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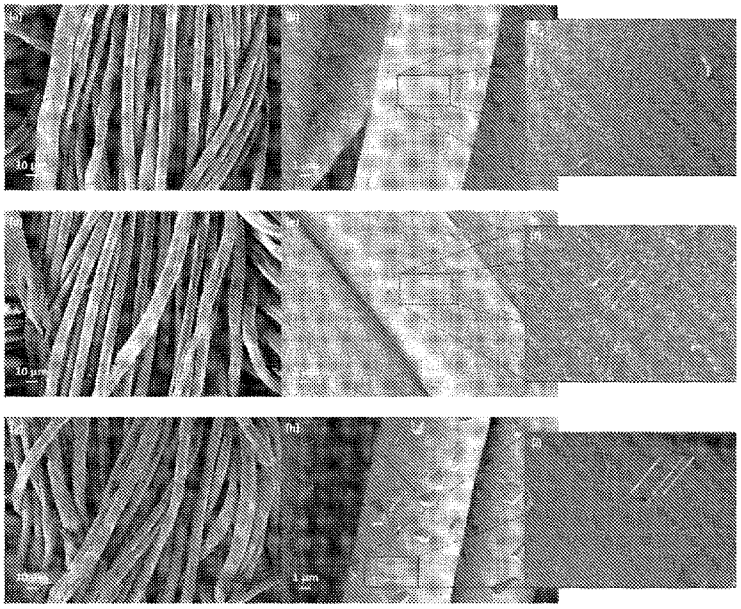
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(54) Title: SILVER-BASED ANTIMICROBIAL AND ANTIVIRAL COMPOSITIONS, TEXTILE MATERIALS COMPRISING THE SAME, METHODS AND USES THEREOF



FIGS. 1A-1I

(57) Abstract: Disclosed herein are silver-based antimicrobial and antiviral composition and textile materials comprising the same. The textile materials disclosed herein are produced from any textile material to which silver-based antimicrobial and antiviral composition is topically applied. Such antimicrobial and antiviral compositions and textile materials have been demonstrated to be effective in inactivating viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The silver-based composition comprises silver particles distributed therethrough and at least one of Methylisothiazolinone (MIT, MI), Chloromethylisothiazolinone (CMIT, CMI, MCI), Benzisothiazolinone(BIT), Octylisothiazolinone (OIT, OI), Dichlorooctylisothiazolinone (DCOIT, DCOI)or Butylbenzisothiazolinone (BBIT).

[Continued on next page]

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SILVER-BASED ANTIMICROBIAL AND ANTIVIRAL COMPOSITIONS, TEXTILE MATERIALS COMPRISING THE SAME, METHODS AND USES THEREOF

Field of the Invention

[0001] This invention relates to silver-isothiazolinone-containing antimicrobial and antiviral compositions and textile materials, as well as uses thereof and methods of obtaining the same. Such materials comprise a topically applied silver-isothiazolinone-based antimicrobial and antiviral and can be used in the manufacturing of any textile product, such as clothes and personal protective equipment.

Background

[0002] Silver-containing antimicrobials have been incorporated into textile substrates for some time and are rapidly gaining acceptance in the medical industry as a safe, effective means of controlling microbial growth.

[0003] In view of the subsequent viral epidemic and pandemic episodes occurred in the last decades, there is a growing need for new antiviral materials, including textile materials. Humanity has experienced epidemic diseases caused by newly recognized viruses, including hepatitis C, HIV/AIDS, SARS-CoV-1, MERS, Lassa fever, Zika virus, and Ebolavirus, as well as Yellow Fever, influenza, and measles virus, which are more common but equally severe. Severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) is a novel coronavirus that causes coronavirus disease 2019 (COVID-19). Since its first detection in December 2019, it has affected millions of people worldwide, carrying a mortality rate much higher than any common flu. These public health outbreaks driven by emerging COVID-19 infectious diseases constitute the forefront of global safety concerns and significant burden on global economies. While there is an urgent need for its effective treatment based on antivirals and vaccines, it is imperative to explore any other effective intervention strategies that may reduce the mortality and morbidity rates of this disease.

[0004] It is likely that not only will an outbreak of infections caused by SARS-CoV-2 take place in the future, within the coming months or years, but also that unknown viruses and/or pathogen will emerge, whose activity, spread, contagion, and mechanism of action will be

unknown. This global crisis allows us to suddenly discover the high value of a solid and precise knowledge as well as the urgent need to prepare us for a new and unpredictable epidemic.

[0005] The human body is a diverse ecosystem that harbors up to 1014 pathogens (bacterial, fungi and virus cells). With our in-depth understanding of the critical relationship between pathogens and diseases, the development of innovative materials capable of avoiding the transmission, spread, and entry of these pathogens into the human body is currently in the spotlight.

[0006] Generally, the surface of a material is the medium by which the human body interacts with them. Therefore, anti-pathogen strategies based on chemical modification of the material surface have been developed. One procedure is to form a layer on the surface of the material, thereby reducing the chance of contact between the pathogen and the surface of the material. And this greatly reduces the number of pathogens adhering to the surface. Another strategy is that killing the adhered pathogen directly by decorating biocide agent on the surface of the material.

[0007] Use of personal protective equipment is considered to be one of the most important strategies for protecting from transmissible pathogens, particularly when no effective treatment or prophylaxis is available for the disease provoked by these pathogens in question. Therefore, the current worldwide public health crisis of COVID-19 has highlighted the particularly emergent need for materials that inactivate enveloped viruses on contact for preventing transmission.

[0008] Inorganic/Organic biocide surfaces and materials have attracted much attention due to their better stability and safety as compared with organic reagents for preventing infections and transmission. Among inorganic agents, silver cation and metal are most widely used. However, Ag cations tend to react with Cl^-, HS^-, and $SO42^-$ in aqueous solution, forming precipitates, thus losing their biocide activity, which affects the practical application of Ag-loaded biocide agents to a certain extent.

[0009] According to the literature, there is a plenty of protocols focused on the production of hybrid/composite materials based on Ag NPs, whose architecture is driven by different synthetic methods and reaction mechanisms.(Abbasi et al. 2016; Fahmy et al. 2020; Iravani et al. 2014; Khatoon, Mazumder, and Sardar 2017; Yamada, Foote, and Prow 2015; Yaqoob, Umar, and Ibrahim 2020; Zhang et al. 2018).

[00010] The hybrid/composite materials based on Ag NPs have been proven to be most useful because they have excellent antimicrobial properties against lethal viruses, microbes/germs, and other nucleus containing microorganisms. These nanoparticles are certainly the most extensively utilized material among all. Thus, it has been used as antimicrobial agent in different textile industries.(Hasan 2014)

[00011] In view of the above, the inventions disclosed herein are aimed to provide innovative products having high bactericide, fungicide and virucide activity for their incorporation and application to textile applications.

Summary

[00012] It has been found that the antimicrobial and antiviral textile material exhibits surprising long-lasting antiviral efficacy against several microorganisms and viruses, including SARS-CoV-2.

[00013] It has also been found that the antimicrobial and antiviral textile materials exhibit a surprisingly durable antiviral efficacy after repeated wash cycles.

[00014] Therefore, the present disclosure refers to antimicrobial and antiviral silver-isothiazolinone-based compositions and textile materials having such surprisingly effective and durable activity against viruses.

[00015] Methods of making and uses of silver-isothiazolinone-containing antimicrobial and antiviral compositions and textile materials are also disclosed herein.

[00016] According to some embodiments disclosed herein, e.g. a textile material, a silver-isothiazolinone-based antimicrobial and antiviral finish is topically applied to a plain fabric comprised of polyester fibers. The treated fabric may ideally be made into a medical garment, such as a white coat. Such medical garment enables the viricidal properties of the silver-isothiazolinone-based antimicrobial and antiviral finish to aid in preventing the transfer of virus from one person to another, for instance, after sharing communal items.

[00017] Therefore, methods of making the silver-containing antimicrobial textile material are provided herein.

[00018] The uses of such compositions on textile materials are also disclosed herein. Textile materials that exhibit long-lasting antiviral efficacy, including against SARS-CoV-2, and also exhibits antiviral efficacy after repeated wash cycles may be obtained by such use.

Brief description of the drawings

[00019] **FIGS. 1A-1I** are FE-SEM images of (**FIGS. 1A-1C**) non-treated polycotton, (**FIGS. 1D-1I**) NanoxClean Ag+Fresh polycotton samples.

[00020] **FIGS. 2A-2F** show AATCC 147 test result against E. Coli for a non-treated polycotton sample as a reference (a) and for the Ag-based antimicrobial treated polycotton (**FIGS. 2B and 2C**: NanoxClean Ag+Fresh) and AATCC 147 test result against S. Aureus for a non-treated polycotton sample as a reference (**FIG. 2A**) and for the Ag-based antimicrobial treated polycotton (**FIGS. 2B and 2C**: NanoxClean Ag+Fresh) exhibiting, respectively, no peripheral inhibition and a measurable zone of inhibition.

[00021] **FIG. 3** is a representative graph of the data obtained in the first experiment, relating the tested products to the viral load found and the percentage of inhibition.

[00022] **FIG 4** : is a representative graph of the data obtained in the second experiment, relating the tested products to the viral load found and the percentage of inhibition.

Detailed description

Antimicrobial and Other Agents

[00023] The particular treatment used herein comprises at least one type of silver containing compositions, or mixtures thereof of different types. The term "silver containing compositions" encompasses compositions of silver compounds (nano-sized silver metal, micro-sized silver metal, silver chlorine and/or silver oxide) finely distributed and homogenously mixed with at least one of isothiazolinone compounds (Methylisothiazolinone (MIT, MI), Chloromethylisothiazolinone (CMIT, CMI, MCI), Benzisothiazolinone (BIT), Octylisothiazolinone (OIT, OI), Dichlorooctylisothiazolinone (DCOIT, DCOI) or Butylbenzisothiazolinone (BBIT)). The preferred silver-ion containing composition for this invention is an antiviral silver/isothiazolinone available from Nanox Company, under the tradename NanoxClean Ag+Fresh® containing nearly 500ppm of silver and 5,000ppm of CMIT and MIT. Other silver ion containing materials and compositions may also be used. Various combinations of these silver containing materials and compositions may be made if it is desired to "tune" the silver release rate over time.

[00024] Generally, such a silver composition or compound is added in an amount from about 0.01% to about 60% by total weight of the particular treatment composition; more preferably, from about 0.05% to about 40%; and most preferably, from about 0.1% to about 30%. Preferably, the metal compound is present in an amount from about 0.001 % to about 6% of the weight of the fabric (owf), preferably from about 0.005% to about 3% owf, more preferably from about 0.01% to about 1% owf

[00025] The binder material provides highly beneficial durability of the antimicrobial compound for the target substrate. Preferably, this component is a polyurethane-based binding agent, although other binders, such as a permanent press type resin or an acrylic type resin, may also be used alone or in combination with other resins. In essence, such resins provide durability by adhering silver/isothiazolinone composition to the target substrate, such as fibers or fabrics, with the polyurethane exhibiting the best overall performance.

[00026] Total add-on levels of silver to the target substrate may be 10 ppm or higher. More preferably, total add-on levels of silver may be 50 ppm or higher. It has not been determined that an upper boundary limit of silver add-on levels to the target substrate exist.

Suitable textile materials for receiving a topically applied silver-based antiviral finish

[00027] Suitable textile materials for receiving a topically applied silver-based antiviral finish include, without limitation, fibers, yarns, and fabrics. Fabrics may be formed from fibers such as synthetic fibers, natural fibers, or combinations thereof. Synthetic fibers include, for example, polyester, acrylic, polyamide, polyolefin, polyaramid, polyurethane, regenerated cellulose, and blends thereof. More specifically, polyester includes, for example, polyethylene terephthalate, polytriphenylene terephthalate, polybutylene terephthalate, polylactic acid, and combinations thereof. Polyamide includes, for example, nylon 6, nylon 6,6, and combinations thereof. Polyolefin includes, for example, polypropylene, polyethylene, and combinations thereof. Polyaramid includes, for example, poly-p-phenyleneteraphthalamid (i.e., Kevlar®), poly-m-phenyleneteraphthalamid (i.e., Nomex®), and combinations thereof. Natural fibers include, for example, wool, cotton, flax, and blends thereof.

[00028] The fabric may be formed from fibers or yarns of any size, including microdenier fibers and yarns (fibers or yarns having less than one denier per filament). The fibers or yarns may have deniers that range from less than about 1 denier per filament to about 2000 denier per filament or

more preferably, from less than about 1 denier per filament to about 500 denier per filament, or even more preferably, from less than about 1 denier per filament to about 300 denier per filament.

[00029] Furthermore, the fabric may be partially or wholly comprised of multi-component or bi-component fibers or yarns which may be splittable along their length by chemical or mechanical action. The fabric may be comprised of fibers such as staple fiber, filament fiber, spun fiber, or combinations thereof.

[00030] The fabric may be of any variety, including but not limited to, woven fabric, knitted fabric, nonwoven fabric, or combinations thereof. The fabric may optionally be colored by a variety of dyeing techniques, such as high temperature jet dyeing with disperse dyes, thermosol dyeing, pad dyeing, transfer printing, screen printing, or any other technique that is common in the art for comparable, equivalent, traditional textile products. If yarns or fibers are treated by the process of the current invention, they may be dyed by suitable methods prior to fabric formation, such as, for instance, by package dyeing or solution dyeing, or after fabric formation as described above, or they may be left undyed. The textile substrate may be dyed or colored with any type of colorant, such as, for example, pigments, dyes, tints, and the like. Other additives may be present on and/or within the textile substrate, including antistatic agents, brightening compounds, nucleating agents, antioxidants, UV stabilizers, fillers, permanent press finishes, softeners, lubricants, curing accelerators, and the like.

[00031] In one embodiment of the invention, a fine-medium weight polyester/cotton woven fabric (plain weave, 120 g/m^2; width 1,60m; ends 35/cm; picks 26/cm; yarn Ne 36 67%Polyester / 33% cotton) is used to form the antiviral medical garment. More specifically, it is believed that any fabric that has been treated with the silver-isothiazolinone-based antimicrobial and antiviral chemistry described herein would fall within the scope of the present disclosure, as well as any of the above-mentioned textile substrate materials.

Method of applying the composition

[00032] The preferred procedure utilizes silver containing compounds and compounds, such as NanoxClean NNXC Ag+Fresh® as the preferred compound (although any similar types of compounds that provide silver/izothiazolinone may also be utilized), which are admixed with a binder to form a bath, into which the target substrate is then immersed.

[00033] It was initially determined that proper binder resins could be selected from the group consisting of nonionic binders (i.e., cross-linked adhesion promotion compounds) or anionic binders (including, without limitation, acrylics). Other nonionics and slightly anionics were also suitable, including melamine formaldehyde, melamine urea, ethoxylated polyesters, and the like. However, it was found that the durability and controlled silver release of such treated substrates was limited.

[00034] It was determined that greater durability and control over silver release was required for this type of antimicrobial textile application. It is desirable that the antimicrobial fabric exhibits a controlled release of silver/isothiazolinone such that the silver/ isothiazolinone composition is slowly released over an extended period of time, rather than being released quickly at one time. Thus, these prior comparative treatments were measured against various other types. Finally, it was discovered that certain polyurethane binders and acrylic binders permitted the best overall durability and controlled release of silver/ isothiazolinone composition.

[00035] With such polyurethane-based binder materials utilized, the antiviral characteristics of the treated substrate remained very effective with regard to the amount of surface available silver/isothiazolinone that could be controllably released to kill virus, without discoloration of the treated substrate. However, while it currently appears that the use of polyurethane based binder resins are preferred due to their silver/isothiazolinone release, in practice essentially any binder resin which is not toxic to the site of skin may be used.

[00036] An acceptable method of providing a durable antimicrobial metal-treated fabric surface, is the application of a silver/isothiazolinone containing compound and polyurethane-based binder resin from a bath mixture. In practice, this mixture of compound and resin may be applied through spraying, dipping, padding, foaming, and the like.

Examples

Example 1. Antimicrobial and antiviral composition

[00037] An antimicrobial and antiviral composition comprising NanoxClean NNXC Ag+Fresh® silver/izothiazolinone compound (available from Nanox Company) was produced for topical application to the target substrate. An exemplary treatment bath of this composition is as follows:

25 g/L Nanox Clean NNXC Ag+Fresh
50 g/L Anionic or nonionic binder
pH: 5 – 6 (acetic acid solution 1% to adjust pH)
Pick-up: 75%
Thermofixation temperature: 170°C

Example 2. Application of chemical finishing composition onto fabrics

[00038] A fine-medium weight 67% polyester /33% cotton woven fabric (plain weave, 120 g/m²; width 1,60m; ends 35/cm; picks 26/cm; yarn Ne 36 67%Polyester / 33% cotton) was used for the application purpose. The antimicrobial product NanoxClean® Ag+Fresh was applied on the polycotton fabric using pad-dry-cure method. The cotton fabric was immersed in the solution containing (5%, % weight basis) of NanoxClean® Ag+Fresh and acrylic binder (6%, % weight basis) and passed through a padder, with a 72% wet pick-up. After drying (80°C, 1 min) the fabric was annealed at 170°C for 2 min.

Characterization

[00039] In order to observe morphological changes in polycotton fibers, FE-SEM measurements were performed (**FIGS. 1A-1I**). Morphologies of the composites were analyzed by Field Emission Scanning Electron Microscopy (FE-SEM) on an FEI instrument (Model Inspect F50) operating at 1 kV. There are no significant differences in the fiber diameters of the samples, being the average diameter values obtained for the non-treated polycotton and NanoxClean Ag+Fresh polycotton samples were 10.62 ± 2.30 and 10.59 ± 2.50 μm respectively. For **FIGS. 1D-1F**, it is possible to observe the formation of small Ag nanoparticles on the polycotton surface, with average size of the 23.51 ± 5.18 nm. Similar behavior was obtained by several other authors in works that incorporated Ag NPS into polycotton in different ways.(Bacciarelli-Ulacha et al. 2014; Chen and Chiang 2008; Ghosh, Yadav, and Reynolds 2010; Maráková et al. 2017; Montazer et al. 2012; Nam et al. 2016; Xu et al. 2019; Zhang et al. 2009) For the **FIGS. 1G-1I**, it is possible to observe the formation of Ag nanoparticles with average size 126.9 ± 19.5 nm. In addition, there is a homogeneous distribution of micrometric crystals of isothiazolinones with well-defined morphology over all polycotton surface fibers, with an average size of 1.62 ± 0.44 μm.

Example 3. Assessment of Allergic Response and Dermatological Photoirrritant and Photosensitive Potential

[00040] A Human Repeat Insult Patch Test (HRIPT) was performed to determine the absence of the potential for dermal irritability and sensitization of the treated fabrics. The study was carried out in maximized conditions, in which semi-occlusive dressings containing the investigational product and controls were applied to the participants' backs. The application of the study dressings occurred for six weeks, with three weeks of application alternately, two weeks of rest and a new application of the dressing containing the product in virgin area in the sixth week (challenge). The readings of the application site were performed at each dressing change according to the reading scale recommended by the International Contact Dermatitis Research Group (ICDRG).(Wilkinson et al. 1970) Dermatological evaluations were carried out at the beginning and end of the study, and the physician was available for evaluation and assistance to the participants in case of positive or adverse reaction. Participants of both genders, with phototypes III to IV (Fitzpatrick),(Fitzpatrick 1988) aged between 21 and 70 were selected. The selected participants were distributed as shown in the **Table 1**.

Table 1. Distribution of selected participants for the HRIPT.

Evaluation Test	Number of Participants	Gender		Age	
		Female	Male	Minimum	Maximum
Primary Dermal Irritability	51	40	11	21	70
Accumulated Dermal Irritability	51	40	11	21	70
Dermal Sensitization	51	40	11	21	70

[00041] Since exposure to solar radiation can trigger or aggravate adverse reactions to topical products, knowing the behavior of the product on human skin stimulated with ultraviolet radiation is of fundamental importance for proof of safety. Therefore, a unicentric, blind, comparative clinical study to assess the photoirritating and photosensitizing potential was also conducted, with the aim of proving the absence of the irritating potential of the product applied to the skin when exposed to ultraviolet radiation. The study was carried out with dressings containing the product,

applied to the participants' skin and, after removal, controlled irradiation with a spectrum of ultraviolet radiation emission was performed. Readings were performed according to the reading scale recommended by the ICDRG. The study with the participants lasted for five weeks, covering 3 phases: induction (FI), rest and challenge (FS). Dermatological evaluations are performed at the beginning and end of the study, or when there is an indication of positivity or adverse reaction. Participants of both sexes, with phototypes III (Fitzpatrick), aged between 21 and 62 were selected. The selected participants were distributed as shown in the **Table 2**.

Table 2. Distribution of selected participants for the photoirritating and photosensitizing clinical study.

Evaluation Test	Number of Participants	Gender		Age	
		Female	Male	Minimum	Maximum
Photosensitization	25	20	05	19	62
Photoirritation	25	20	05	19	62

[00042] Both the Human Repeat Insult Patch Test and the clinical study to assess the photoirritating and photosensitizing potential were conducted according to the Cosmetic Product Safety Assessment Guide, published by the Brazilian regulatory agency ANVISA(Agência Nacional de Vigilância Sanitária 2012), by the ECOLYZER Group (São Paulo/SP, Brazil), an independent and ISO certified laboratory. For the HRIPT, Primary Dermal Irritability, Accumulated Dermal Irritability and Dermal Sensitization potential were determined. The clinical evaluation criterion was the observation of clinical signs or symptoms such as swelling (edema), redness (erythema), papules and vesicles according to the reading scale recommended by the ICDRG. No adverse reactions (erythema, edema, papules or vesicles) were detected in the product's application areas, in the analysis of primary and accumulated irritability, sensitization, during the study period. The same clinical evaluation criterion was used to determine the Dermal Photoirritation (FI) and Dermal Photosensitization (FS) in the clinical study. As in the HRIPT, on this study no adverse reactions (erythema, edema, papules or vesicles) were detected in the product's application areas during the study period. According to the results obtained from the sample of participants studied, we can conclude that the treated fabrics did not induce a photoirritating, photosensitizing, irritation or sensitization process and, therefore, can be

considered hypoallergenic and dermatologically tested and approved, being considered safe, according to ANVISA's Guide for Cosmetic Product Safety.

Example 4. Antimicrobial and antiviral activities

[00043] As Ag-based antimicrobial additives caused distinct surface interactions in cotton fibers, it is expected that this difference will be reflected in their physical and chemical and biological properties. In this way, experiments were carried out to evaluate the biological properties of composites obtained through the allergenic response to humans and microbicidal activity against *E. Coli), S. Aureus, C. Albicans* and SARS-CoV-2.

Assessment of Antimicrobial Activity

[00044] The AATCC 147 Parallel Streak Standard Method(Anon 2006b) was used as a qualitative method to evaluate antibacterial activity of the treated fabrics. Sterile plate count agar was dispensed in petri plates. 24 hours broth cultures of the test organisms (Escherichia Coli (E. coli - ATCC8739) and Staphylococcus aureus (S. aureus - ATCC6538) were used as inoculums. Using a 10μL inoculation loop, 1 loop full of culture was loaded and transferred to the surface of the agar plate by making 7.5cm long parallel streaks 1 cm apart in the center of the plate, refilling the loop at every streak. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with the agar surface. The plates were incubated at 37°C for 18-48 hours. After incubation, a streak of interrupted growth underneath and along the side of the test material indicates antibacterial effectiveness of the fabric.

[00045] The quantitative antimicrobial activity assessment of the treated polycotton fabrics was determined according to AATCC Test Method 100(Anon 2006a). Fabric specimens (circular swatch 4.8 cm in diameter) were impregnated with 1.0 mL of inoculum in a 250 mL container. The inoculum was a nutrient broth culture containing 2.0~3.0 · 105/mL colony forming units of microorganisms. E. coli and S. aureus were used as a reference for gram-negative and gram-positive bacteria, respectively, and C. albicans (ATCC 10231) as a reference for fungus. The microorganisms counted on the treated polycotton fabric and those on a controlled sample were determined after a 24-hour incubation period at 37°C. The antimicrobial activity was expressed in terms of percentage reduction of the microorganism after contact with the test specimen compared

to the number of microbial cells surviving after contact with the control. The results are expressed as percent reduction of microorganisms by Eq. (1).

$$\text{Reduction (\%)} = [(B-A)/B] \times 100 \qquad (1)$$

where A and B are the numbers of bacteria or fungus recovered from the antimicrobial-treated and untreated polycotton fabrics in the jar incubated over the desired contact period, respectively.

[00046] The AATCC 147 test results against S. Aureus (gram positive) and E. Coli (gram negative) bacteria for the non-treated and the Ag-based antimicrobial treated polycotton samples are shown in **FIGS. 2A-2F** and **Table 3**. For the control polycotton, growth of E. Coli and S. Aureus was observed under the specimen while no growth appeared for the treated fabric. The zone of inhibition for the control sample was 0 mm, in comparison to 2-3 mm for the treated fabric. It can be seen from these results that the Ag-based antimicrobials treated fabrics displayed a high level of antibacterial performance.

[00047] **FIGS. 2A-2F** show AATCC 147 test result against E. Coli for a non-treated polycotton sample as a reference (**FIG. 2A**) and for the Ag-based antimicrobial treated polycotton (**FIGS. 2B and 2D**: NanoxClean Ag+Fresh) and AATCC 147 test result against S. Aureus for a non-treated polycotton sample as a reference (**FIG. 2D**) and for the Ag-based antimicrobial treated polycotton (**FIGS. 2E and 2F**: NanoxClean Ag+Fresh) exhibiting, respectively, no peripheral inhibition and a measurable zone of inhibition.

Table 3. AATCC 147 tests results against *S. Aureus* and *E. Coli* for non-treated (control) and Ag-based antimicrobials treated fabric samples.

	E. Coli	***S. Aureus***
Antimicrobial Product / Concentration of Antimicrobial in impregnation bath[a] (% weight)	**Growth under the specimen**	
Non-treated control polycotton / 0	Yes	Yes
Ag+Fresh treated polycotton / 5%	No	No
Ag+Fresh treated polycotton / 6%	No	No
	Zone of inhibition (mm)	
Non-treated control polycotton / 0	0	0
Ag+Fresh treated polycotton / 5%	3	2.5
Ag+Fresh treated polycotton / 6%	2	2

[a] Treatment condition: soaking in 5% solution; 72% wet pick-up (padder); 80°C, 2 min drying; annealing at 170°C / 2 min

[00048] The quantitative antimicrobial activities of finished textiles treated with the Ag-based antimicrobial (NanoxClean Ag+Fresh) according to the AATCC 100 standard are shown in **Table 4.** All the Ag-based polycotton samples had efficient antimicrobial activities, displaying a 99.99% reduction in all tested samples.

Table 4. Qualitative antibacterial results according to the AATCC 100 standard.

Antimicrobial Product / Concentration of Antimicrobial in impregnation bath[a] (% weight)	**Microbial Reduction[b] (%)**								
	S. aureus ATCC 6538			**E. coli ATCC 8739**			**C. albicans ATCC 10231**		
	Zero-time bacteria	Bacteria count after 24 hours	***% Reduction***	Zero-time bacteria count	Bacteria count after 24 hours	***% Reduction***	Zero-time Fungi count	Fungi count after 24 hours	***% Reduction***
Non-treated Control	2.1×10^5	2.2×10^5	-	2.3×10^5	2.2×10^5	-	2.0×10^5	2.2×10^5	-
Ag+Fresh / 5%	2.1×10^5	1.6×10	**99.99%**	2.3×10^5	1.3×10	**99.99%**	2.0×10^5	1.3×10	**99.99%**
Ag+Fresh / 6%	2.1×10^5	1.1×10	**99.99%**	2.3×10^5	1.1×10	**99.99%**	2.0×10^5	1.4×10	**99.99%**

[a] Treatment condition: soaking in 5% solution; 72% wet pick-up (padder); 80°C, 2 min drying; annealing at 170°C / 2 min;
[b] Percent bacterial reduction as measured against a non-treated control.

Assessment of Antiviral activity against SARS-CoV-2

[00049] An adaptation of ISO 18184 Determination of antiviral activity of textile products Standard Method (https://www.iso.org/standard/71292.html) was used as a reference for a

quantitative method to evaluate the treated polycotton's ability to inactivate the SARS-CoV-2 virus particles, under the tested conditions, at two different time intervals (2 and 5 minutes of contact time). For the application purposes, a fine-medium weight 67% polyester /33% cotton woven fabric (plain weave, 120 g/m²; width 1,60m; ends 35/cm; picks 26/cm; yarn Ne 36 67%Polyester / 33% cotton) was used. The antimicrobial product NanoxClean® Ag+Fresh was applied on the cotton fabric using pad-dry-cure method. The polycotton fabric was immersed in the solution containing (2%, % weight basis for sample G and 5%, % weight basis for sample H) of NanoxClean® Ag+Fresh and acrylic binder (6%, % weight basis in both samples) and passed through a padder, with a 72% wet pick-up. After drying (80°C, 1 min) the fabric was annealed at 170°C for 2 min.

[00050] The virus was inoculated into liquid media containing no fabric, treated (samples G and H) and non-treated polycotton samples and incubated for 2 different time periods. Then, they were plated onto tissue cultures of Vero CCL-81 cells. After the incubation, the viral genetic material was quantified in each condition using real-time quantitative PCR, and based on the control samples, the ability of each sample to inactivate SARS-CoV-2 was determined.

[00051] Vero CCL-81 cells were plated onto 24-well plates containing 1x105 Vero cells per well. The cells were maintained in DMEM high glucose culture medium (Sigma, 51435C) supplemented with 10% fetal bovine serum, 100 units/mL of Penicillin, and 100 µg/mL of Streptomycin. The plate was incubated at 37 °C, 5% CO2 atmosphere for 24 h. Following this period, the medium was removed and replaced with 666.7µL of DMEM High Glucose/well without supplementation.

[00052] Three test specimens, non-treated polycotton control and Ag-isothiazolinone-based antimicrobial treated polycotton samples (samples G and H), measuring 6,25cm2 apiece, were tested. Each test specimen was placed into a different tube and 1.33mL of DMEM high glucose medium without supplementation was added to each tube. In parallel, 500µL of culture medium containing SARS-CoV-2 was diluted in 4.5mL of DMEM high glucose culture medium without supplementation, and then 333.4µL of this viral suspension was added to each of the tubes containing the pieces of cloth. The mixtures were incubated with the virus for 2 minutes and the tubes were homogenized every 30 seconds. After this period, 166.7µL of each sample was transferred to different wells of the plates containing the cells previously seeded. After a total of 5 min of incubation, an additional 166.7µL aliquot was removed from each tube and incubated in

other wells on the same plate. As control, the viral suspension was incubated in media without supplementation, with samples collected at 2 and 5 min used to infect Vero cells on the same plate.

[00053] The plate was incubated for 2h at 37 °C, 5% CO2 for viral adsorption, and after this period, 166.6µL of DMEM High Glucose medium containing 12% fetal bovine serum were added to each well, making to a final volume of 1ml of medium/well containing 2% serum. Immediately after adding the medium, the plate was further incubated at 37 °C, 5% CO2 for 48h.

[00054] After 48 hours post infectious, the plate was removed from the incubator and 100µL of the medium from each well (each well a different condition) was removed and placed in lysis buffer to proceed with the viral RNA extraction. For the extraction, the MagMAX ™ CORE Nucleic Acid Purification Kit (Thermo Fisher) was used, following the manufacturer's instructions, on the semi-automated platform MagMAX Express-96 (Applied Biosystems, Weiterstadt, Germany).

[00055] The detection of viral RNA was carried out using the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems) on an ABI 7500 SDS real-time PCR machine (Applied Biosystems), using a published protocol and sequence of primers and probe for E gene (Corman, V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real time RT-PCR. Euro Surveill. 25, 1–8 (2020)). The viricidal activity, or viral inactivation, was determined as a percentage related to the control (media without fabric specimen).

[00056] The experiment was repeated using the same experimental conditions, but with media incubated in two pieces of test specimens (instead of one) per condition.

[00057] The Antiviral Activity test was designed to determine the inactivation of viral particles upon short exposure to the products, which in this case were the Ag-based treated polycotton samples incubated in liquid media. After a short period of incubation, the media were transferred to a tissue culture, where viable virions would be able to enter cells and replicate within. The supernatant of tissue cultures was recovered after 48 h and the viral load was determined by RT-qPCR, resulting in the determination of number of viral particles per mL.

[00058] **Table 5** shows the number of copies of the control media without any fabric sample, non-treated polycotton, and the two Ag-based treated polycotton samples at the two different tested time periods. With the result of the number of copies of each sample, the viral inactivation effect of each cloth was calculated, using media without any fabric sample as a control.

Table 5. Copies per mL of SARS-CoV-2 at different times in the first experiment.

Antimicrobial Product / Concentration of Antimicrobial in impregnation bath[a] (% weight)	Copies/mL (SARS-CoV-2)	Viral Inactivation (%)	Incubation
Media without any fabric sample	1.85×10^9	-	2 min
Non-treated Control (Blank)	1.55×10^9	16.57%	
Ag+Fresh (Sample G) / 2%	2.48×10^8	86.65%	
Ag+Fresh (Sample H) / 5%	7.39×10^6	99.60%	
Media without any fabric sample	1.26×10^9	-	5 min
Non-treated Control (Blank)	9.87×10^9	21.99%	
Ag+Fresh (Sample G) / 2%	2.14×10^8	83.12%	
Ag+Fresh (Sample H) / 5%	5.50×10^7	95.65%	

[a] Treatment condition: soaking in 5% solution; 72% wet pick-up (padder); 80°C, 2 min drying; annealing at 170°C / 2 min.

[00059] Regarding the second experiment, the number of copies per milliliter in each sample was also obtained and the percentage of inhibition of the products was calculated from the control media without any fabric sample. The obtained results were summarized in **Table 6**.

Table 6. Copies per mL of SARS-CoV-2 at different times in the second experiment.

Antimicrobial Product / Concentration of Antimicrobial in impregnation bath[a] (% weight)	Copies/mL (SARS-CoV-2)	Viral Inactivation (%)	Incubation
Media without any fabric sample	4.15×10^9	-	2 min
Non-treated Control (Blank)	3.27×10^9	21.28%	
Ag+Fresh (Sample G) / 2%	6.82×10^8	83.57%	
Ag+Fresh (Sample H) / 5%	2.72×10^5	99.99%	
Media without any fabric sample	3.03×10^9	-	5 min
Non-treated Control (Blank)	2.34×10^9	22.86%	
Ag+Fresh (Sample G) / 2%	3.60×10^8	88.12%	
Ag+Fresh (Sample H) / 5%	1.04×10^5	99.99%	

[a] Treatment condition: soaking in 5% solution; 72% wet pick-up (padder); 80°C, 2 min drying; annealing at 170°C / 2 min.

[00060] The graphs in **FIGS. 3 and 4** represent the data described in **Tables 5 and 6**, of the control media without any fabric sample, non-treated polycotton, and the two Ag-based treated polycotton samples.

[00061] **FIGS. 3 and 4** show the results of the first and second experiments, respectively, indicating the number of viral copies per mL and the percentage of inhibition of each compound above the bar referring to it. Inhibition was calculated for each treatment using its respective control.

[00062] **FIG. 3** shows a representative graph of the data obtained in the first experiment, relating the tested products to the viral load found and the percentage of inhibition.

[00063] **FIG. 4** shows a representative graph of the data obtained in the second experiment, relating the tested products to the viral load found and the percentage of inhibition.

[00064] In both experiments, in the two time periods tested, the untreated polycotton showed a subtle activity, which was already expected by data already published by Chin and colleagues (Chin, Alex, et al. "Stability of SARS-CoV-2 in different environmental conditions." medRxiv (2020)). The Ag+Fresh treated polycotton sample G showed a high viricidal activity when incubated with the virus. At both time periods in both experiments, the Ag+Fresh treated polycotton sample H obtained a higher rate of viral inactivation compared to Ag+Fresh sample G.

[00065] In short, both treated polycotton samples were effective in viral inhibition in 2 and 5 minutes in two different experiments, where there was variation in the amount of virus per cm^2 of fabric (4x less virus/cm^2 in the second experiment). The Ag+Fresh treated polycotton sample H showed the best activity, reaching 99.99% within two minutes of incubation with the virus in the second experiment. The Ag+Fresh treated polycotton sample G, despite being less effective than the Ag+Fresh treated polycotton sample H, showed high anti-SARS-CoV-2 activity, with more than 80% inhibition rate in all tests performed.

[00066] The differential capabilities of this product are the prevention of cross infection caused by pathogens, such as opportunistic bacteria and fungi, responsible for the worsening of COVID-19 and other types of viruses.

CLAIMS

What is claimed is:

1. A composition having antimicrobial and antiviral activity comprising silver particles, wherein the composition comprises at least one type of silver-containing compounds or mixtures thereof, the silver-containing compounds being comprising nano-sized silver metal, micro-sized silver metal, silver chlorine and/or silver oxide.

2. The composition of claim 1, wherein the silver particles are finely distributed and homogenously mixed with at least one of isothiazolinone compounds (Methylisothiazolinone (MIT, MI), Chloromethylisothiazolinone (CMIT, CMI, MCI), Benzisothiazolinone (BIT), Octylisothiazolinone (OIT, OI), Dichlorooctylisothiazolinone (DCOIT, DCOI) or Butylbenzisothiazolinone (BBIT).

3. The composition of claim 1, wherein the silver particles are present in an amount from about 0.01% to about 60% by total weight of the particular treatment composition, optionally, from about 0.05% to about 40%; optionally, from about 0.1% to about 30%.

4. The composition of claim 1, wherein the silver particles are present in an amount from about 0.001 % to about 60% of the weight of the fabric (owf), optionally from about 0.005% to about 30% owf, more preferably from about 0.01% to about 1% owf,

5. The composition of claim 1 further comprising a binder material.

6. The composition of claim 5 wherein the binder material is selected from a polyurethane-based binding agent or other binders, such as a permanent-press type resin or an acrylic type resin, or combinations thereof.

7. A textile material comprising the composition having antimicrobial and antiviral activity of claims 1-6.

8. A method of applying a composition having antimicrobial and antiviral activity to a textile material, the method comprising applying the composition of claims 1-6 to the textile material.

9. Method, according to claim 8, further comprising the steps of passing the textile material through a padder, drying the textile material, annealing the textile material and washing and drying the textile material.

10. The method of claims 8, wherein the composition is applied through spraying, dipping, padding, foaming, and the like.

11. The method of claim 8, wherein the amount of silver to the target substrate is 10 ppm or higher.

12. The method of claim 8, wherein the amount of silver to the target substrate is 50 ppm or higher.

13. Use of the composition having antimicrobial and antiviral activity according to claims 1-6 for providing topical antimicrobial and antiviral finish to textile materials.

14. Suitable textile materials of claim 7 for receiving a topically applied silver-based antiviral finish include, without limitation, fibers, yarns, and fabrics. Fabrics may be formed from fibers such as synthetic fibers, natural fibers, or combinations thereof.

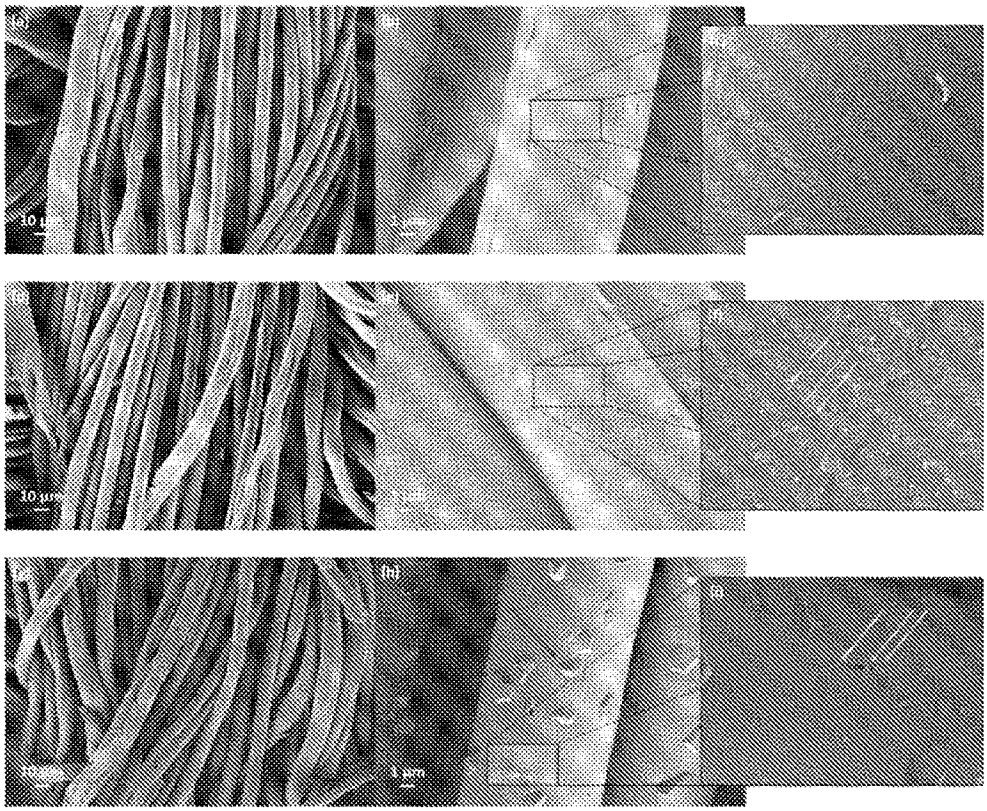
15. Suitable fabrics of claim 13 for receiving a topically applied silver-based antiviral finish may be formed from fibers or yarns of any size, including microdenier fibers and yarns (fibers or yarns having less than one denier per filament). The fibers or yarns may have deniers that range from less than about 1 denier per filament to about 2000 denier per filament or more preferably, from less than about 1 denier per filament to about 500 denier per filament, or even more preferably, from less than about 1 denier per filament to about 300 denier per filament.

16. Suitable fabrics of claim 13 for receiving a topically applied silver-based antiviral finish may be partially or wholly comprised of multi-component or bi-component fibers or yarns which may be splittable along their length by chemical or mechanical action. The fabric may be comprised of fibers such as staple fiber, filament fiber, spun fiber, or combinations thereof.

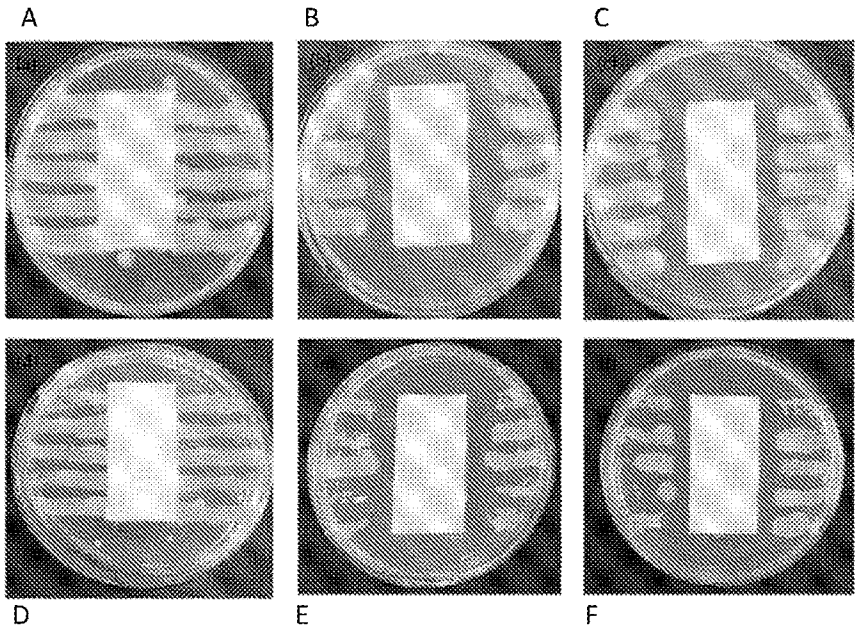
17. Suitable fabrics of claim 13 for receiving a topically applied silver-based antiviral finish may be of any variety, including but not limited to, woven fabric, knitted fabric, nonwoven fabric, or combinations thereof.

18. Suitable fabrics of claim 13 for receiving a topically applied silver-based antiviral finish may optionally be colored by a variety of dyeing techniques, such as high temperature jet dyeing with disperse dyes, thermosol dyeing, pad dyeing, transfer printing, screen printing, or any other technique that is common in the art for comparable, equivalent, traditional textile products.

19. Use of the textile material of claim 7 for manufacturing clothes, personal protection equipment and other textile-based products.



FIGS. 1A-1I



FIGS. 2A-2F

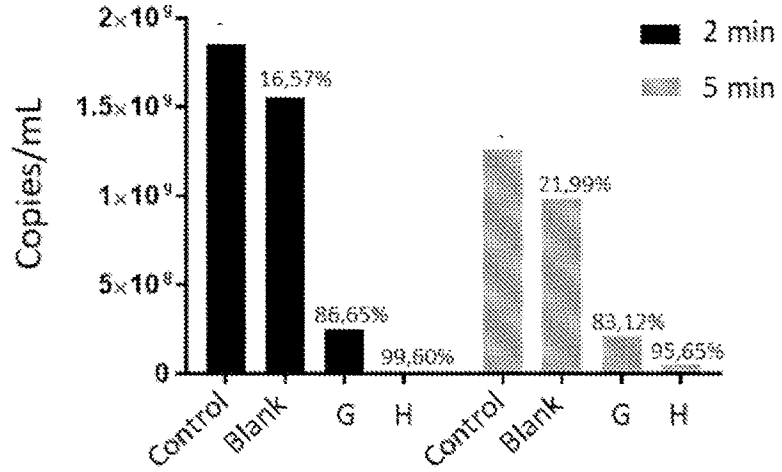

2×10⁹
1.5×10⁹
1×10⁹
5×10⁸
0
Copies/mL
2 min
5 min
16,57%
86,65%
99,60%
21,99%
83,12%
99,65%
Control
Blank
G
H
Control
Blank
G
H

FIG. 3

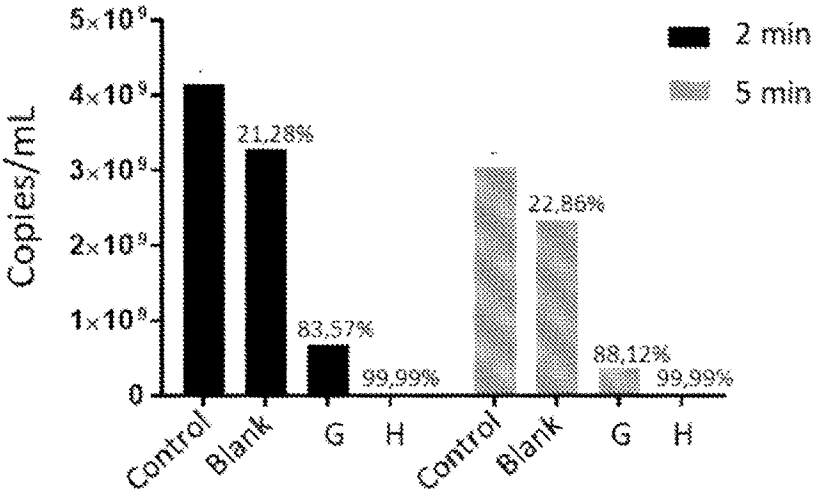

5×10⁹
4×10⁹
3×10⁹
2×10⁹
1×10⁹
0
Copies/mL
2 min
5 min
21,28%
83,57%
99,99%
22,88%
88,12%
99,99%
Control
Blank
G
H
Control
Blank
G
H

FIG. 4

RELATÓRIO DE PESQUISA INTERNACIONAL

Depósito internacional Nº
PCT/BR2021/050256

A. CLASSIFICAÇÃO DO OBJETO
(IPC) A01N 43/80 (2006.01), A01N 59/16 (2006.01), A61K 33/38 (2006.01)
(CPC) A01N 43/80, A01N 59/16, A61K 33/38
De acordo com a Classificação Internacional de Patentes (IPC) ou conforme a classificação nacional e IPC

B. DOMINIOS ABRANGIDOS PELA PESQUISA

Documentação mínima pesquisada (sistema de classificação seguido pelo símbolo da classificação)

A01N, A61K

Documentação adicional pesquisada, além da mínima, na medida em que tais documentos estão incluídos nos domínios pesquisados

INPI-BR Patents Database, GOOGLE

Base de dados eletrônica consultada durante a pesquisa internacional (nome da base de dados e, se necessário, termos usados na pesquisa)

EPODOC

C. DOCUMENTOS CONSIDERADOS RELEVANTES

Categoria*	Documentos citados, com indicação de partes relevantes, se apropriado	Relevante para as reivindicações Nº
X	BR PI0615286 A2 (THOR GMBH [DE]) 17 de maio de 2011 (17.05.2011) Ver pag. 3, l. 27 - pag. 4, l. 5; pag. 5, l. 31 – pag. 6, l. 4; pag. 6, l. 10-16; pag. 8, l. 6-12.	1, 2, 3, 4

X Y	US 2006068024 A1 (SCHROEDER [US]) 30 March 2006 (2006-03-30) See par. [0017], [0019], [0021], [0022], [0025], [0031], [0041] Claims 41, 43.	5, 6, 8, 14 7, 10

X Y	EP 1490543 A2 (CC TECHNOLOGY INVEST CO LTD [CN]) 29 de dezembro de 2004 (29.12.2004) Ver par. [0001], [0020], [0021], [0047], [0048], [0049], [0054], [0072].	4, 8, 9, 13, 14, 17, 18 7, 10

☒ Documentos adicionais estão listados na continuação do Quadro C ☒ Ver o anexo de família da patentes

* Categorias especiais dos documentos citados:

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"&" documento membro da mesma família de patentes.

Data da conclusão da pesquisa internacional	Data do envio do relatório de pesquisa internacional:
09/08/2021	31/08/2021
Nome e endereço da ISA/**BR** Nº de fax:	Funcionário autorizado Nº de telefone:

Categoria*	Citação do documento com indicação de partes relevantes, quando apropriado	Relevante para as reivindicações Nº
X Y	São Paulo Government, "Pesquisa da Unicamp cria tecido que reduz casos de infecção hospitalar". Available in: https://saopaulo.sp.gov.br/ultimas-noticias/pesquisa-da-unicamp-cria-tecido-que-reduz-casos-de-infeccao-hospitalar/ 06/11/2007. acesso em 09/08/2021.	19 7

Y	SBT Brasil TV News: "Novo tipo de tecido é capaz de matar o novo coronavírus em até 15 minutos", exibihited in 11/06/2020. Transcription available in https://www.sbt.com.br/jornalismo/sbt-brasil/noticia/143188-novo-tipo-de-tecido-e-capaz-de-matar-o-novo-coronavirus-em-ate-15-minutos acesso em 09/08/2021.	7

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		US 2008227766 A1	2008-09-18
		WO 2007026004 A2	2007-03-08
		ZA 200801849 B	2009-10-28
US 2006068024 A1	2006-03-30	EP 1793861 A1	2007-06-13
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		US 2008026028 A1	2008-01-31
		WO 2006036909 A1	2006-04-06
EP 1490543 A2	2004-12-29	EP 1490543 B1	2006-05-10
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		CN 100460590 C	2009-02-11
		DE 60305172 D1	2006-06-14
		TW 200504262 A	2005-02-01
		TW I244522 B	2005-12-01
		US 2003190851 A1	2003-10-09
		US 6979491 B2	2005-12-27
		WO 03080911 A2	2003-10-02

INTERNATIONAL SEARCH REPORT

International application N°
PCT/BR2021/050256

A. CLASSIFICATION OF SUBJECT MATTER

(IPC) A01N 43/80 (2006.01), A01N 59/16 (2006.01), A61K 33/38 (2006.01)
(CPC) A01N 43/80, A01N 59/16, A61K 33/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01N, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

INPI-BR Patents Database, GOOGLE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claims N°
X	BR PI0615286 A2 (THOR GMBH [DE]) 17 May 2011 (2011-05-17) See pag. 3, l. 27 - pag. 4, l. 5; pag. 5, l. 31 – pag. 6, l. 4; pag. 6, l. 10-16; pag. 8, l. 6-12.	1, 2, 3, 4
X Y	US 2006068024 A1 (SCHROEDER [US]) 30 March 2006 (2006-03-30) See par. [0017], [0019], [0021], [0022], [0025], [0031], [0041] Claims 41, 43.	5, 6, 8, 14 7, 10
X Y	EP 1490543 A2 (CC TECHNOLOGY INVEST CO LTD [CN]) 29 December 2004 (2004-12-29) See par. [0001], [0020], [0021], [0047], [0048], [0049], [0054], [0072].	4, 8, 9, 13, 14, 17, 18 7, 10

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

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"&" document member ot the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09/08/2021	31/08/2021

Name and mailing address of the ISA/**BR**	Authorized officer
INPI INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL Rua Mayrink Veiga nº 9, 6º andar cep: 20090-910, Centro - Rio de Janeiro/RJ	**Paula Salles de Oliveira Martins**
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claims N°
X Y	São Paulo Government, "Pesquisa da Unicamp cria tecido que reduz casos de infecção hospitalar". Available in: https://saopaulo.sp.gov.br/ultimas-noticias/pesquisa-da-unicamp-cria-tecido-que-reduz-casos-de-infeccao-hospitalar/ 06/11/2007. Access in 09/08/2021.	19 7
Y	SBT Brasil TV News: "Novo tipo de tecido é capaz de matar o novo coronavírus em até 15 minutos", exibihited in 11/06/2020. Transcription available in https://www.sbt.com.br/jornalismo/sbt-brasil/noticia/143188-novo-tipo-de-tecido-e-capaz-de-matar-o-novo-coronavirus-em-ate-15-minutos Access in 09/08/2021.	7

Patent documents cited in search report	Publication date	Patent family members	Publication date
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		CN 101252841 A	2008-08-27
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		US 2008227766 A1	2008-09-18
		WO 2007026004 A2	2007-03-08
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		TW 200624627 A	2006-07-16
		US 2008026028 A1	2008-01-31
		WO 2006036909 A1	2006-04-06
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