diseases that had appeared recently.1,2 In March 2020, the World Health Organization’s director general has declared the COVID-19 outbreak a global pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).3 According to the WHO’s operational report on February first, 2021 the pandemic has caused 102,399,513 infected cases and 2,217,005 deaths globally.4 The rapid geographical spread of coronavirus among people caused by several reasons, namely, physical contact between infected and uninfected individuals, respiratory droplets generated from infected people during talking, sneezing and coughing, and small droplets from infected individuals that are circulated by air currents and inhaled by uninfected persons.5,6 SARS-CoV-2 virus diameter ranges between 60 and 140 nm, and it is smaller than bacteria, dust, and pollen; thus, the infectious respiratory aerosols of diameter ≤5 nm may play a key role in spreading the virus.7,8 The WHO had recommended several prevention actions and hygiene practices for reducing the transmission of the virus including physical distancing, wearing face masks, eye protection, and disinfection of surfaces that are regularly touched such as doorknobs, switches, and tabletops.3,5,9

Face masks can provide protection from transmission of airborne particles, droplets, pathogens, and body fluids by filtering them from the breathable air.10,11 Nevertheless, face masks may allow air and microorganisms leakages and may cause choking sensation. Also, they are costly and not environmentally friendly under the current crisis of coronavirus pandemic.12 Chu et al.13 had carried out 172 observational studies across 16 countries, and found that using face masks and eye protection and keeping physical distance had decreased SARS-CoV-2 virus transmission between individuals. According to ASTM F2100 and EN 14683 standards, the performance requirements of the materials used in medical face masks are based on testing bacterial filtration efficiency (BFE), particulate filtration efficiency (PFE), fluid resistance, differential pressure (breathability), and flammability.11,14 Surgical face masks and N95 respirators are the most common types that have been used to prevent SARS-CoV-2 virus transmission. Many countries mandated wearing face masks in public areas to decrease the infection rate, which leads to high demands for the medical face masks causing shortage of masks for the healthcare
workers. Consequently, researchers and manufacturers had worked on developing reusable face masks to meet the market needs. Also, many people have resorted to make their household masks, recycling used masks, or using masks that offer less protection. In this context, research work has been done to enhance the performance afforded by cloth face masks through finding new materials with sufficient filtration efficiency, breathability, and comfort, in addition to imparting new properties such as hydrophobicity, antimicrobial, and antiviral properties.

Hydrophobic material is needed in face masks, as the moist environment is well-known for its support to the growth of bacteria, dermal infection, and unpleasant odor which may cause health issues to the wearers when used for long periods of time. Air permeability and breathability are significant properties that provide comfort to the persons wearing face masks. Increasing the mask porosity will increase the air permeability but will decrease the protection level since the infectious aerosols can easily pass through the fabric openings. As well as, higher values of differential pressure (breathability resistance) indicate that the mask is hard to breathe through and lower values indicate that the breathing effort is normal. Cloth face masks can be made using woven or knitted fabrics, as knitted fabrics are characterized by their excellent comfort properties, they possess high extensibility allowing a comfortable fit on the user’s face. Several materials can be used in producing cloth masks such as cotton, chiffon, silk, synthetics, or a combination of them which can increase their filtration efficiency. Davies et al. found that surgical and household cotton masks greatly reduced the expelled bacterial and viral aerosols from personal coughs. Disposable face masks cannot block all pathogens and do not kill them, which makes the discarded mask a route for the disease as the pathogens multiply in the mask fibers. Accordingly, utilizing anti-pathogens methods based on chemical treatment of the material surface have been studied to enhance the masks with a biocide effect.

Metal nanoparticles such as silver nanoparticles (AgNPs) are extensively used in various biomedical and textile applications, as they offer high antibacterial and antifungal activities against pathogens due to the inhibition of respiratory enzymes through releasing the Ag⁺ ions. They intercalate into the bacterial DNA once entering the cell and inhibit the proliferation of the pathogen. Also, AgNPs are environmentally friendly and nontoxic to humans. Several in vitro and in vivo studies concerned with investigating the cytotoxicity of AgNPs. Hiragond et al. found that soaking face masks in a colloidal solution of starch-capped silver nanoparticles showed effective inhabitation to the growth of Staphylococcus and Escherichia bacteria. Silver nanoparticles had revealed to have an antiviral activity through penetrating the cell membrane of the virus, and interacting with the viral genome to prevent the cell from replicating. The efficiency of AgNPs against influenza A, human immunodeficiency virus-1 (HIV-1), monkeypox, influenza A virus subtype H1N1 (H1N1), and hepatitis B viruses have been investigated recently. It was observed that the size of the nanoparticles exhibited a significant effect on their antiviral activity. Also, AgNPs possess potent antiviral activity against feline coronavirus (FCoV), adenovirus, herpes simplex virus, norovirus, bovine herpesvirus, human parainfluenza virus type 3 (HPIV3), and Middle East respiratory syndrome coronavirus (MERS-CoV). Cloth masks treated with silver ions could offer a
potent perception tool against viral infection, where the virus can be destroyed by the following methods; the metal nanoparticles create a free-radical like-OH ion which is toxic to the virus’s cell, the metal combines with N, O, and S in the virus’s cell to damage it by chelation, or the metal damages the electron transport chain because silver belongs to the transition element having d-block.20

On the other hand, the physiological effects of wearing face masks during doing various activities had been considered recently. During respiration, gases exchange occur where oxygen is inhaled and carbon dioxide exhaled.35,36 While wearing the face mask, there is a possibility that a great amount of carbon dioxide is inhaled as a result of being trapped between the face and the mask, causing hypercapnia hypoxia due to the increase in arterial carbon dioxide that displaces oxygen from hemoglobin.9,37–39 Pulse oximeter device is used to monitor the arterial blood oxygen saturation level of hemoglobin (SpO2) and it is based on photoplethysmography. It measures the SpO2 (%) through the difference in the absorption spectrum of oxyhemoglobin and deoxyhemoglobin in the visible and infrared spectral regions and also, it measures the heart pulse rate.40 Several researches have studied the physiological effects of wearing surgical face masks during work in terms of their effect on SpO2, heart rate, and CO2 levels.9,35,37,41–45

It worth mentioning here, that the breathability performance is considered one of the most important aspects that the ASTM and ISO standards referred to, as it may cause serious problem to the wearer. Several publications in face masks were ignoring this test, and here, we are focusing on the breathability performance of cloth masks and its effect on the oxygen saturation percentage and heart rate in order to raise the awareness and the importance of the breathability of face masks. So, the aim of the present work is to produce cloth face masks made from different combinations of cotton and polyester knitted fabrics treated with silver nanoparticles to be used as an antiviral face mask during COVID-19 pandemic. The prepared AgNPs were characterized using SEM, TEM, and EDX, and their antiviral activity was assessed against SARS-CoV-2 virus. K/S and color fastness properties of the treated dyed fabrics were determined. Fabric porosity was measured using optical image analysis. The effect of using different fabric materials, number of layers, and hybrid layers on the air permeability and breathability of the cloth masks was investigated to evaluate their comfortability. Also, the physiological impacts of wearing the proposed face masks were evaluated through measuring the oxygen saturation of hemoglobin (SpO2%) and heart rate of the wearers during doing normal activities such as performing office work and walking.

Materials and methods

Materials

Fabrics. Weft knitted fabrics are characterized by their high extensibility under low load allowing comfortable fit on any part of the body, so these fabrics can adapt easily with face movement during wearing the masks. Therefore, in this work different types of cotton and polyester weft knitted fabrics were used in the study. The fabrics were produced with single jersey structure on circular knitting machine (SINTELLI, Fujian, China). The
fabric and the machine specifications are; 13 inches’ diameter, machine gauge is 28 needle/inch, and the machine is equipped with 38 feeders for cotton yarn and 38 feeders for elastomer yarn. Cotton yarns of count 30/1 Ne, polyester yarns of count 30/1 Ne, and spandex yarns of counts 20 and 70 Denier were used in manufacturing of the fabrics. Half and full spandex’s yarns feeders were used to fabricate the stretchy cotton and polyester samples. The percentage of cotton/polyester with spandex yarn of 20D ranges between 2 and 4%, while with using count 70D it ranges between 6 and 12%. Table 1 lists the specifications of the produced knitted fabric samples.

### Chemicals

Silver nitrate (AgNO₃) was purchased from Fisher scientific and trisodium citrate (Na₃C₆H₅O₇) was purchased from Aldrich chemical. C.I Reactive Green and C.I. Disperse Blue 56 dyes were supplied from some private sector dye house companies. Sodium chloride, sodium carbonate, acetic acid, hydrosulfite, sodium hydroxide, and Ciba Pone R detergent were purchased from domestic chemical company.

### Methods

**Preparation of silver nanoparticles.** Waste cotton yarns were used as a reducing agent in the preparation of silver nanoparticles in order to carry out the in situ synthesis of the nanoparticles. No surfactant was used during the AgNPs preparation. In this method, 250 g of weft knitted cotton fabric was immersed in 15 L of 0.2 g/L silver nitrate solution with 1:50 liquor ratio at room temperature. An aqueous solution of 22.5 g in 200 mL water

| Code | Material | Spandex count (den.) | Spandex feeders | Wales/cm | Courses/cm | Loop length (mm) | Stitch density (cm²) | Basis weight (g/m²) |
|------|----------|----------------------|-----------------|----------|------------|------------------|---------------------|-------------------|
| S1   | Cotton 100% | 20 | Half | 16 | 23 | 2.92 | 368 | 224.3 |
| S2   | Cotton/spandex | 20 | Full | 17 | 28 | 2.80 | 476 | 308.7 |
| S3   | Cotton/spandex | 70 | Full | 18 | 31 | 2.9 | 558 | 381.7 |
| S4   | Cotton/spandex | 20 | Half | 17 | 23 | 2.68 | 391 | 206.7 |
| S5   | Cotton/spandex | 20 | Full | 18 | 28 | 2.61 | 504 | 279.7 |
| S6   | PET/spandex | 70 | Full | 18 | 31 | 2.78 | 589 | 385.7 |
| S7   | PET/spandex | 20 | Half | 17 | 28 | 2.84 | 476 | 269 |
| S8   | PET/spandex | 20 | Full | 18 | 28 | 2.61 | 504 | 279.7 |
| S9   | PET/spandex | 70 | Half | 17 | 28 | 2.84 | 476 | 269 |
of trisodium citrate was added drop wise to the mixture, then the temperature was raised up to 100°C and was kept at 100°C for further 45 min; afterward, the solution was kept to cool down to room temperature, and the obtained brown solution of 200 ppm AgNPs was collected and then the fabric was rinsed with tap water and hot air dried (Table 2).46,47

**Treatment of cotton and polyester fabrics with AgNPs.** A colloidal solution of AgNPs with a concentration of 100 ppm was used at room temperature (25°C). The polyester and cotton fabric samples were soaked in the colloidal solution of AgNPs for 30 s followed by squeezing to 90% wet pick-up using a laboratory padder at constant pressure. The samples were dried at 70°C and cured at 150°C for 2 min.48,49

**Dyeing of the treated cotton fabrics.** Cotton fabrics were dyed with reactive green dye at 1% shade (owf), with liquor ratio 1:20, the dye and NaCl 30 g/L were dissolved at room temperature, the temperature was slowly raised to 60°C, then after 15 min 15 g/L sodium carbonate was added and dying was continued for further 60 min. After though, the dyed cotton samples were rinsed and neutralized by acetic acid (1 g/L) and soaped using (Ciba Pone R) detergent followed by hot rinsing and air drying. Finally, the dyed samples were dried and assessed for color strength and overall fastness properties.50

**Dyeing of the treated polyester fabrics.** The polyester fabrics were dyed using high temperature dyeing process. C.I. Disperse Blue 56 dye was used for dyeing the fabrics. Two grams of the fabric samples was introduced in IR dyeing machine’s cup containing 1% shade (owf) dye with 1:50 liquor ratio, the pH was adjusted at 4–5 and the temperature was raised and kept at 130°C for 60 min. At the end of the dyeing process, the temperature was released to cool down and the dyed samples were removed, dyed samples were removed, rinsed in warm water and treated in 1:40 liquor ratio of a solution containing 2 g/L sodium hydrosulftite and 2 g/L sodium hydroxide for 10 min at 60°C, then the samples were neutralized at 40°C for 5 min in a solution of 1 g/L acetic acid., then the dyed samples were removed, rinsed with tap water and allowed to dry at room temperature for further testing.51

**Analysis and testing**

**Characterization of the AgNPs.** The morphological features of the cotton and polyester fabric samples treated with silver nanoparticles and their elemental composition were
studied using scanning electron microscope (SEM) (TESCAN-VEGA 3, Czech Republic) with 30 kv scanning voltages coupled with an energy dispersive X-rays (EDX) analyzer unit. Transmission electron microscope (TEM) (JEOL-JEM-1200, Japan) was used to determine the size and shape of the synthesized silver nanoparticles.

**MTT cytotoxicity assay for the AgNPs.** To assess the half maximal cytotoxic concentration (CC$_{50}$), stock solutions of the test compounds were prepared in 10% dimethyl sulfoxide (DMSO) in double-distilled water (ddH$_2$O), and diluted further to the working solutions with Dulbecco’s Modified Eagle’s Medium (DMEM). The cytotoxic activity of the extracts was tested in VERO-E6 cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with minor modifications. Briefly, the cells were seeded in 96 well plates (100 μL/well at a density of $3 \times 10^5$ cells/ml), and incubated for 24 h at 37°C in 5% CO$_2$. After 24 h, the cells were treated with various concentrations of the treated compounds in triplicates. 24 h later, the supernatant was discarded and cell monolayers were washed with sterile 1× phosphate buffer saline (PBS) 3 times, and MTT solution (20 μL of 5 mg/mL stock solution) was added to each well and incubated at 37°C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 μL of acidified isopropanol (0.04 M HCL in absolute isopropanol = 0.073 mL HCL in 50 mL isopropanol). Absorbance of formazan solutions was measured at $\lambda_{max}$ 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (TC$_{50}$)$^{52}$

\[
\text{Cytotoxicity(%) = } 100 \times \frac{\text{(Absorbance of cells without treatment} - \text{Absorbance of cells with treatment})}{\text{Absorbance of cells without treatment}}
\]  

(1)

**Inhibitory concentration 50 (IC$_{50}$) determination for the AgNPs.** In 96-well tissue culture plates, $2.4 \times 10^4$ VERO-E6 cells were distributed in each well and incubated overnight in a humidified 37°C incubator under 5% CO$_2$ condition. The cell monolayers were then washed once with 1× PBS and subjected to virus adsorption (hCoV-19/Egypt/NRC-03/2020 (Accession Number on GSAID: EPI-ISL-430820)) for 1 h at room temperature (RT). The cell monolayers were further overlaid with 50 μL of DMEM containing varying concentrations of the test sample. Following incubation at 37°C in 5% CO$_2$ incubator for 72 h, the cells were fixed with 100 μL of 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet in distilled water for 15 min RT. The crystal violet dye was then dissolved using 100 μL absolute methanol per well and the optical density of the color is measured at 570 nm using Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC$_{50}$ of the compound is that required to reduce the virus-induced cytopathic effect (CPE) by 50%, relative to the virus control. The obtained results from this test present the minimum amount needed to keep the cell alive and to be used for the antiviral activity test. The graph will be drawn to present the cell viability (%) vs the concentration in volume (μL/mL).
Color measurement of the treated dyed fabrics. UltrascanPro (HunterLab) Spectrophotometer was used in the color measurements of the treated dyed fabrics. The geometry of this instrument is d/8° reflectance and the Port Size/Measured Area in RSIN/RSEX reflectance modes are as follows:

- Large Area View (LAV): 25 mm (1 in) illuminated/19 mm (0.75 in) measured.
- Medium Area View (MAV): 13 mm (0.5 in) illuminated/9 mm (0.35 in) measured.
- Small Area View (SAV): 7 mm (0.25 in) illuminated/4 mm (0.16 in) measured.

All the measurements were occurred at 625 nm wavelength. The corresponding color strength value (K/S) was evaluated by applying the KubelkaMunk equation\(^5^3\) as follows

\[
\frac{K}{S} = \frac{(1 - R)^2}{2R} - \frac{(1 - R_0)^2}{2R_0}
\]

(2)

where \(R\) is the decimal fraction of the reflectance of the dyed fabric, \(R_0\) is the decimal fraction of the reflectance of the undyed fabric, \(K\) is the absorption coefficient, and \(S\) is the scattering coefficient.\(^5^4\)

CIE lab difference. The color measurements of the treated fabrics were tested according to the CIE (L*, a*, b*) system to evaluate the color coordinates, where the L* value refers to lightness/darkness from 100 to 0 representing white to black, a* values applied from green (negative) to red (positive), while the b* values from negative (blue) to positive (yellow). The fabric specimen is folded twice to prevent the penetration of incident light. Measurements were recorded at three different positions for each dyed fabric and their average was the final K/S value.

Color fastness. The treated fabrics were tested after washing-off using 3 g/L non-ionic detergent (Hostapal CV) at 60°C for 30 min, according to ISO standard methods. The specific tests were ISO 105-X12: 2016 for color fastness to rubbing; ISO 105-C10:2006 for color fastness to washing; and ISO 105-E04:1994 for color fastness to perspiration. The light fastness was tested on the treated fabrics using samples of size 10×10 cm mounted on a white chart paper and irradiated using a XENOTEST 1200 apparatus at a relative air humidity of 65% and 50°C with duration 4 h.

Fabric porosity. Fabric porosity is the percentage of the voids space to a given fabric volume. The fabrics porosity percentage was determined using digital image analysis technique. The image of one layer of each type of the knitted fabrics was captured using Stereozoom microscope (Optika SZN-6, Italy) with achromatic zoom 0.67×–4.5×. The images were taken at zoom 0.7×. ImageJ as an image processing and analysis software inspired by NIH image was used for analyzing the fabric images to determine the porosity in each fabric sample. The fabric images were saved in gray scale and then converted to black and white monochromatic images in order to determine the porosity (%) through calculating each color pixels’ ratio.
**Cloth mask samples testing protocol**

The comfortability performance of treated fabric samples was evaluated in terms of their air permeability and breathability properties. Air permeability test is performed as a primary test to evaluate the performance of the fabric samples in order to determine the right samples to be suitable for the breathability test with respect to EN 14683:2019 standard.

**Air permeability.** Air permeability is the measure of the air flow rate that passes perpendicularly through a given area of fabric. The fabrics air permeability was determined according to ASTM D-737–18. The test was carried out on Toyoseiki device model no 869 Permeameter. The test was carried out on 1, 2, and 3 layers of fabric samples (Table 3).

**Breathability (differential pressure).** The differential pressure test is an indicator of the breathability comfort for the face mask user. The breathability is measured by the differential pressure between the two sides of the mask as the air flows through them in a rate similar to breathing. The used apparatus was designed according to the standard EN 14683:2019-Annex C14 (Figure 1). An electric vacuum pump was used to draw air through both sides of a measured surface area of the test specimen at a constant flow rate 8 L/min which corresponds to the normal breathing rate and air velocity of 27.2 cm/s according to EN 14683:2019. A differential manometer was used to measure the air pressure difference and obtained the results directly. The differential pressure (\(\Delta P\)) of the tested fabrics was calculated according to the following equation

\[
\Delta P = \frac{(X_1 - X_2)}{4.9}
\]  

(3)  

where \(\Delta P\) is the differential pressure per cm\(^2\) of the tested specimen expressed in (Pa/cm\(^2\)), \(X_1\) is the low side pressure of the test specimen in Pa, \(X_2\) is the high pressure side of the test specimen in Pa, and 4.9 is the area of the test specimen in cm\(^2\).

The test protocol was setup to conduct the test on 1, 2, and 3 layers of fabric samples (Table 3). As the breathability test is the major test to meet the requirements by the EN 14683:2019 standard, all the samples that did not meet the standard for type I, II, and IIR masks in 1 layer test were discarded from the following tests. Then the 2 layers’ test was conducted on 2 layers of the same samples and on 2 hybrid layers of different samples specifications. After performing the 2 layers’ test, all the 2 layers’ samples that did not meet the requirement of the EN 14683 standard were discarded (Table 3). Then, to provide a higher level of protection and adequate level of breathability, a 3 hybrid layers’ samples were prepared using the breathable 2 layers’ samples with adding one more layer and then tested for their air permeability and breathability.

From the results of the 3 layers’ test, high breathability hybrid samples that meet the standard were chosen to be tested for their physiological impacts on the wearers. Blood oxygen saturation (SpO\(_2\)%) and heart rate (HR) were evaluated on the users during doing various activities such as performing of office work and walking with a medium activity...
which corresponds to 5 km/h speed.\textsuperscript{55} Table 3 illustrates the protocol of testing the performance of fabric samples.

| No. of layers | Samples code | Air permeability test | Breathability test | Discarded samples | Physiological tests |
|---------------|--------------|----------------------|--------------------|-------------------|--------------------|
| 1             | S1           | √                    | √                  | —                 | —                  |
| 1             | S2           | √                    | √                  | —                 | —                  |
| 1             | S3           | √                    | √                  | —                 | —                  |
| 1             | S4           | √                    | √                  | √                 | —                  |
| 1             | S5           | √                    | √                  | —                 | —                  |
| 1             | S6           | √                    | √                  | —                 | —                  |
| 1             | S7           | √                    | √                  | —                 | —                  |
| 1             | S8           | √                    | √                  | —                 | —                  |
| 1             | S9           | √                    | √                  | —                 | —                  |
| 2             | S1/S1        | √                    | √                  | —                 | —                  |
| 2             | S2/S2        | √                    | √                  | —                 | —                  |
| 2             | S6/S6        | √                    | √                  | —                 | —                  |
| 2             | S7/S7        | √                    | √                  | —                 | —                  |
| 2             | S8/S8        | √                    | √                  | —                 | —                  |
| 2             | S2/S6        | √                    | √                  | —                 | —                  |
| 2             | S1/S2        | √                    | √                  | —                 | —                  |
| 2             | S7/S1        | √                    | √                  | —                 | —                  |
| 2             | S8/S1        | √                    | √                  | —                 | —                  |
| 3             | S8/S8/S1     | √                    | √                  | —                 | √                  |
| 3             | S7/S1/S1     | √                    | √                  | —                 | —                  |
| 3             | S6/S6/S1     | √                    | √                  | —                 | √                  |
| 3             | S6/S1/S1     | √                    | √                  | —                 | √                  |
| 3             | S8/S1/S1     | √                    | √                  | —                 | —                  |
| 3             | S1/S1/S1     | √                    | √                  | —                 | —                  |
| 3             | S8/S8/S1     | √                    | √                  | —                 | —                  |

\textit{Evaluation of the physiological impacts of wearing the produced cloth face masks.} Based on the breathability test results, three fabric combinations were chosen to produce the masks, each one composed of three fabric layers treated with AgNPs. The inner layer of the mask is made from cotton fabric to offer comfort and absorb moisture, while the outer layer is made from polyester fabric which is a naturally hydrophobic material to block any droplets, while the middle layer is made either from cotton or polyester fabrics based on the test results (Figure 2). The commercial surgical face mask was also tested for the sake of the comparison with our developed cloth masks. Members of the work team involved in the study work wore the proposed cloth masks to perform the physiological evaluation. This physiological evaluation was carried out using Pulse oximeter device type (CONTEC CMS50D, China) to monitor the arterial blood oxygen saturation of
hemoglobin (SpO₂) and the heart rate (HR) under various conditions such as performing of office work and walking with medium activity. The readings were recorded with and without wearing the cloth face mask at different time intervals (0, 10, 20, 30, 40, 50, and 60) minutes to determine whether wearing these masks affected the oxygenation level (SpO₂%) or not over the specified time.

**Results and discussion**

**Mechanism of in situ AgNPs incorporation into fabric matrix**

Sodium citrate as a reducing and stabilizing agent has been previously reported in the preparation of metal nanoparticles, especially preparation of silver nanoparticles. Excess amount of trisodium citrate controls the particle growth of the Ag particles and form a stable nano-silver colloid. Adding silver nitrate to the fabric, generate an ion exchange interaction and/or complexation between the chelating (COOH and OH groups) of cotton and silver ions. After sodium citrate addition, citrates are oxidized to acetone carboxylate and Ag⁺ is in turn reduced to Ag⁰ nanoparticles at high temperature. Then, both of citrate and building units of fabrics acts as stabilizer for the formed nanoparticles as they both chelate the NPs (i.e., Citrate–AgNPs–Fabrics). Accordingly, nanoclusters grow up and avoiding of aggregation could be resulted from chemical coordination between AgNPs and fibers building units (OH/COOH group) of cotton.⁵⁶–⁶⁴

**Characterization of the AgNPs**

The surface morphology of the cotton and polyester samples treated with silver nanoparticles is shown in Figures 3 and 4, respectively. It can be observed that the cotton and polyester fabrics are covered with the silver nanoparticles. TEM image in Figure 5(a) depicts that the AgNPs are irregular in shape and some are spherical and semi-spherical.
The selective area electron diffraction (SAED) pattern of single silver nanoparticle is shown in Figure 5(a) confirming the crystallinity of the silver nanoparticles. Figure 5(b) reveals that the AgNPs have an average particle size ranging between 6 and 70 nm with a major diameter ranging between 10 and 20 nm.

The elemental composition analyzed by EDX for cotton and polyester samples treated with AgNPs is shown in Figures 6 and 7, respectively. It is clear from the EDX spectra in both figures the presence of Ag metal peaks at 3 KeV which confirm the presence of silver nanoparticles in the samples, as silver nanoparticles generally show typical optical absorption peak approximately at 3 KeV due to their surface plasmon resonance.55

MTT cytotoxicity assay (TC50) and antiviral activity of AgNPs against SARS-CoV-2

The prepared AgNPs cytotoxicity and antiviral activity against SARS-CoV-2 virus replication efficiency in vitro in VERO-E6 cell culture were examined and the results are illustrated in Figure 8(a) and (b). The AgNPs’ cytotoxic effect was determined by the cell viability (%) which is the percentage of cells survived after applying an antiviral agent. Figure 8(a) indicates that the AgNPs cytotoxic concentration (CC50) is 142.5 μL/mL, which revealed that the prepared silver nanoparticles have low toxic effect. So, it will be safe while used in face masks. Due to the less toxic effect of the prepared AgNPs, the amount needed of AgNPs to have a 21.49% virus inhibition is 25 μL/mL. The relation between virus inhibition (%) and effective AgNPs concentration is illustrated in Table 4.

The AgNPs were effective in inhibiting extracellular of SARS-CoV-2 virus, this could be attributed to the smaller size of the silver nanoparticles which have a strong activity, due to the large specific surface area that regulate the oxidation stress and dissolution rate of silver nanoparticles into ions depending on the interfacial interaction.65,66 As well, the cell uptake of silver nanoparticles is highly depending on the size of the nanoparticles which results in different concentrations of the silver nanoparticles inside the cells.67 Silver nanoparticles of size around ≤10 nm have an antiviral effect; this was confirmed in a previous study done by Jeremiah et al.,67 who found that the antiviral effect of AgNPs against SARS-CoV-2 was observed with particles of size ranges from 2 to 15 nm. The AgNPs exert their antiviral effect on SARS-CoV-2 through binding to the viral surface.
Figure 3. SEM images for the cotton fabric treated with AgNPs at (a) 500×, (b) 2500×, and (c) 5000× magnifications.

Figure 4. SEM images for the polyester fabric treated with AgNPs at (a) 500×, (b) 2500×, and (c) 5000× magnifications.

Figure 5. TEM images: (a) Silver nanoparticles and (inset) shows selected area electron diffraction (SAED) pattern of AgNPs and (b) nanoparticles size distribution histogram.
Figure 6. EDX spectrum of the cotton sample treated fabric with AgNPs.
Figure 7. EDX spectrum of the polyester sample treated fabric with AgNPs.
proteins rich in sulfhydryl groups, and cleave the disulfide bonds on the spike protein and ACE2 receptors, destabilizing the protein and thereby affecting the viral infectivity.68–70

Colorimetric analysis of the treated fabrics

The coloration properties are among the main aesthetics properties of fabrics. In this study, the influence of AgNPs treatment and fabric dyeing on their coloration properties was examined and the results are presented in Tables 5 and 6, respectively. Table 5 shows the properties of the dyed cotton and polyester fabrics using disperse and reactive dyes. Colorimetric values show that both cotton and polyester fabric exhibited deep, brilliant, and homogeneous color yields with good K/S of 7.84 and 15.56, respectively, as indicated in Table 5.

The fastness properties of the dyed fabrics are presented in Table 6. To assess the fastness properties of the dyed fabrics, a rating scale of 1 (poor) to 5 (excellent) was used, while light fastness was tested after 4 h irradiation of the dyed samples using XENOTEST 1200 apparatus at a relative air humidity of 65°C and 50°C. It can be noticed from Table 6 that the dyed cotton and polyester fabrics show a very good to excellent fastness.
properties to rubbing, washing, acid, and alkali perspiration ranging from 4 to 5. On the other hand, light fastness with value 6 indicating a long term and very good fading stability and light resistance.

Table 6. Fastness properties of the fabrics.

| Fabric type | Washing | Rubbing | Perspiration |
|-------------|---------|---------|--------------|
|             | St.*    | St.**   | Alt | Dry | Wet | St.* | St.** | Alt | Light |
| Cotton      | 4–5     | 4–5     | 4–5 | 4    | 3–4 | 4–5  | 4–5   | 4–5 | 4     |
| Polyester   | 4–5     | 4–5     | 4–5 | 4    | 4   | 4–5  | 4–5   | 4–5 | 6     |

St.*: Staining on cotton; St.**: Staining on wool; Alt.: Alteration in color.

Figure 9. Macroscopic images of the fabric samples (S1–S9) at 0.7× magnification.
Fabric porosity

Fabric porosity is a property that significantly affects the thermo-physiological comfort of clothing. It is influenced by several parameters such as yarn count, yarns density, fabric structure, and pore size. Spandex yarns have been added to the cotton and polyester fabrics to maintain the fabrics’ dimensions with repeated use and to reduce fabric porosity. Figure 9 shows the macroscopic images of one layer of the fabric samples (S1 to S9) with single jersey structure, and Figure 10 shows the inverted black and white images of the fabric samples to determine the porosity (%). ImageJ as an image processing program was used to analyze the black and white pixels’ ratio to calculate the porosity percentage.

It can be observed from Table 1 and Figures 9 and 10 that the samples with lower course density showed the highest porosity percentages. Sample S1 made of 100% cotton exhibited the highest loop length due to the natural twists found in the cotton fibers. The polyester samples showed the lowest loop length values compared to the cotton samples. Also, sample S1 showed the highest porosity of 50.80%, followed by sample S6 and sample S2 with porosity 49.21% and 46.79%, respectively. It is worth mentioning that
samples S6 and S2 are made of polyester and cotton fabrics with spandex yarn count 20 denier and half spandex feeders’ ratio.

Whereas, samples S5 and S9 made from cotton and polyester fabrics with spandex yarn 70 denier and full spandex feeders ratio showed the lowest porosity of 28.40% and 26.49%, respectively. Changing in fabric porosity could be attributed to the presence of spandex yarns with different count and number of feeders. Utilization of spandex yarn also caused increase in the fabrics weight and increase in the wales and courses density gradually. Thus, the loops contracted to each other, and their length decrease, which caused the fabrics to compact and resulted in decreasing the porosity.

Air permeability

The influence of changing fabric material, number of layers and layer’s combinations on the air permeability of the fabric samples for masks is shown in Figures 11–13. Regarding the one layer test, Figure 11 revealed that sample S1 (100% cotton) shows the highest air permeability value when compared with all samples. Sample S6 made of polyester with half feeders of spandex yarns of count 20 denier and sample S8 made of polyester with half feeders of spandex yarn of count 70 denier come follow sample S1. On the other hand, sample S5 made of cotton with full feeders of spandex yarn of count 70 denier showed the lowest air permeability value. This variation in the air permeability values may be attributed to the fact that the sample S1 has the lowest stitch density, longest loop length, and highest porosity compared with the other fabric samples blended with spandex yarns. The same can be observed for polyester fabric samples that showed higher air

![Figure 11. Air permeability values for one and two layers’ fabric samples of the same material.](image-url)
permeability values compared to cotton samples with spandex yarns, due to that polyester fibers have smooth surface which increased the air gaps in the fabric compared to cotton fibers which characterized by natural crimps in the fibers. Therefore, the fabrics produced with cotton yarns have more intra-yarn air paths with lower inter-yarn air spaces that caused a reduction in the air permeability of fabrics.71 The addition of spandex with different yarn count and ratios causing contraction of the loops to each other, increasing the stitches density and decreasing the porosity of the fabrics particularly with using spandex yarns at high feeding percentage 100%.72

Figure 12. Air permeability values for two hybrid fabric layers of different materials.

Figure 13. Air permeability values for three hybrid fabric layers of different materials.
Moreover, as assumed increasing the number of fabric layers in the face mask can enhance filtration efficiency, but it may negatively affect the user’s breathing comfort. So, it can be indicated from Figure 11 that increasing the number of fabric layers of the same material leads to decreasing the air permeability values of all samples in the same manner as in one layer samples’ test results. As, sample S1 recorded the highest air permeability value and sample S5 recorded the lowest value of air permeability. Figures 12 and 13 show the air permeability values of testing two layers and three layers of hybrid fabric samples combinations. The samples that achieved better results in one layer test were chosen for two and three hybrid layers’ test. It can be observed that the two layers of hybrid samples showed also lower values of air permeability. The hybrid sample (S8/S1) showed the higher air permeability value of 45.4 cm³/cm²/s, followed by sample (S7/S1) with a value of 31.3 cm³/cm²/s. This could be related to utilization of polyester fabrics in the outer layer, as S7 and S8 recorded higher air permeability values in one layer test. As well, using sample S1 (100% cotton) as the inner layer enhanced the air flow passed through the air gaps in fabrics.

The hybrid cotton sample (S5/S4) showed the lowest air permeability due to the low porosity and air permeability values of samples S5 and S4 which are made with spandex yarns of count 70 denier. The three layers’ sample (S1/S1/S1) composed of 100% cotton showed the highest air permeability value of 61 cm³/cm²/s, followed by sample (S6/S6/S1) of 36.3 cm³/cm²/s, and sample (S6/S1/S1) of 32.9 cm³/cm²/s. This could be related to the high porosity of sample S1 which increased the air flow passing from one layer to another. Finally, the combination of sample (S8/S8/S1) showed the lowest air permeability of 27.41 cm³/cm²/s compared to all samples of three layers. The standard error in

Figure 14. Breathability values of one and two layers fabric samples of the same material.
Figure 11 shows that there is significant difference between all the one layer and the two layers’ samples, which indicated that doubling the layers of each fabric decreased the air permeability value by approximately 50%. On the other hand, Figure 12 shows that there is a significant difference between hybrid samples S2/S6, S1/S2, S7/S1, and S8/S1 and these samples recorded an air permeability values over 15 cm³/cm²/s.

In Figure 13, from the standard error it can be observed that sample S1/S1/S1 is significantly different than the other samples with value of 50 cm³/cm²/s. It worth mentioning here that when one layers of these three layers S1 used to make samples S6/S1/S1 and S8/S1/S1, it is found that the air permeability value changed significantly and dropped form 50 cm³/cm²/s to 35 cm³/cm²/s.

Breathability (Differential pressure)

According to the EN 14683:2019 standard, surgical face masks are classified into two types (I and II) depending on their bacterial filtration efficiency (BFE) and breathability. Type I masks are recommended for use by patients and other persons to reduce risk of spreading infections in pandemic situations, and are not intended for use by healthcare professionals. While type II masks with high BFE are intended for use by healthcare professionals. Type IIR masks are resistant to splashes with high BFE to protect against exposure to blood and other body fluids. Both type I and II masks require breathability value <40 Pa/cm² and type IIR requires breathability value <60 Pa/cm². In this test, the influence of using different fabric materials, number of layers, and layers’ combinations on their breathability is shown in Figures 14–16. Also, the breathability levels for types I,
II, and IIR masks according to the EN 14683 standard are illustrated in the secondary y-axis in the same figures in order to compare the breathability values of standard’s requirements and the cloth face masks.

It is indicated from Figure 14 that the samples S1 made of 100% cotton and S6 made of polyester with half feeders of spandex yarns of count 20 denier achieved the best breathability values according to the standard levels for type I and II masks. The samples showed the lowest differential pressure values of 10.20 and 20.41 Pa/cm² in testing one and two layers of fabrics, respectively, followed by sample S8 made of polyester with half feeders of spandex yarns of count 70 denier which achieved 20.41 and 30.61 Pa/cm², respectively. Whereas sample S5 made of cotton with full feeders of spandex yarns of count 70 denier showed the highest differential pressure of 293.37 and 306.12 Pa/cm² which indicates it will cause a difficulty in breathing. These results are consistent with the air permeability test results. This could be attributed to the fact that using more than one layer of fabric from same material caused decreasing in the porosity of the fabrics which obstacles the air flow to pass through the air gaps where it faces higher frictional forces from the fibers in the fabrics. In Figure 15, for testing a combination of two hybrid layers of fabrics from different materials, it was found that the hybrid samples (S8/S1) and (S7/S1) achieved the best values of differential pressure of 35.71 and 51.02 Pa/cm² according to the standard. This could be related to the high porosity of sample S1 in the inner layer that facilitates passing of the air flow.

So from the results of testing one and two layers, samples S1, S6, S7, and S8 were chosen for the three layers’ test. Figure 16 shows the breathability values of testing three hybrid layers of different combinations from the mentioned samples. It is revealed that all the fabric samples combinations achieved high values of breathability according to the
standard for type I and II masks except the hybrid sample S7/S1/S1 which showed differential pressure of 56.12 Pa/cm² which could be suited for type IIR masks. Thus, the combination of using polyester samples S6 or S8 in the outer layer and sample S1 (100% cotton) in the inner layer enhanced the breathability of the masks due to their high porosity and low compactness. Standard error in Figure 14 shows that all single and double layers’ samples are significantly different regarding the breathability value except for the one and two layers of sample 5. This could be due to the close structure of the one layer of S5 as it recorded the highest differential pressure.

Physiological impacts of wearing masks (blood oxygen saturation and heart rate)

This test was carried out to make sure that wearing the cloth face masks will not negatively affect the oxygenation level and heart rate of the users. The normal range of oxygen saturation level of hemoglobin (SpO₂) is between (95–100%) and heart rate (HR) between (60–100 bpm). Low oxygen level can cause hypoxemia and it is noted when there is a decrease in SpO₂ % ≥3% from baseline to a value of ≤94%. Also it worth mention that walking quickly requires at least increase in heart rate about 10 bpm.⁴⁵ Table 7 illustrates the results of the oxygen level (SpO₂ %) and heart rate (bpm) of the users without and with wearing the surgical and the proposed cloth face masks. Firstly, during carrying out all the tests there were no signs of hypoxemia observed on the users. Secondly, from Table 7 it can be found that while performing the office work, there was no significant changes in the SpO₂ level with wearing the surgical and cloth masks compared to the baseline (with no mask) which showed SpO₂ of (98.5–99%). The oxygenation level SpO₂% of the surgical mask ranges between (98–98.5%) and for the three cloth masks ranges between (98–99%). Also the heart rate during putting on the cloth masks are close to the baseline (with no mask) which showed (70–80 bpm) and the surgical mask showed (72.5–78.5 bpm). The heart pulse during wearing the cloth masks ranged between (73–83.5 bpm) for sample S6/S6/S1, (70–78 bpm) for sample S6/S1/S1, and (70–85 bpm) for sample S8/S8/S1.

On the other hand, during walking while doing medium activity, it was found that there are no significant changes in the SpO₂ level when wearing the cloth masks compared to the baseline (with no mask) and the surgical mask, it ranges between (98–98.5%). The
heart rate during walking without the mask ranged between (90–131 bpm), while it ranges between (89–117.5 bpm) with wearing the surgical mask. There is an increase in the heart rate results during walking compared to the results of performing office work which is normal due to the higher intensity of physical activity. The heart rate values obtained during walking with wearing the cloth masks were (90.5–118 bpm), (94–120 bpm), and (97.5–130 bpm) for samples S6/S6/S1, S6/S1/S1, and S8/S8/S1, respectively. So it can be indicated that wearing the proposed face masks during doing normal activities does not affect their oxygenation level and comfort.

It is worth to mention that this test was applied in a limited range across the team members, but in future studies some points will be considered such as conducting the tests on a large number of volunteers to include different categories during the performance of more activates. Also the walking speed could be controlled better by using a treadmill and test duration can be increased.

Conclusions

The breathability performance is one of the most significant aspects that should be considered in producing face masks. Most of face mask producers neglect the importance of breathability comfort of the wearer, so they produce the mask with a high number of tight fabric layers to provide more protection from virus transmission. The consequences of this behavior in designing the face mask may cause people to suffer from breathing problems and uncomfortability while wearing the mask. In this work, different types of cotton and polyester knitted fabrics were produced with spandex yarns of different yarn count and ratios. The fabrics were treated with silver nanoparticles to be used as antiviral cloth face mask during COVID-19 pandemic. The main objective of this work is to provide the adequate level of breathability with high number of face mask layers, which raise the awareness regarding the importance of the breathability comfort of face masks.

The results revealed that the AgNPs have an antiviral activity along with low cytotoxicity against SARS-CoV-2 virus. The 100% cotton sample achieved the highest air permeability and breathability values for one and two layers’ tests, followed by the polyester sample made with half feeders of spandex yarns of count 20 denier. The three layers’ masks made of polyester using half feeders of spandex yarns in the outer and middle layers with 100% cotton whether in the inner layer or in both middle layer and inner layer, showed high breathability and comfortability due to their high porosity and low compactness. Moreover, wearing these masks during performing office work and walking showed no significant effect on the blood oxygen saturation (SpO2%) and heart rate of the wearers compared with the surgical face masks. Hence, the produced cloth face masks are cost-effective, comfortable to wear and offer adequate protection from SARS-CoV-2 virus.

Our future work will focus on drawing a correlation between the breathability test and filtration efficiency to may have a model that can predict the filtration efficiency based on breathability performance. Also, future studies may consider conducting the oxygen saturation (SpO2%) and heart rate tests on a large number of volunteers to include
different categories during doing the activities. Also the walking speed could be controlled better by using a treadmill and test duration can be increased.

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