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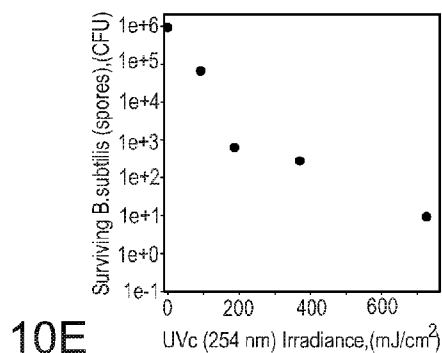
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(57) Abstract: A method of sterilising a polymer comprises providing a polymer and exposing the polymer to UVc radiation to reduce the bioburden, for example to reduce the bioburden to a SAL value of 10^{-6} . The method may be used to sterilise polymers, particularly biopolymers, against bacterial and fungal cells and spores. An apparatus for sterilising a polymer and a packaged polymer is also described.



STERILISATION

This invention relates generally to sterilisation. More specifically, although not exclusively, this invention relates to a method of sterilising polymers, e.g. biopolymers.

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Biopolymers, e.g. hydrogels, have shown promising potential for use in many medical and pharmaceutical applications such as wound care, drug delivery systems, and tissue regeneration. However, a barrier to the adoption of biopolymers as components in these applications is the difficulty and lack of appropriate and available methods for their sterilisation.

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Sterilisation is defined as a process intended to remove or destroy all viable forms of microbial life, including bacterial spores. In contrast, disinfection is the destruction of pathogenic and other kinds of microorganisms by thermal or chemical means. Disinfection is a less lethal process than sterilisation because it destroys most recognised pathogenic microorganisms, but not necessarily all microbial forms, such as bacterial spores. Disinfection processes do not ensure the margin of safety associated with sterilisation processes (Disinfection, Sterilization, and Preservation by Seymour Stanton Block, Lippincott Williams & Wilkins, Fifth Edition, 2001).

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The terms 'sterilisation' and 'disinfection' are often wrongly interchanged, which leads to their incorrect usage in the prior art.

Components and materials for use in medical and pharmaceutical applications such as implantable or topical treatments must be completely sterile to receive appropriate regulatory approval to bring the product to market. In general, components and materials for use in implantable or topical applications, require a sterility levels of SAL (Sterility Assurance Level) of 10^{-6} , the SAL being the probability that a single unit that has been subjected to sterilization nevertheless remains nonsterile. As such, a SAL of 10^{-6} is the probability that there is a one in a million (1:1000000) chance of a non-sterile unit.

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The conventional methods of sterilisation include the use of heat, pressure, filtering, chemicals, or irradiation. However, some polymers, e.g. biopolymers, are thermally and chemically unstable in the presence of heat and ionising radiation, and consequently, such conventional methods are not appropriate for their sterilisation.

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Moreover, it is of interest to be able to suspend actives, *e.g.* therapeutics such as proteins and/or small molecules, in polymers such as biopolymers, *e.g.* in hydrogel matrices, for medical and for pharmaceutical uses. Conventional methods of sterilisation often lead to chemical damage or destruction of such actives suspended in, for example, biopolymers, which alters, *e.g.* inhibits, their activity and/or degrades their structure into toxic products, precluding such use in the manufacture of commercial products.

Autoclaving is a conventional sterilisation process that uses elevated temperatures. However, biopolymers often comprise cleavable bonds that are thermally unstable, which leads to thermal degradation, rendering them unsuitable for use in the intended medical application. In addition, it is known that hydrogel systems mechanically breakdown in the presence of elevated temperatures.

The use of ionising radiation such as gamma radiation (*e.g.* at 12 to 25 kGy) and X-rays (*e.g.* at 3 kGy) in sterilisation procedures of biopolymers leads to the formation of free radicals, which causes chemical damage such as cleavage of monomers and/or oxidation of the biopolymer molecular structure.

The use of ethylene oxide (ETO) for sterilisation relies on contact of ETO with microorganisms. Consequently, this process is inconvenient because the biopolymer component must be unpackaged before undergoing sterilisation and must be repackaged after the process has finished. The process of repacking must be performed in an aseptic environment to ensure that the biopolymer is not contaminated post-sterilisation. Moreover, care must be taken to ensure that no significant amounts of ETO remain in the biopolymer, which may be toxic and/or cause undesired side-effects.

Therefore, it is known that conventional sterilisation methods are unsuitable for use on many types of biopolymers, which poses a major barrier to the translation of say new wound care devices and/or drug delivery vehicles and/or treatment regimes.

An alternative method of achieving sterility of components for use in medical devices is sterile manufacture, which requires elaborate technical equipment in a dedicated facility, and the implementation of stringent processes to ensure that the sterile environment within the facility is maintained. Therefore, sterile manufacture has huge set-up costs, is complicated and expensive to run, and requires specific levels of expertise to maintain.

It is therefore a first non-exclusive object of the invention to provide a method of sterilising polymers, e.g. biopolymers, particularly those polymers such as biopolymers that are or may be thermally and chemically unstable to conventional sterilisation techniques, which does not result in thermal and/or chemical degradation of the polymer, and that achieves a SAL value of 10^{-6} .

Accordingly, a first aspect of the invention provides a method of sterilising a substance, for example a polymer, the method comprising providing substance, for example a polymer, exposing the substance, for example the polymer, to UVc radiation for sufficient time to reduce the bioburden, for example to reduce the bioburden to a SAL value of 10^{-6} .

The polymer may be a biopolymer. In this specification, the term “biopolymer” is taken to mean a polymer comprising monomers wherein said monomers are derived from molecules produced naturally and/or by living organisms. The biopolymer may be naturally-derived (e.g. present in or produced by a living organism) or may be a synthetic biopolymer (e.g. chemically synthesised from naturally occurring monomers and/or modified naturally occurring monomers). The biopolymer may comprise both naturally occurring monomers and synthetically modified naturally occurring monomers. An example of a biopolymer is polysaccharide.

UV light has been used as a disinfection method, for example, in microbiology protocols, in lab water systems, and in food production. The use of UV light in these applications is known to reduce the viability of most recognised pathogenic microorganisms, but not all microbial forms, such as bacterial spores. Therefore, this known use of UV light in disinfection is not a method of sterilisation.

Moreover, the use of UV light in disinfecting polymers in prior art applications is performed as a precautionary measure. Disinfection using UV light is performed on biomaterials that are already expected to be substantially sterile, for example, having been previously manufactured under GMP (good manufacturing practice) using microbial filters, but may have become trace contaminated since being produced and/or unpackaged. The maximum bioburden expected in these scenarios may be in the range of say less than or equal to 10 CFU/100 mL (0.1 CFU/mL) (CFU = colony-forming units).

In contrast, the method of the invention is for use in sterilising polymers that have not undergone other disinfection or sterilisation procedures and/or have not been manufactured in a sterile environment. In this application, we call these 'nascent polymers'. Accordingly, the invention provides a method of sterilising a nascent polymer, the method comprising
5 providing a nascent polymer, exposing the nascent polymer to UVc for sufficient time to reduce the bioburden to a SAL value of 10^{-6} .

It has been surprisingly found by the inventors that the method of the invention may be used to sterilise polymers, e.g. nascent polymers, particularly those polymers, e.g. biopolymers,
10 that are thermally and chemically unstable to conventional sterilisation techniques, which does not result in thermal and/or chemical degradation of the polymer, and that achieves a SAL value of 10^{-6} .

Therefore, the molecular structure of the polymer provided may be substantially unchanged
15 after exposing the polymer to UVc for sufficient time to reduce the bioburden to a SAL value of 10^{-6} .

More surprisingly, the method of the invention may be used to achieve a SAL value of 10^{-6} when starting from a high bioburden, for example, a bioburden of 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , or
20 10^6 CFUs (colony-forming units). At least the upper ranges of such a bioburden represents a significantly higher level of contamination than one would ever expect to be encountered during manufacture of GMP (good manufacturing practice) polymers and/or polymeric materials.

Therefore, the method of the invention has been shown to be highly effective at sterilising
25 polymers, e.g. nascent polymers, to a SAL value of 10^{-6} , without leading to degradation, even when targeting a polymer with a high bioburden, say of 10^6 CFUs.

For example, the bioburden of the polymer may be greater than 1, for example, greater
30 than 10, or greater than 10^2 , or greater than 10^3 , or greater than 10^4 , or greater than 10^5 , or greater than or equal to 10^6 CFU.

As we expect that bioburdens of, say, 10^6 CFU are rare in manufactured polymers, the sterilisation of such polymers to a SAL value of 10^{-6} clearly demonstrates the efficacy of the
35 method.

Moreover, the need to use sterile manufacturing methods is avoided in the production of sterile polymers.

5 This is particularly surprising because the use of UV light as a method of sterilisation, which can achieve a SAL of 10^{-6} has never been disclosed. For example, the UK MHRA (Medicines & Healthcare products Regulatory Agency) does not recognise UV light as a suitable method of sterilising components for use in the production of pharmaceuticals or medical devices.

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The UV light used in the present invention is UVc, which has a wavelength of between 100 to 280 nm. For example, the wavelength may be between any one of 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270 nm, to any one of 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110
15 nm. Preferably from 220 to 280 nm, say 240 to 270 nm. In a preferred embodiment, the UVc wavelength is about 254 nm, which is the wavelength of UVc emission of a mercury vapour lamp such that the invention is inexpensive and easy to operate.

Advantageously, the method of the invention is effective for the sterilisation of a wide range
20 of polymers, for example, biopolymers, e.g. nascent biopolymers.

Advantageously, the method of the invention may be used to sterilise transparent materials comprising or consisting of synthetic polymers. For example, the polymer may comprise poly(2-hydroxyethyl methacrylate) (pHEMA) or derivatives thereof, a silicone based
25 polymer, e.g. polydimethylsiloxane (PDMS) or blends thereof, (PDMS), and/or poly(methyl methacrylate) (PMMA) or derivatives thereof.

In particular, the method is applicable to shear thinning polymers, e.g. shear thinning nascent polymers. Shear thinning polymers may have particular utility in medical
30 applications because, as the name implies, shear thinning polymers thin (become less viscous) when subjected to shear strain.

The polymer may be non-ionic or ionic, e.g. cationic or anionic.

35 The polymer may be a biopolymer.

The biopolymer may be a polysaccharide, *i.e.* a polymeric carbohydrate comprising monosaccharide monomers, *e.g.* glucose, fructose, rhamnose, glucuronic acid, mannuronate, and/or guluronate.

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The biopolymer may comprise one or more of a natural, synthetic, or synthetically modified agar, agarose, arabinoxylan, carrageenan, gelatin, gellan gum, glucan, carboxy cellulose, curdlan, pectin, xanthan gum, gum arabic, guar gum, locust bean gum, gum tragacanth, gum karaya, cellulose and derivatives thereof, alginate, fibrin, starch, chitosan, dextran, collagen and hyaluronic acid, or salts (*e.g.* mono or polyvalent, *e.g.* divalent or trivalent, salts) or derivatives of the above and/or combinations thereof.

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The biopolymer may comprise or consist of gellan gum. Gellan gum is a water-soluble anionic polysaccharide comprising two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid.

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The biopolymer may comprise or consist of alginate. Alginate is an anionic polysaccharide comprising homopolymeric blocks of (1-4)-linked β -D-mannuronate (M) and its C-5 epimer α -L-guluronate (G) residues.

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The biopolymer may comprise or consist of cellulose. Cellulose is an anionic polysaccharide comprising D-glucose residues.

The biopolymer may comprise chitosan. Chitosan is a linear polysaccharide composed of randomly distributed β -(1 $\rightarrow$ 4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

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The biopolymer for use in the method of the invention may comprise one chemically distinct biopolymer, *e.g.* gellan gum, or may comprise more than one chemically distinct biopolymer, *e.g.* a mixture of gellan gum and agarose.

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The biopolymer may comprise polymer chains that may be crosslinked or crosslinkable. The biopolymer may comprise polymer chains that may be chemically crosslinked (*i.e.* by ionic or covalent bonds) or physically crosslinked (*i.e.* by intermolecular interactions such as hydrogen bonds).

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The polymer, e.g. biopolymer, may be a quiescent gel, that is, a gel that has not been sheared on gelling and that make a single continuous gelled matrix.

5 The polymer, e.g. biopolymer, may be a hydrogel. The polymer may be a polymer gel, for example a polymer gel constructed of a network of crosslinked hydrophilic polymer chains. The polymer may be a fluid gel. Fluid gels are formed by the application of a shear force to a gelling solution. For polymers that are subject to thermally reversible transitions it is possible to control the final physical properties of the fluid gel by applying shear and
10 controlling the rate of cooling. Thus, there is a competition between the formation of the gel and the physical destruction of the gel which can alter the as-formed particle size. Fluid gels, may have a network of physically crosslinked hydrophilic polymer chains, *i.e.* a network of polymer chains that are crosslinked by electrostatic interactions, that is capable of flowing as a fluid.

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The hydrogel, polymer gel or fluid gel may comprise 0.1 to 5.0 wt.% (e.g. 0.1 to 3.5 wt.%, 0.1 to 2.5wt.%) polymer.

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In some embodiments the polymer is dehydrated. For example, the polymer may be a dehydrated gel. Surprisingly, it has been found that UV light is able to penetrate into the collapsed polymer network of a dehydrated gel and provide sterilisation.

Preferably, the polymer is a fluid gel. Preferably the gel is crosslinked prior to be subjected to sterilisation.

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The polymer may comprise an active substance, *e.g.* loaded, suspended or located in and/or bonded to the polymer. The active substance may comprise a small molecule, *e.g.* a pharmaceutical drug, and/or a protein, *e.g.* an enzyme, a co-factor.

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The active substance may be a medicament, *i.e.* an active substance used for medical treatment. The medicament may be an antimicrobial agent, an anti-inflammatory agent, an antipyretic, an analgesic, an antimalarial, an antiseptic, a mood stabiliser, and/or a hormonal agent, *e.g.* a contraceptive medicament or a hormone replacement. The medicament may be one or more of a small molecule pharmaceutical compound, an
35 antibody, a vaccine, or any other substance used for medical treatment that may be capable

of being absorbed or adsorbed into a polymer. In embodiments, the active substance is decorin.

It has been surprisingly found that the method of the invention may successfully be used to
5 sterilise a polymer, e.g. a nascent polymer, containing an active substance without causing physical or chemical alteration of the active substance, such that the activity of the active substance is substantially the same as prior to being irradiated with UVc.

The polymer may be filtered, for example filtered prior or subsequent to contact with an
10 active substance or medicament. The filter may comprise a sterile filter. For example, a filter arranged to remove colony forming units and/or other foreign bodies. The polymer and active or medicament may be contacted together and stirred to form a solution, for example a homogenous solution. The polymer or the solution may be conveyed to packaging to provide a packaged polymer. The packaged polymer may be exposed to the UVc radiation
15 to effect sterilisation. Additionally or alternatively, the polymer may be conveyed along an at least partially UVc-transparent conduit and sterilisation effected as the polymer flows along the conduit, whereupon it is packaged in packaging to provide a packaged polymer. In either case, the polymer is contained when exposing the polymer to UVc for sufficient time to reduce the bioburden to a SAL value of 10^{-6} .

20 The method of the invention is a sterilisation method. Therefore, all microbial forms including spores are destroyed. Surprisingly, embodiments of the method of the invention has been shown to destroy, for example, *Bacillus pumilus* spores, which are particularly difficult to destroy using radiation due to the creation of a shadow during the microbial
25 growth of this microorganism.

The method may comprise exposing the polymer to UVc at a sufficient irradiance (measured in mW/cm^2) to reduce the bioburden to an SAL value of 10^{-6} .

30 The method may comprise irradiating the polymer with sufficient energy (measured in mJ/cm^2) to reduce the bioburden to an SAL value of 10^{-6} . It will be appreciated that if the polymer is inside a container or package while it is exposed to UVc radiation, the energy received by the polymer must be sufficient to reduce the bioburden to an SAL value of 10^{-6} . Accordingly, the energy delivered must be adjusted in order to take into account any
35 absorption by the container or packaging.

As is known in the art, the D value is the exposure time required, at a set irradiance (energy (J) per cm² per second), to reduce the number of CFUs in the polymer by 1-log. This may also be expressed as the energy (i.e. dose) required to reduce the number of CFUs in the polymer by 1-log. Therefore, to reduce the bioburden to a SAL value of 10⁻⁶ from a starting bioburden of 1x10^x, the amount of energy that must be delivered to the polymer will be approximately equal to: (6+x) multiplied by the D value. For example, if it is assumed that the bioburden prior to sterilisation is 10⁶, then in order to achieve a SAL value of 10⁻⁶ (i.e. a reduction of 12-log), the polymer must be exposed to energy equivalent to the D value multiplied by 12.

In some embodiments, the polymer is irradiated with UVC such that the polymer receives a radiant exposure of at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 750, at least 1000, at least 1500, at least 2000, at least 2500, at least 3000, at least 3500, or at least 4000 mJ/cm² (e.g. about 4092 mJ/cm²).

By 'radiant exposure', we mean the radiant energy received by a surface per unit area.

The time, irradiance and/or energy sufficient to reduce the bioburden of the polymer to an SAL value of 10⁻⁶ may vary depending on several factors including the initial bioburden of the polymer, the type(s) of microorganism to be destroyed, the type, shape, and/or construction of the polymer, and/or composition of the polymer.

In some embodiments, the polymer is irradiated with UVC for at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8 minutes, at least 9 minutes, at least 10 minutes, at least 11 minutes or at least 12 minutes.

In addition, the time and/or irradiance sufficient to reduce the bioburden of the polymer to an SAL value of 10⁻⁶ will vary depending on whether the polymer is irradiated directly with UVC. Alternatively, the polymer may be irradiated during a manufacturing process, wherein the UVC also needs to penetrate further layers of material, e.g. a vessel or container.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) by removal of any microbes which may be present, such as bacteria, fungi and/or spores.

In some embodiments, the method is for sterilising a polymer against bacteria. The method may be for sterilising a polymer against Gram negative bacteria, e.g. *E. coli*. The method may additionally, or alternatively, may be for sterilising a polymer against Gram positive bacteria, e.g. *Bacillus* species, or *Staphylococcus aureus*.

In some embodiments, the method is for sterilising a polymer against fungi, including yeast, e.g. *C. albicans*.

In some embodiments, the method is for sterilising a polymer against fungal and/or bacterial spores, such as *Bacillus subtilis* and/or *Bacillus pumilus* spores.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) against *Bacillus pumilus* to a sterility assurance level (a SAL value) of 10^{-6} . In some embodiments, the method may comprise delivering a radiant exposure (i.e. total energy) (to the polymer itself, i.e. taking into account any absorption by packaging which may be present) of greater than or equal to 290, 300, 330, 350, 450 or 500 mJ/cm² (e.g. greater than or equal to 501 mJ/cm²). The radiant exposure may be no more than 1500, 1200, 1000, 800 or 600 mJ/cm². In some embodiments, this may be achieved by exposing the polymer to UVC for a period of time of greater than or equal to 9 minutes (e.g. greater than or equal to 9 minutes and 28 seconds).

For *Bacillus pumilus*, the D value may be greater than or equal to 10, 20, 30 or 40 mJ/cm² (e.g. greater than or equal to 42 mJ/cm²). The D value may be no more than 100, 80 or 60 mJ/cm². In some embodiments, this may correspond to exposing the polymer to UVC for a period of time of greater than or equal to 40 seconds, or 45 seconds, or 47 seconds.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) against *Escherichia coli* (*E. coli*) to a sterility assurance level (a SAL value) of 10^{-6} . In some embodiments, the method may comprise delivering a radiant exposure to the polymer of greater than or equal to 300, 320, 350, 400, 450, 480, 500, 530 or 550 mJ/cm² (e.g. greater than or equal to 552 mJ/cm²). The radiant exposure may be no more than 1500, 1200, 1000, 800 or 600 mJ/cm². In some embodiments, this may correspond to exposing the polymer to UVC for a period of time of greater than or equal to 10 minutes (e.g. greater than or equal to 10 minutes and 13 seconds).

For *Escherichia coli* (*E. coli*), the D value may be greater than or equal to 10, 20, 30 or 40 mJ/cm² (e.g. greater than or equal to 42, 44 or 46 mJ/cm²). The D value may be no more than 100, 80, 60 or 50 mJ/cm². In some embodiments, this may correspond to exposing the polymer to UVC for a period of time of greater than or equal to 45 seconds, or 47 seconds, or 49 seconds, or 50 seconds, or 51 seconds.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) against *Candida albicans* (*C. albicans*) to a sterility assurance level (a SAL value) of 10⁻⁶. In some embodiments the method may comprise delivering a radiant exposure to the polymer of greater than or equal to 280, 320, 350, 400, 430, 450, 470 or 490 mJ/cm² (e.g. greater than or equal to 492 mJ/cm²). The radiant exposure may be no more than 1500, 1200, 1000, 800 or 600 mJ/cm². In some embodiments, this may be achieved by exposing the polymer to UVC for a period of time of greater than or equal to 9 minutes (e.g. greater than or equal to 9 minutes and 16 seconds).

For *Candida albicans* (*C. albicans*), the D value may be of greater than or equal to 10, 20, 30 or 40 mJ/cm² (e.g. greater than or equal to 41 mJ/cm²). The D value may be no more than 100, 80, 60 or 50 mJ/cm². In some embodiments, this may correspond to exposing the polymer to UVC for a period of time of for greater than or equal to 40 seconds, or 42 seconds, or 44 seconds, or 46 seconds.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) against spores of *Bacillus subtilis* (*B. subtilis*) to a sterility assurance level (a SAL value) of 10⁻⁶. In some embodiments the method may comprise delivering a radiant exposure to the polymer of greater than or equal to 1200, 1500, 1800, 2000, 2100 or 2150 mJ/cm² (e.g. greater than or equal to 2171 mJ/cm²). The radiant exposure may be no more than 4000, 3000, 2500, 2300 or 2200 mJ/cm². In some embodiments, this may be achieved by exposing the polymer to UVC for a period of time of greater than or equal to 4 minutes (e.g. greater than or equal to 4 minutes and 46 seconds).

For *Bacillus subtilis* (*B. subtilis*) spores, the D value may be greater than or equal to 100, 130, 150, 170 or 180 mJ/cm² (e.g. greater than or equal to 181 mJ/cm²). The D value may be no more than 300, 250 or 200 mJ/cm². In some embodiments, this may correspond to

exposing the polymer to UVc for a period of time of greater than or equal to 20 seconds, or 21 seconds, or 22 seconds, or 23 seconds, or 23.8 seconds.

The method of the invention may be suitable for sterilising a polymer (e.g. a biopolymer) against spores of *Bacillus pumilus* (*B. pumilus*) to a sterility assurance level (a SAL value) of 10^{-6} . In some embodiments the method may comprise delivering a radiant exposure to the polymer of greater than or equal to 2300, 2500, 2700, 3000, 3500, 3800, or 4000 mJ/cm² (e.g. greater than or equal to 4092 mJ/cm²). The radiant exposure may be no more than 8000, 6000, 5000, 4500 or 4200 mJ/cm². In some embodiments, this may be achieved by exposing the polymer to UVc for a period of time of greater than or equal to 10 minutes (e.g. greater than or equal to 11 minutes).

For *B. Pumilus*, the D value of greater than or equal to 250, 270, 300, 330 mJ/cm² (e.g. greater than or equal to 340, or 341 mJ/cm²). The D value may be no more than 600, 500, 400, 300 or 350 mJ/cm². In some embodiments, this may correspond to exposing the polymer to UVc for a period of time of greater than or equal to 50 seconds, or 51 seconds, or 52 seconds, or 53 seconds, or 54 seconds, or 54.9 seconds.

Advantageously, a method suitable for sterilising a polymer (e.g. a biopolymer) against spores of *Bacillus pumilus* (*B. pumilus*) to a sterility assurance level (a SAL value) of 10^{-6} is believed to be effective for sterilisation of the polymer against substantially all microorganisms. This is because *B. pumilus* and its spores are particularly difficult to destroy using radiation due to the creation of a shadow during the microbial growth of this microorganism. Therefore, the energy required to sterilise the polymer (e.g. the biopolymer) is higher than for other microorganisms. Consequently, the method according to this embodiment may be assumed to be effective for sterilisation against substantially all microbial growth within in a polymer (e.g. a biopolymer).

In embodiments, the polymer is contained within a container, for example, a hermetically sealed container. In embodiments, the polymer is a biopolymer and/or a fluid gel and/or a hydrogel.

The radiation source may be a monochromatic UVc radiation source.

The UVc source may be a germicidal lamp, or a UV sterilisation chamber.

Other factors include whether the UVc sterilisation occurs as a terminal sterilisation process, that is, sterilisation in a primary packaging and/or a secondary packaging. The composition, shape, dimensions of the primary and/or secondary packaging, the fill volume, the orientation of the primary and/or secondary packaging, the spatial arrangement of the primary and/or secondary packaging, the ability of the primary and/or secondary packaging to absorb light, and/or the potential of the packaging to create a shadow over the polymer are all factors that will all affect the time and/or irradiance sufficient to reduce the bioburden of the polymer to an SAL value of 10^{-6} .

Alternatively, the polymer may be sterilised in bulk form, before further processing, e.g. as a bulk drug product. The path length of the polymer, e.g. the depth of the polymer material, the transparency of the gel, the incorporation of other substances and species that may scavenge, absorb, and/or scatter light are all factors that will all affect the time and/or irradiance sufficient to reduce the bioburden of the polymer to a SAL value of 10^{-6} .

The method of sterilisation may be performed as a batch process, e.g. on a bulk polymer material. The method of sterilisation may be performed as an inline and/or continuous process. In a preferred embodiment, the polymer is a fluid gel that is able to flow inline through a reactor.

A further aspect of the invention provides an apparatus for sterilising a polymer, e.g. a nascent polymer, the apparatus comprising a vessel for locating a polymer therein, and a source of UVc light.

The apparatus may be used to perform the method according to the first aspect of the invention.

The vessel may be a tube, e.g. a tube with a first end for locating unsterilised polymer, an intermediate portion for locating the polymer to be exposed to UVc, and a second end for locating sterilised polymer.

The apparatus may further comprise a means to convey the polymer from the first end of the tube to the second end of the tube, e.g. a pump, such that the method of the invention may be performed as a continuous process.

The apparatus may comprise a first holding tank for use in housing unsterilised polymer, and a second holding tank for use in housing the sterilised polymer, having undergone UVc treatment within the apparatus of the invention.

5

In a further aspect, there is provided an apparatus for sterilising a polymer to a sterility assurance level (a SAL value) of 10^{-6} , the apparatus comprising a (e.g. monochromatic) UVc light source configured to provide UVc radiation (e.g. at a wavelength of between 240-280 nm, for example, 254 nm) to the polymer.

10

The apparatus may be for sterilising a polymer against the spores of *Bacillus pumilus* to a sterility assurance level (a SAL value) of 10^{-6} . In some embodiments, the UVc light source is configured to deliver to the polymer a radiant exposure (e.g. total energy) of at least 3600, at least 3800, or at least 4000 mJ/cm² (e.g. about 4092 mJ/cm²).

15

Advantageously, delivering an energy of at least 4092 mJ/cm² to the polymer ensures that the spores of *Bacillus pumilus* are eradicated. It is believed that an apparatus that is suitable for use in eradication of the spores of *Bacillus pumilus*, is also suitable for use in eradication of substantially all other microorganisms.

20

The apparatus may comprise a housing for location of the polymer. The polymer may be located in the housing whilst UVc radiation is delivered to the polymer.

The apparatus may further comprise a means to convey the polymer from the first end of the tube to the second end of the tube.

25

The apparatus may further comprise a controller for controlling the energy delivered by the UVc light source to the polymer. The controller may control the duration and/or irradiance of the UVc radiation such that a desired total energy is delivered.

30

A further aspect of the invention provides a polymer having a SAL of 10^{-6} . Preferably, the polymer has located therein an active substance.

Within the scope of this application it is expressly intended that the various aspects, embodiments, examples and alternatives set out in the preceding paragraphs, in the claims

35

and/or in the following description and drawings, and in particular the individual features thereof, may be taken independently or in any combination. That is, all embodiments and/or features of any embodiment can be combined in any way and/or combination, unless such features are incompatible. For the avoidance of doubt, the terms “may”, “and/or”, “e.g.”, “for example” and any similar term as used herein should be interpreted as non-limiting such that any feature so-described need not be present. Indeed, any combination of optional features is expressly envisaged without departing from the scope of the invention, whether or not these are expressly claimed. The applicant reserves the right to change any originally filed claim or file any new claim accordingly, including the right to amend any originally filed claim to depend from and/or incorporate any feature of any other claim although not originally claimed in that manner.

Embodiments of the invention will now be described by way of example only with reference to the accompanying drawings in which:

Figure 1 is a flow diagram showing a method of UVc sterilisation of gellan fluid gels, according to Examples 1 to 4 of the invention; and

Figure 2 is a series of photographs of culture plates corresponding to Examples 1 to 4, and Controls 1 to 4 of the invention;

Figure 3A is a series of photographs of a gellan patch corresponding to Example 5 of the invention after irradiation with UVc at time points between 0 to 5 minutes;

Figure 3B is a series of graphs used to calculate the D value for UVc sterilisation of the gellan patch of Example 5;

Figure 4A is a schematic diagram of a UVc light box containing a rack of packaged biopolymers for use in the method of the invention;

Figure 4B is an intensity map showing the UVc light detected in the UVc light box by sensors within the packaged biopolymers of Figure 4A;

Figure 4C is a graph showing the UVc energy transmitted through the packaging of the packaged biopolymers at various locations within the rack of the UVc light box;

Figure 4D is a graph showing the UVc energy transmitted through the primary packaging and the secondary packaging of the packaged biopolymer;

Figure 4E shows photographic images of the experimental set up for the graph of Figure 4D;

Figure 4F is a graph showing the UVc energy transmitted through the packaging of the packaged biopolymers, when the packaging is empty and contains the biopolymer;

Figure 5 is a schematic illustration of an apparatus for use in a procedure for the fabrication of a gellan composition containing decorin of Example 6;

Figure 6A is a series of circular dichroism spectra showing the effects of UVc on the protein structure of decarin of Example 7;

Figure 6B is a series of dose-response curves showing the effects of UVc on the ability of decarin to control collagen fibrillogenesis of Example 7;

Figure 7A is a gel permeation chromatogram (GPC) for a control of gellan gum and gellan gum post-sterilisation with gamma radiation, according to Comparative Examples of the invention;

Figure 7B is a mass spectrum of gellan gum after irradiation with gamma rays, according to Comparative Examples of the invention;

Figure 7C is a series of selected enlarged regions of the mass spectrum shown in Figure 7B;

Figure 8 is a graph comparing the rheological behavior of a UV treated polymer sample with that of an untreated control sample;

Figure 9 is a graph comparing the viscoelastic behavior of a UV treated polymer sample with than of an untreated control sample;

Figure 10 is a series of dose-response curves which were used to calculate the D value for UVc sterilisation of a biopolymer against different microorganisms; and

Figure 11 is a series of microbiology plates containing different species of microorganisms, before and after exposure to UVc for 300 seconds.

The invention will now be exemplified with the following non-limiting Examples.

The following Examples 1 to 5 of the invention describe direct inoculation studies, which were performed to gain proof of concept that the method of the invention is a viable option to sterilise biopolymers. As an example, we used a gellan gel as a model.

Examples 1 to 4

Referring now to Figure 1, there is shown a flow diagram 10 showing a method of validation for the sterilisation of Examples 1 to 4 of the invention. In the flow diagram 10, there is shown Steps 1 to 9, which are described in more detail as follows:

Step 1: Culture microbes.

Referring now to Table 1, there is shown the microbes that were cultured, according to Examples 1 to 4 of the invention:

5

Table 1		
Example	Microbe	Description
1	<i>E. Coli</i>	Gram negative
2	<i>B. Subtilis</i>	Gram positive
3	<i>P. Aeruginosa</i>	Gram negative
4	<i>Candida Albicans</i>	Yeast

Step 2: Count and serial dilute microbes.

This was performed using either optical density (OD) measurements or using a haemocytometer. The microbes were serial diluted in order to obtain countable colony forming units (CFUs). A range from 10^4 to 10^2 was used. In other embodiments we use up to 10^6 .

10

Step 3: Centrifuge microbes into a pellet.

The microbes of Step 2 were centrifuged into a pellet.

15

Step 4: Prepare fluid gels.

Gellan fluid gels were used as a model biopolymer, which were prepared using sterile materials on lab bench in the following procedure using HyPure Cell Culture Grade Water.

20

- A gellan sol (1% (w/v)) was produced by adding 0.9 g of gellan to 90 ml of water.
- Using heated agitation (70 °C, 800 rpm for 1 hour) the gellan was allowed to dissolve.
- Once fully dissolved the heating was removed and 5 ml of NaCl (0.2 M) added.
- Following 5 ml of PBS was added to the solution.
- The system was allowed to cool under shearing (800 rpm) to result in a fluid gel with final compositions of 0.9% w/v polymer, 10 mM NaCl and 5% v/v PBS.

25

Step 5: Resuspend microbial pellet in fluid gel.

The microbial pellet comprising the respective microbe of Example 1 to 4 was resuspended individually in the gellan fluid gel prepared in Step 4 using a vortex mixer for 30 seconds.

30

This spiked the fluid gel with known colony forming units (CFUs) of microbes, ranging from 10^4 to 10^2 . The resulting spiked fluid gels were left for 24 hours.

Step 6: Package.

- 5 The spiked fluid gels comprising microbes were packaged into plastic tubes (REDIPAC(TM) single use polypropylene tubes manufactured by STELLA from Adelphi Healthcare Packaging of West Sussex, UK).

Step 7: Sterilisation: Irradiate using UVc (254 nm).

- 10 The plastic tubes containing the spiked fluid gels of Examples 1 to 4 were separately irradiated with UVc at 254 nm for a total of 5 minutes, 10 minutes, and 15 minutes (bioburden of 10^4 CFU).

- A set of identical spiked fluid gels were used as controls (bioburden of 10^4 CFU), which
15 were not irradiated with UVc. These correspond to Controls C1 to C4.

Step 8: Plate and incubate.

- The post-sterilisation spiked fluid gels of Examples 1 to 4, and of Controls C1 to C4, were plated onto TSA (trypticase soy agar) for Examples 1 to 3, and both TSA and SDA
20 (sabouraud dextrose agar) for Example 4.

The plates were incubated for 7 days.

Step 9: Image.

- 25 The plates of Examples 1 to 4 and Controls C1 to C4 were imaged by photography.

- Referring now to Figure 2, there is shown a series of images 20, of Examples 1 to 4, and Controls C1 to C4, from Step 9 of the flow diagram 10 of Figure 1. There is shown images 1 to 4, corresponding to Examples 1 to 4, and images C1 to C4, corresponding to Controls
30 C1 to C4.

The images 1 to 4 of Examples 1 to 4 show the microbial growth after incubation for 7 days, having been irradiated for a total of 10 minutes under UVc at 254 nm.

The images C1 to C4 of Controls 1 to 4 show the microbial growth with no sterilisation procedure.

It is shown in the images 1 to 4 of Examples 1 to 4 that there are no viable counts of microbes after 10 minutes of UVc irradiation. In contrast, the images C1 to C4 show considerable microbial growth.

In addition, the method of validation for the sterilisation shown in Figure 1 proves that the UVc radiation penetrates packaging, e.g. the plastics tubes used in step 6.

Example 5

The UV sterilisation of the invention was tested using the following procedure.

A gellan patch was fabricated in the following procedure.

1. Gellan gum (6 g) was dissolved in water (285 mL) under stirring and heating.
2. Once dissolved, Dulbecco's PBS (15 mL) was added.
3. The resulting solution (20mL) was cast into a square shaped tissue culture dish measuring 10cm x 10cm laterally.
4. The dish was swirled gently and placed on an orbital mixer plate to ensure even coating of the dish.
5. The resulting cast material was transferred to a vacuum oven pre-heated to 50 °C for 17 hours. The resulting thin dry film of polymer was lifted from the dish.
6. The cast dried dressings were individually packed in polyethylene envelopments and heat sealed.

The gellan patch was spiked with a bioburden of 10^{-6} CFU/ml of *B. pumilus*. Two methods of spiking were used to achieve a bioburden of 10^{-6} CFU. In the first method, the Dulbecco's PBS of Step 2 of the procedure described above contained 10^{-6} CFU/ml of *B. pumilus*. In the second method, the gellan patch was rehydrated with PBS comprising a bioburden of 10^{-6} CFU/ml of *B. pumilus* in a separate step (Step 7()) of the method described above.

B. pumilus is a gram-positive spore-forming bacillus, which is known to be both UV and desiccant resistant. It is used as a biological indicator for determining the efficacy of ionising sterilisation such as gamma irradiation.

The gellan patch was irradiated with UVc radiation at 254 nm for a total of 0 minutes, 0.5 minutes, 1 minute, 3 minutes, and 5 minutes.

Referring now to Figure 3A, there is shown a series of images 3A for the gellan patch after 0 minutes (image 31), after 0.5 minutes (image 32), after 1 minute (image 33), after 3 minutes (image 34), and after 5 minutes (image 35) of UVc irradiation. The images 3A were obtained by placing the sterilised gellan patch in sterile PBS. An aliquot of the PBS containing the sterilised gellan was then plated and cultured to determine the efficacy of the UVc sterilisation method.

The images were used to construct a series of lethality curves which are shown in the series of graphs of Figure 3B. The series of graphs 36-39 show the decrease in the number of surviving *B. pumilus* (measured in colony forming units, CFU) subsequent to irradiation with UVc.

There is provided graphs 36, 37 showing the decrease in the number of surviving *B. pumilus* as a function of irradiance (mW/cm²).

The graphs 36, 37 can be used to calculate that the irradiance must be 1961 mW/cm² or higher to reduce the bioburden to 10⁻⁶ CFU.

There is also shown graphs 38, 39 showing the decrease in the number of surviving *B. pumilus* as a function of time (minutes).

The MHRA require a defined D value, which is the time (or dose) required at a given condition (e.g. temperature), or set of conditions, to kill 90% of the exposed microorganisms. In other words, the D value is defined as the time (or dose) required to reduce the microbial population being considered by one logarithmic unit.

Using standard sterilisation kinetic theory, the D value was calculated for the system of Example 5.

$$\frac{N}{N_0} = 10^{-kt}$$

wherein:

N_0 = initial number of microorganisms

N = number of microorganisms after the exposure time, t

t = elapsed exposure time

k = reaction rate constant

The reaction rate constant, k , depends on the species of microorganism and the conditions for sterilisation.

In this specification, the D-value provided is the energy delivered to the polymer itself. All D-values have been corrected for packaging absorbance.

It is shown from the graphs 38, 39 that, using an irradiance of 3974.8 mW/cm², it takes 46 seconds to reduce the microbial population by one logarithmic unit, from 10⁶ CFU to 10⁵ CFU.

Therefore, using the log/linear relationship described above, it is calculated that the time required to reduce the microbial population from 10⁶ to 10⁻⁶ is 9 minutes and 26 seconds (47.22 seconds x 12 logarithmic units) for the gellan patch of Example 5.

Table 2				
logY	Time (mins)	Time (mm:ss)	t-interval between on log y	
6	0.00	0	0.79	00:47.2
5	0.79	00:47.2	0.79	00:47.2
4	1.57	01:34.5	0.79	00:47.2
3	2.36	02:21.7	0.79	00:47.2
2	3.15	03:09.0	0.79	00:47.2
1	3.94	03:56.2	0.79	00:47.2
0	4.72	04:43.5	0.79	00:47.2
-1	5.51	05:30.7	0.79	00:47.2
-2	6.30	06:17.9	0.79	00:47.2
-3	7.09	07:05.2	0.79	00:47.2
-4	7.87	07:52.4	0.79	00:47.2
-5	8.66	08:39.7	0.79	00:47.2
-6	9.45	09:26.9	0.79	00:47.2

Therefore, it has been surprisingly found that the method of the invention is able to sterilise a gellan patch containing 10⁶ CFU of *B. pumilus* to a sterility level (SAL) of 10⁻⁶ CFU.

This is surprising because *B. pumilus* is particularly difficult to destroy because a shadow is created by this type of microorganism, which, we believe, creates difficulties with radiation (and especially UV radiation) successfully reaching the bacteria at an energy that is high enough to destroy the bacterium completely.

Referring now to Figure 4A, there is shown a schematic diagram of a UVc light box 40 containing a rack 41 of packaged biopolymers 42. In this instance, the packaged biopolymers 42 comprise a biopolymer held in a sealed plastics tube. The UVc light box 40 comprises four UVc lamps, with two at the top of the chamber 43a and two at the bottom of the chamber 43b.

Referring also to Figure 4B, there is shown an intensity map 4B of the UVc light box 40 of Figure 4A. The intensity map 4B shows the UVc light detected by the sensors within the light box 40 and indicates that the intensity of light is not uniform across the light box 40 at various locations of the packaged biopolymers 42 on the rack 41. Indeed, in graphs 44a to 44d there is shown the effect of having a packaged biopolymer 42 at one of the indicated locations. The UVc light was detected at various locations on the rack 41 by sensors that were located both 'uncovered' on the rack 41, *i.e.* the sensor was not covered by any packaging, and sensors that were 'covered' by the packaging of the packaged biopolymer 42 on the rack 41. The graphs 44a to 44d show the UVc irradiance detected within the UVc light box 40 by both the uncovered and covered sensors and thus indicated the available UVc radiation to sterilise the sample biopolymer held within the packaged biopolymers 42.

Referring now to Figure 4C, there is shown a graph 4C showing the UVc irradiance detected within the UVc light box 40 by both the uncovered and covered sensors versus time, for various locations within the rack 41.

It is shown by graphs 44a to 44d, and graph 4C that, although the uncovered sensors detect a higher intensity of UVc light, the sensors covered by the packaging of the packaged biopolymers 42 detect sufficient UVc light energy to effect sterilisation irrespective of the position within the light box 40.

Referring now to Figure 4D, there is shown a graph showing the UVc irradiance detected within the UVc light box 40 by both the uncovered and covered sensors versus time, for

experimental runs in which a sensor S is covered by a single wall thickness of plastic material (47a) and for experimental runs in which the sensor S is covered by a packaging (empty) for a packaged biopolymer 42 (47b). The 'uncovered' left hand side of the graph shows that with the sensor uncovered the irradiance is, unsurprisingly, the same whereas in the 'covered' state' the single wall thickness of plastics material SL attenuates less radiation than the packaging for the packaged biopolymer 42, with the measured irradiance being one third for the complete package (ca 1 mW/cm²) as compared to irradiance for the single wall thickness (SL) of plastics material (ca 3 mW/cm²).

Figure 4E shows the single wall thickness SL of plastics material (47a) and the packaging for the packaged biopolymer 42 (47b), each being mounted on a sensor S.

Therefore, it is shown that although a single wall of the packaging materials (47a) attenuates some of the incident UVC radiation, and that the packaging for the packaged biopolymer 42 attenuates even more of the radiation sufficient UVC light energy is able to penetrate the packaging for the packaged biopolymer 42 to effect sterilisation.

Referring now to Figure 4F, there is shown a graph 4F showing the UVC irradiance detected within the UVC light box 40 for both the uncovered sensor and a covered sensors. The experimental runs for the covered sensor were broken into two, one for the packaging for the packaged biopolymer 42 which was empty (48a) and one for the packaging for the packaged biopolymer 42 which contained 0.5ml of biopolymer gel. The results show that the presence of 0.5ml biopolymer gel within the packaging for the packaged biopolymer 42 does not significantly attenuate the incident UVC radiation,

Therefore, it has been surprisingly shown that sterilisation of the biopolymer packaging for the packaged biopolymer 42 is possible. Moreover, it has also been shown that irradiation through a single wall thickness is possible (Figure 4E).

Example 6

Referring now to Figure 5, there is shown a schematic illustration of a procedure 50 for the fabrication and UVC sterilisation of a gellan composition containing decorin.

Decorin was used as an active for suspension in the biopolymer. Decorin is a naturally occurring anti-fibrotic small leucine-rich proteoglycan that is naturally present at high levels

bound to collagen in the corneal stroma and which, when released, tightly regulates TGF β activity by binding the growth factor and sequestering it within the ECM. Decorin regulates cell proliferation, survival and differentiation by modulating numerous growth factors as well as directly interfering with collagen fibrillogenesis. Human recombinant (hr)Decorin is now
5 available in GMP form and it has been shown is functional in minimizing fibrosis in the brain and spinal cord.

In the procedure 50 of Figure 5, there is shown Steps [1] to [5].

10 There is shown a stirrer hotplate 51, sterile filters 52, a peristaltic pump 53, a syringe pump 54, sterile connectors 55, a pin stirrer 56, and a UVc cabinet 57.

The pin stirrer 56 comprises detachable and sterilisable parts.

15 In Step [1] of the procedure 50, a gellan sol comprising NaCl, which was fabricated under GMP (good manufacturing practices), was stirred on the stirrer hotplate 51 at 95°C.

The resulting gellan sol composition was pumped through the sterile filters 52 by the peristaltic pump 53, and through the sterile connectors 55, into the pin stirrer 56.

20

In Step [2], GMP grade sterile decorin was pumped using the syringe pump 54 from a vial (not shown) into the pin stirrer 56.

25

In Step [3], the decorin was mixed in the pin stirrer 56 with the gellan sol composition of Step [1].

In Step [4], the resulting gellan sol and decorin mixture was either: (i) dispensed in an isolator; or (ii) pumped into primary packaging via a closed sterile fill system (not shown).

30

In Step [5], the gellan compositions containing decorin of Step [4] underwent UVc sterilisation in the UVc cabinet 57.

Example 7

Gellan compositions containing decorin were fabricated in the following procedure.

35

Low acyl gellan gum (Kelco gel CG LA, Azelis, UK) was dissolved in deionized water. Gellan powder was added to deionized water at ambient temperature in the correct ratio to result in a 1% (w/v) solution. The sol was heated to 70 °C under agitation, on a hotplate equipped with a magnetic stirrer, until all the polymer had dissolved. Once dissolved, gellan sol was added to the cup of a rotational rheometer (AR-G2, TA Instruments, UK) equipped with cup and vane geometry (cup: 35 mm diameter, vane: 28 mm diameter). The system was then cooled to 40 °C. hrDecorin (Galacarin™; Catalent, USA) in PBS (4.76 mg/ml) and aqueous sodium chloride (0.2 M) was then added to result in final concentrations of 0.9% (w/v) gellan, 0.24 mg/ml hrDecorin and 10 mM NaCl. Following this, the mixture was cooled at a rate of 1 °C/min under shear (450 /s) to a final temperature of 20 °C. The sample was then removed and stored at 4 °C until further use.

The gellan composition containing decorin was irradiated using UVc for 0 minutes (control), 5 minutes, 10 minutes, and 30 minutes.

Referring now to Figure 6A, there is shown a series of overlaid spectra 6A showing the results of circular dichroism (CD) experiments on the UVc treated decorin after 0 minutes (control), 5 minutes, 10 minutes, and 30 minutes. Circular dichroism (CD) is used to probe the 3D structure of proteins. Consequently, any changes in the spectra of decorin after 0 minutes (control), 5 minutes, 10 minutes, and 30 minutes show the effect of UVc treatment on decorin by whether the protein structure has changed.

Surprisingly, it is shown from the spectra 6A that the plots for decorin after each of 0 minutes (control), 5 minutes, 10 minutes, are substantially the same, which indicates that UVc has had a negligible impact on the protein structure of decorin, with some minor changes after the sample has been irradiated for 30 minutes.

Referring also to Figure 6B, there is shown a series of dose-response curves 6B determined using collagen fibrillogenesis assay, which was performed using the UVc treated decorin after 0 minutes (control), 5 minutes, 10 minutes, and 30 minutes.

Decorin regulates cell proliferation, survival and differentiation by modulating numerous growth factors as well as directly interfering with collagen fibrillogenesis. Therefore, the process of collagen fibrillogenesis was monitored to determine the effect of UVc treatment on the ability of decorin to control this process.

It is shown in Figure 6B that the dose-response curves 6B for collagen fibrillogenesis performed using the UVc treated decorin after 0 minutes (control), 5 minutes, 10 minutes, and 30 minutes are substantially the same, which also indicates that UVc has had a negligible impact on the protein structure of decorin.

Therefore, it has been surprisingly found that the method of sterilisation according to the invention is able to sterilise a biopolymer, e.g. gellan gum, containing an active, e.g. decorin, without changing the molecular structure of either the biopolymer or the active.

Moreover, the method of sterilisation may be performed in a continuous process. For example, the biopolymer to be sterilised may flow or be pumped through a vessel, e.g. a tube, adjacent a suitable UVc source such as adjacent a UVc lamp, e.g. in a UVc chamber, to effect sterilisation. It has been surprisingly shown in Figures 4A to 4E that sufficient UVc light energy is able to penetrate a single wall thickness of plastic packaging of a packaged biopolymer to effect sterilisation. Therefore, the biopolymer may flow or be pumped in a continuous process, through a vessel, e.g. a tube, comprising a single wall thickness of plastic, the vessel being adjacent a suitable UVc source, such that the biopolymer is sterilised in the process.

Comparative Examples

Referring now to Table 3, there is shown a list of qualitative results for the sterilisation of the gellan gum of Procedure 1 and the gellan patch of Procedure 2 using conventional, prior art, methods of sterilisation.

Table 3				
Method	Gellan Patch		Gellan Fluid Gel	
	Material Properties	Bioactivity	Material Properties	Bioactivity
Autoclave	Rehydrates the patch	Denatures decorin	Thermal reversal of crosslinking.	Denatures decorin
Gamma radiation (12 and 25 kGy)	The formation of radicals during the sterilisation process results in the breakdown of the polysaccharide into toxic products, and also effects the activity of the decorin.			
X-Ray (3 kGy)	Reduction in pH post-sterilisation	Lowers activity of decorin	Uneven physical and chemical changes	Lowers activity of decorin
Ethylene oxide (ETO)	This was observed to have cytotoxic effects post-sterilisation. Additionally, the products need to be unpackaged before undergoing sterilisation and repackaged afterwards.			

Referring also to Figure 7A, there is shown a gel permeation chromatogram (GPC) for a control of gellan gum 71, and gellan gum post-sterilisation with gamma radiation 72. It is shown that the GPC of the control of gellan gum 71 has a sharp peak, indicating a pure product, whereas the GPC of the gellan gum post-sterilisation with gamma radiation 72 shows that degradation has taken place.

Referring also to Figures 7B and 7C, there is shown a mass spectrum 73 for the gellan gum post-sterilisation with gamma radiation (25 KGy), and selected enlarged regions of the mass spectrum 73, shown as spectra 74 to 77.

The mass spectrum 73, 74 to 77 showed the degradation products of the gellan gum post-sterilisation with gamma-radiation to be 1-hydroxy propanone (spectra 74), acetic acid (spectra 75), formic acid (spectra 76), and furfuryl alcohol (spectra 77). This is because exposure of gellan gum to gamma radiation leads to the breakdown of the material at a molecular level.

Therefore, the method of sterilisation according to the invention has surprisingly been found to sterilise a biopolymer, e.g. gellan gum, without leading to the degradation products observed from other prior art methods of sterilisation, e.g. gamma radiation.

Referring now to Figure 8, there is shown a graph 80 showing a comparison of rheological data (shear rate vs. viscosity) for a UV treated polymer sample 81 and an untreated control

polymer sample 82. The polymer sample used was gellan gum, which was prepared in the same manner as described in Step 4 of Examples 1 to 4.

5 The UV treated polymer sample 81 was irradiated under UV light (254 nm) for 10 minutes according to the method of the invention.

Surprisingly, it is shown that the rheology of the UV treated polymer sample 81 is substantially unchanged after sterilisation, when compared with the untreated control polymer sample 82.

10

Referring now to Figure 9, there is shown a graph 90 showing a comparison of the viscoelastic behaviour (strain vs. storage modulus and strain vs. loss modulus) for a UV treated polymer sample and an untreated control polymer sample.

15 The UV treated polymer sample was irradiated under UV light (254 nm) for 10 minutes according to the method of the invention.

There is shown data for the strain vs. storage modulus for a UV treated polymer sample 91, the strain vs. storage modulus for an untreated control polymer sample 92, the strain vs.
20 loss modulus for a UV treated polymer sample 93, the strain vs. loss modulus for an untreated control polymer sample 93.

Surprisingly, it is shown that the viscoelastic behaviour of the UV treated polymer sample is substantially unchanged after sterilisation, when compared with the untreated control
25 polymer sample.

Example 8

Referring now to Figure 10, there is shown a series of dose-response curves which were used to calculate the D values for the sterilisation of a biopolymer against different
30 microorganisms. In this series of Examples, the biopolymer was low acyl gellan. There is shown a graph for the sterilisation against *C. albicans* (10A), *B. pumilus* (10B), *E. coli* (10C), spores of *B. pumilus* (10D), and spores of *B. subtilis* (10E).

The polymers for *C. albicans* (10A), *B. pumilus* (10B), and *E. coli* (10C) were provided as a
35 packaged fluid gel. These were made and sterilised using the following methods:

Preparation of fluid gels

Fluid gels were prepared by:

- Addition of gellan powder to water with 5% PBS to form a 1% polymer solution.
- 5• Heating the solution above the gelling point.
- Adding crosslinker (sodium chloride added (10 mM final concentration))
- Cooling the solution through the gelling point whilst constantly shearing.

Preparation of microbes

- 10• *C. albicans*, *E. coli* and *B. pumilus* were cultured in either YM (*C. albicans*) or Nutrient II (*E. coli*, *B. pumilus*) broth at either 25 °C (*C. albicans*) or 30 °C (*E. coli*, *B. pumilus*).
- Microbes were counted via Helber counter or Haemocytometer.
- Microbes were then diluted in PBS to a concentration of ca. $\times 10^6$ CFU/ml.

15 Preparation of inoculated fluid gel:

- Microbes in PBS were centrifuged at 4000 g to form a pellet.
- The supernatant was removed and pellet resuspended in fluid gel to maintain a concentration of ca. $\times 10^6$ CFU/ml.
- 0.5 ml of the inoculated fluid gel was then pipetted into a single use dropper (poly
- 20 propylene based) and sealed.

Irradiation of samples:

- Samples were placed in a UVpro EKB 100 irradiation chamber in which was housed a 254 mm germicidal lamp. Specifications of the chamber are as follows:
- 25 Input power: 100 W (4 x 25W bulbs)
- Current flow: 450 mA
- Total UVc: 32 W (4 x 8 W)
- Intensity at 10 cm: 10.7 $\mu\text{W}/\text{cm}^2$
- A UV sensor (254 nm) was placed next to the sample.
- 30• The chamber was switched on and irradiated (254 nm) for varying exposure times.
- Irradiance was calculated as the cumulative energy detected by the sensor. This value was corrected for loss due to absorbance by the plastic container (determined by placing a container over the sensor and measuring the energy difference).

Determination of microbial reduction:

- Irradiated samples were opened and 100 µl of the inoculated gel plated on TSA and spread. Where necessary, gels were serial diluted in PBS prior to plating.
- After incubation of up to 14 days at either 25 °C (*C. albicans*) or 30 °C (*E. coli*, *B. pumilus*) plates were removed and colonies counted.

The energy input of these dose-response curves was corrected such that the packaging absorption was removed from the total energy input to leave the energy that reached the material. This was performed using a sensor for 254nm UV light. The irradiance detected with the packaging and without the packaging was compared to calculate the ratio of loss due to the absorption of irradiance by the packaging. This was used to calculate the actual amount of UV light energy that the microorganisms are exposed to in the polymer. This may be performed for different packaging types and thicknesses.

The polymers for spores of *B. pumilus* (10D), and spores of *B. subtilis* (10E) were provided as a dehydrated hydrogel. These were made and sterilised in the following method:

Preparation of gels

Gels were prepared by:

- Addition of gellan powder to water with 5% PBS to form a 2% polymer solution.
- Heating the solution above the gelling point.
- Adding crosslinker (sodium chloride added (10 mM final concentration))
- Casting the sol into moulds and cooling the solution through the gelling point.
- Dehydration of the hydrogels was achieved by oven drying at 50 °C.

Preparation of microbes

- *B. pumilus* and *B. subtilis* were cultured in Nutrient II broth at 30 °C.
- Microbe concentration was determined by optical density.
- Spores were formed via heat induced stress.
- Spores were then concentrated in PBS to a concentration of ca. $\times 10^6$ CFU/ml.

Preparation of inoculated gel

- Inoculation of the patches was achieved by application of spores in liquid onto the dried hydrogel.
- Absorption of the water was allowed followed by drying in air (under flame).

- Samples were placed in polypropylene pouches.

Irradiation of samples

- Samples were placed in a UVpro irradiation chamber as described above.
- A UV sensor (254 nm) was placed next to the sample.
- The chamber was switched on and irradiated (254 nm) for varying exposure times.

Determination of microbial reduction:

- Irradiated samples were opened and mixed with PBS until saturated.
- Gels were mechanically broken down and serial diluted.
- The liquid was filtered to remove large particles and 100 µl of the liquid plated on TSA and spread.
- After incubation of up to 14 days at either 30 °C plates were removed and colonies counted.

The D-values and the energy required to obtain an SAL of 10^{-6} for each microbe is shown in Table 4. The D-values are calculated as a function of energy delivered to the microbe itself (that is, corrected for packaging absorbance). Therefore, the D-value represents the dose to reduce the bioburden by one logarithmic unit.

Table 4		
<u>Microbe</u>	<u>D-value (mJ/cm²)</u>	<u>Energy (mJ/cm²) to obtain a SAL $\times 10^{-6}$</u>
<i>B. pumilus</i> *	42 (47 s)	501 (9 mins 28 s)
<i>B. pumilus</i> (spores)	341 (54.9 s)	4092 (11 mins 0 s)
<i>B. subtilis</i> (spores)	181 (23.8 s)	1800 (4 mins 46 s)
<i>E. coli</i> *	46 (51.1 s)	552 (10 min 13 s)
<i>C. albicans</i> *	41 (46 s)	492 (9 mins 16 s)

* Irradiated in packaging, therefore energy values have been corrected for packaging absorbance.

It is shown that a method that is suitable for obtaining an SAL of 10^{-6} against spores of *B. pumilus* is effective against all other microbes tested.

It was found that the gellan did not absorb much of the light. Therefore, the D-values stated above are believed to represent the amount of energy needed to kill the microbe. In other systems, the amount of energy actually applied may need to be greater to take into account absorbance by the polymer any packaging.

5

Referring now to Figure 11, there is shown a series of microbiology plates data. There is show a series of images (11A) showing *B. pumilus* at varying time intervals from 0 seconds to 300 seconds after UV exposure to the plate. There is also shown an image at 0 seconds and an image at 300 seconds after UV exposure to the plate for the following
10 microorganisms: *B. subtilis* (11B); *E. coli* (11C); *C. albicans* (11D); and *P. aeruginosa* (11E).

Advantageously, the method of the invention has been shown to be effective for species of microorganisms include Gram positive (*B. subtilis*), Gram negative (*E. coli*), yeast (*C. albicans*) and spore forming (*B. subtilis*). The UV light is able to penetrate both hydrated
15 and dehydrated polymer, e.g. low acyl gellan, to sterilise the polymer.

The total viable count has been reduced to zero in the method of the invention by application of 254 nm UV light.

20 More advantageously, the method of the invention does not result in the breakdown of the low acyl gellan. Fluid gels are biopolymer-based materials with 99% water. It is usually challenging to sterilise fluid gels by irradiation (gamma, e beam, x ray) because radicals are formed which cleave the polymer via different mechanisms, but in sugar chains, it is often due to oxidative cleavage. However, using this method, it has been shown that no
25 substantial degradation is observed. Additionally, it has been found that the UV light was able to penetrate the entire volume of the fluid gel and also the collapsed polymer network of the dehydrated hydrogel. Therefore, the method of the invention is effective in sterilising both hydrated and dehydrated polymer gels.

30 It will also be appreciated by those skilled in the art that any number of combinations of the aforementioned features and/or those shown in the appended drawings provide clear advantages over the prior art and are therefore within the scope of the invention described herein.

CLAIMS

1. A method of sterilising a polymer, the method comprising providing a polymer, e.g. a biopolymer, exposing the polymer to UVc radiation to reduce the bioburden, for example to reduce the bioburden to a SAL value of 10^{-6} .
5
2. A method according to Claim 1, comprising exposing the polymer to UVc for sufficient time and/or to sufficient energy to reduce the bioburden to a SAL value of 10^{-6} .
- 10 3. A method according to Claim 1 or 2, wherein the initial bioburden of the polymer being provided is greater than 0.1 CFU/mL, for example, greater than 1 CFU/mL, e.g. greater than 10, or greater than 10^2 , or greater than 10^3 , or greater than 10^4 , or greater than 10^5 , or greater than or equal to 10^6 CFU/mL.
- 15 4. A method according to any preceding Claim, wherein the molecular structure of the polymer provided is substantially unchanged after exposing the polymer to UVc to reduce the bioburden to a SAL value of 10^{-6} .
- 20 5. A method according to any preceding Claim, comprising locating the polymer in a container and exposing the container to the UVc radiation to reduce the bioburden to a SAL value of 10^{-6} .
6. A method according to Claim 5, comprising locating the polymer in a sealed container.
- 25 7. A method according to any preceding Claim, comprising exposing the polymer to UVc having a wavelength of between 210 to 280 nm, e.g. between 220 to 270 nm, or 230 to 260 nm, e.g. 254 nm.
8. A method according to any preceding Claim, wherein the polymer is charged, e.g. anionic and/or cationic.
30
9. A method according to any preceding Claim, wherein the polymer is a polysaccharide.
10. A method according to any any preceding Claim, wherein the polymer comprises one or more of a natural, synthetic, or synthetically modified agar, agarose, arabinoxylan,
35

carrageenan, gelatin, gellan gum, glucan, curdian, pectin, xanthan gum, gum arabic, guar gum, locust bean gum, gum tragacanth, gum karaya, cellulose and derivatives thereof, alginate, fibrin, starch, chitosan, dextran, collagen and hyaluronic acid, or combinations thereof.

5

11. A method according to any preceding Claim, wherein the polymer comprises polymer chains that are crosslinked or crosslinkable.

12. A method according to Claim 11, wherein the polymer comprises a shear thinning gel.

10

13. A method according to any preceding Claim, comprising locating within the polymer an active substance, e.g. a small molecule, e.g. a pharmaceutical drug, and/or a protein, e.g. an enzyme, a co-factor prior to exposing the polymer to the UVc radiation to reduce the bioburden to a SAL value of 10^{-6} .

15

14. A method according to Claim 13, wherein the molecular structure of the active is substantially unchanged after exposing the polymer to UVc for sufficient time to reduce the bioburden to a SAL value of 10^{-6} .

20

15. A method according to any preceding Claim, wherein the polymer is a nascent polymer.

16. A method according to any preceding Claim, wherein method is suitable for sterilising the polymer against spores of *Bacillus pumilus* to a sterility assurance level (an SAL value) of 10^{-6} , and wherein the method comprises delivering a radiant exposure to the polymer of greater than or equal to about 4000 mJ/cm² (e.g. greater than or equal to 4092 mJ/cm²).

25

17. A packaged polymer, comprising a package containing a polymer having a SAL value of 10^{-6} .

30

18. A packaged polymer according to Claim 17, wherein the polymer is a shear thinning gel.

19. A packaged polymer according to Claim 17 or 18, further comprising a medicament or other active.
- 5 20. A packaged polymer according to any of Claims 17, 18, or 19, wherein the package is a sealed dispenser, from which the polymer may be dispensed.
21. A packaged polymer according to any of Claims 17 to 20, wherein the package comprises an individual doses of polymer.
- 10 22. An apparatus for sterilising a polymer against the spores of *Bacillus pumilus* to a sterility assurance level (an SAL value) of 10^{-6} , the apparatus comprising a UVC light source configured to deliver to the polymer a radiant exposure (e.g. total energy) of at least about 4000 mJ/cm² (e.g. greater than or equal to 4092 mJ/cm²).

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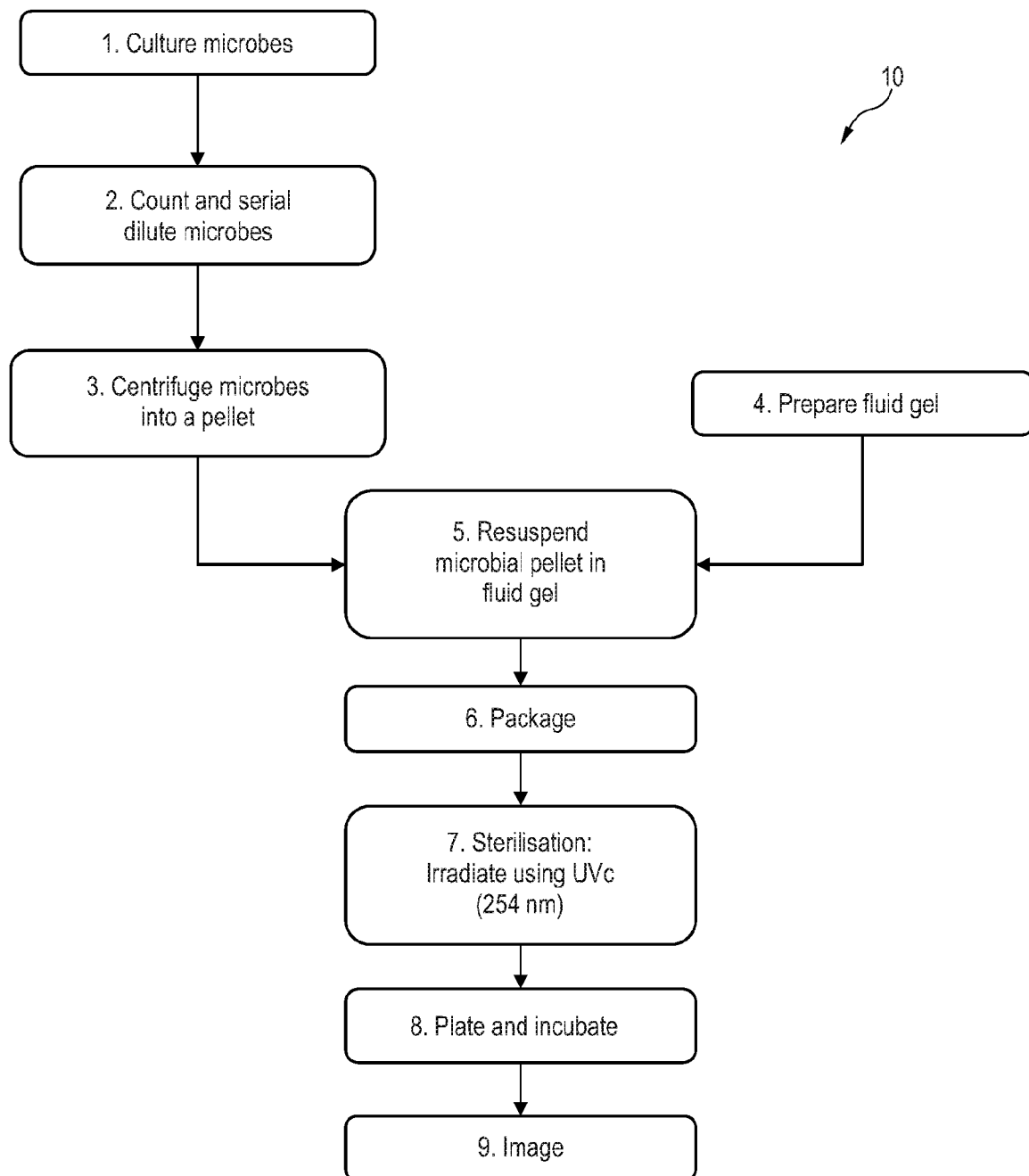


Fig. 1

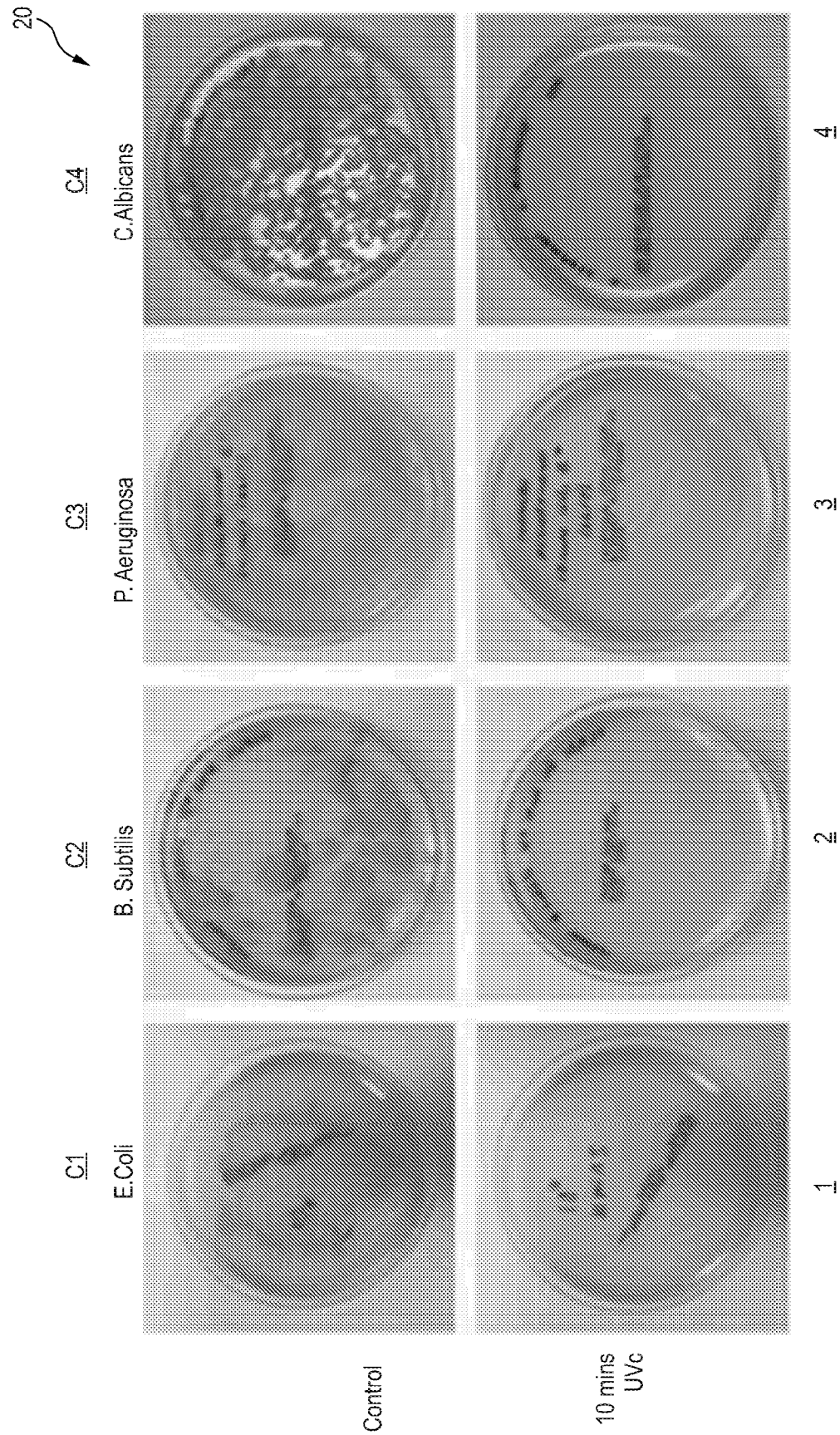


Fig. 2

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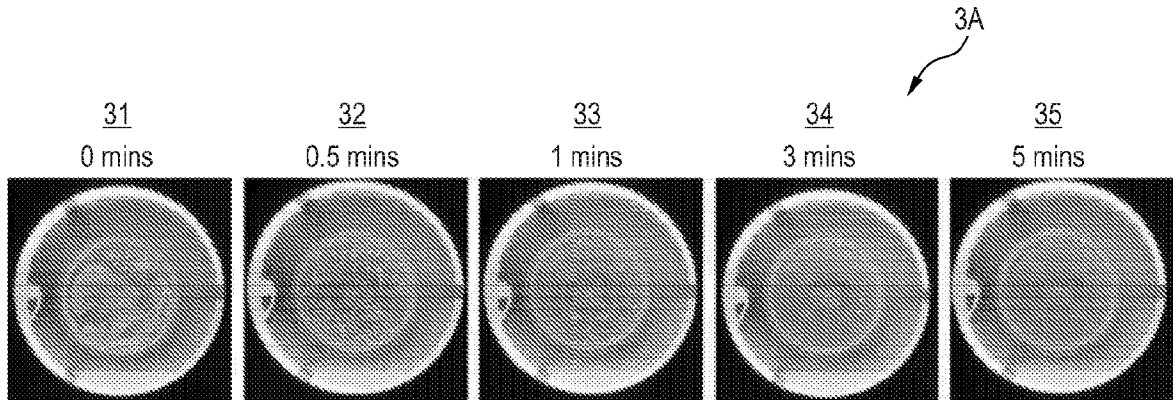


Fig. 3A

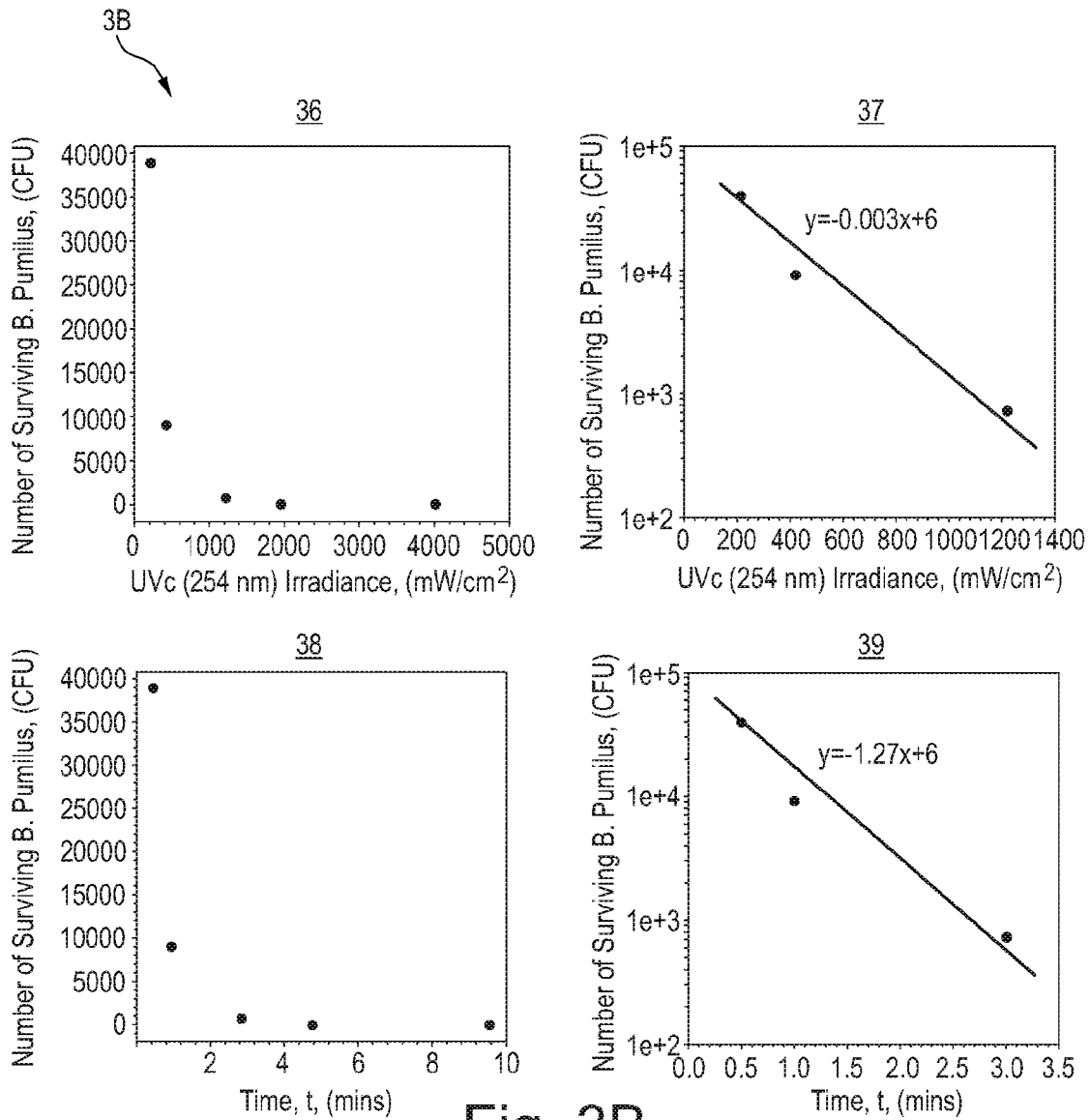


Fig. 3B

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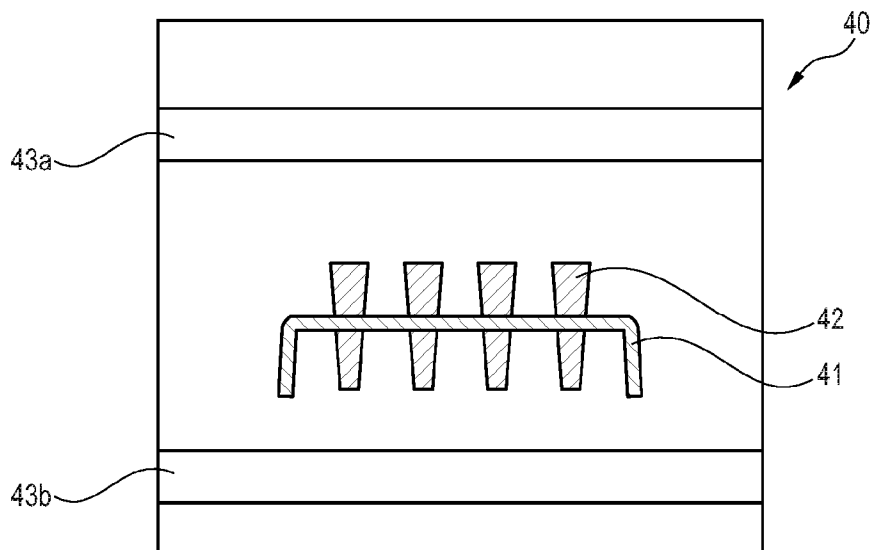


Fig. 4A

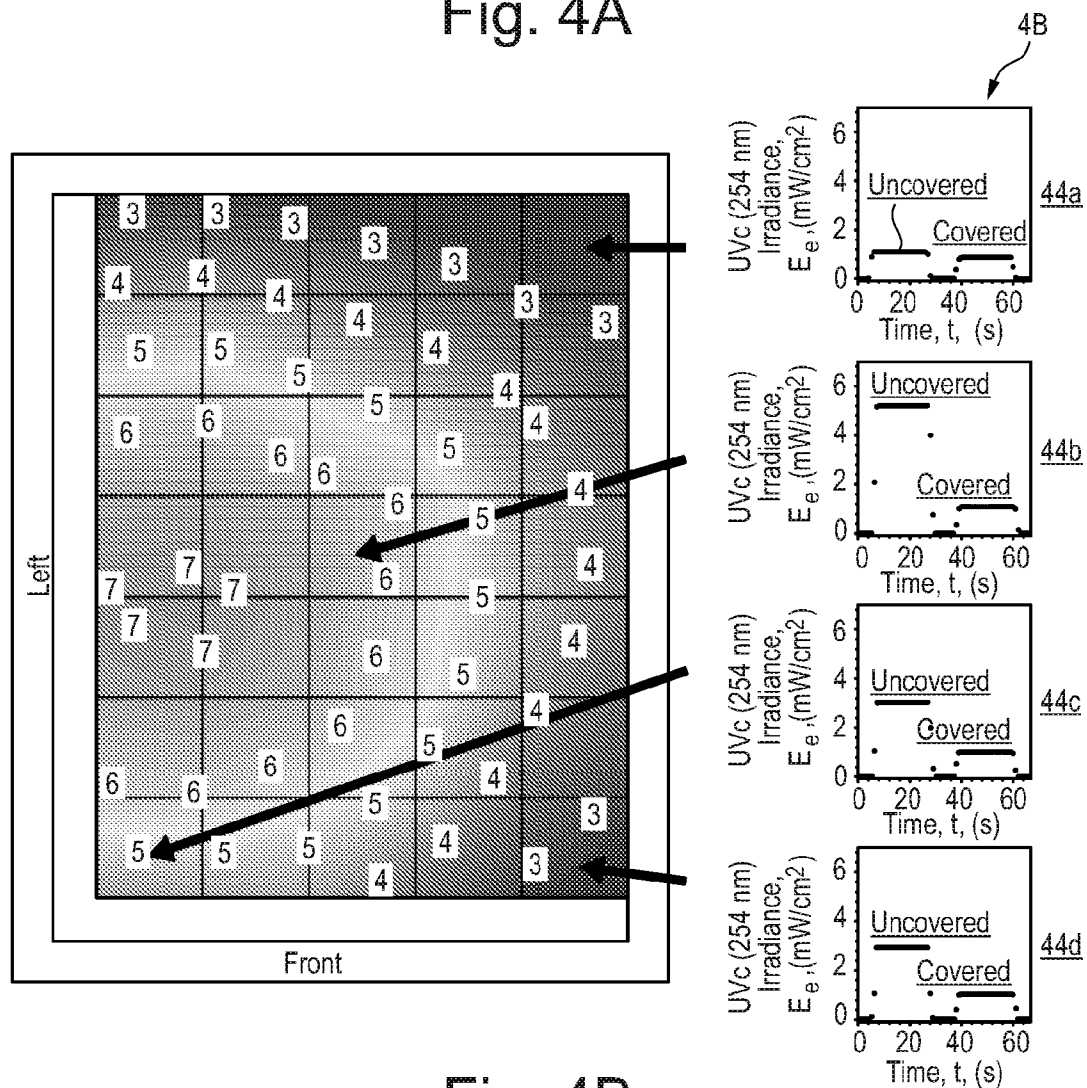


Fig. 4B

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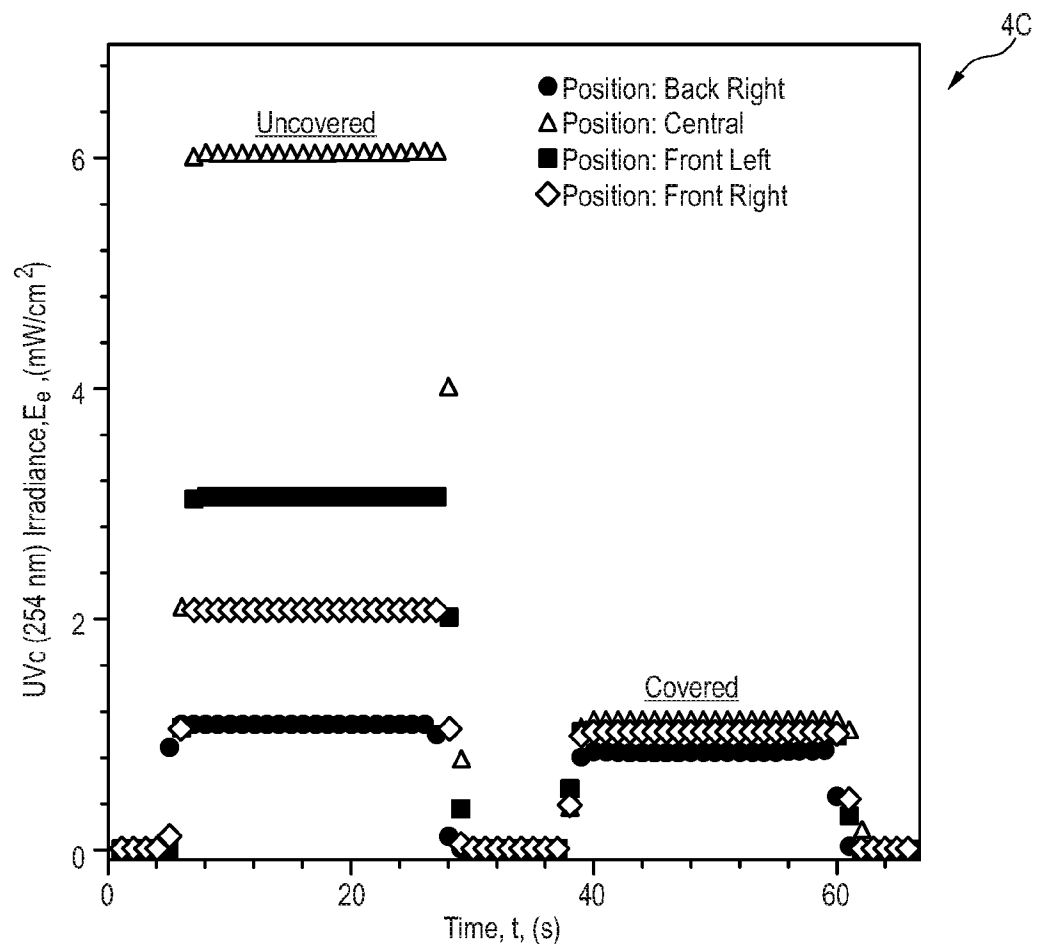


Fig. 4C

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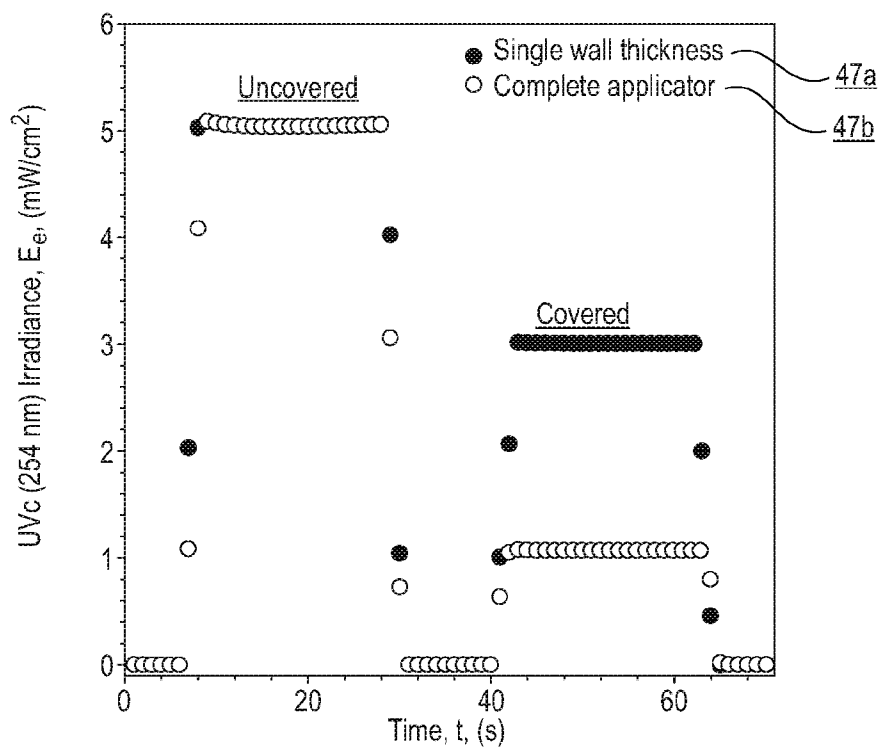


Fig. 4D

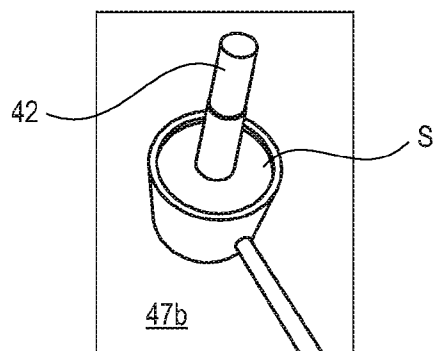
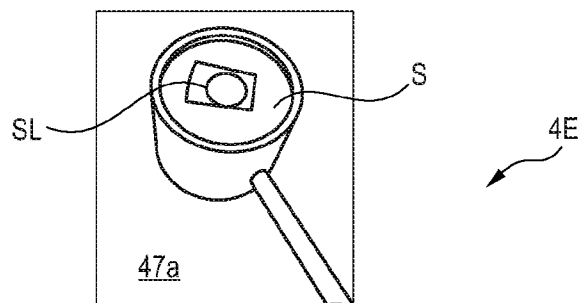


Fig. 4E

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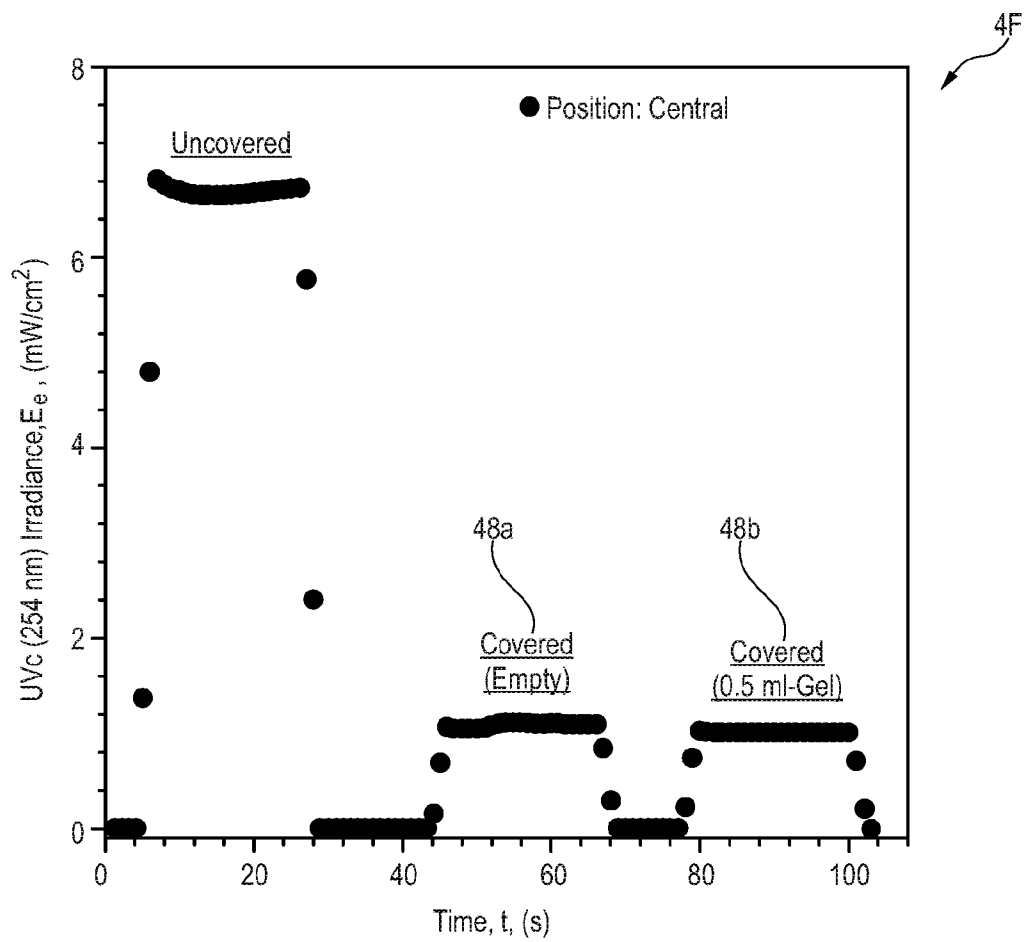


Fig. 4F

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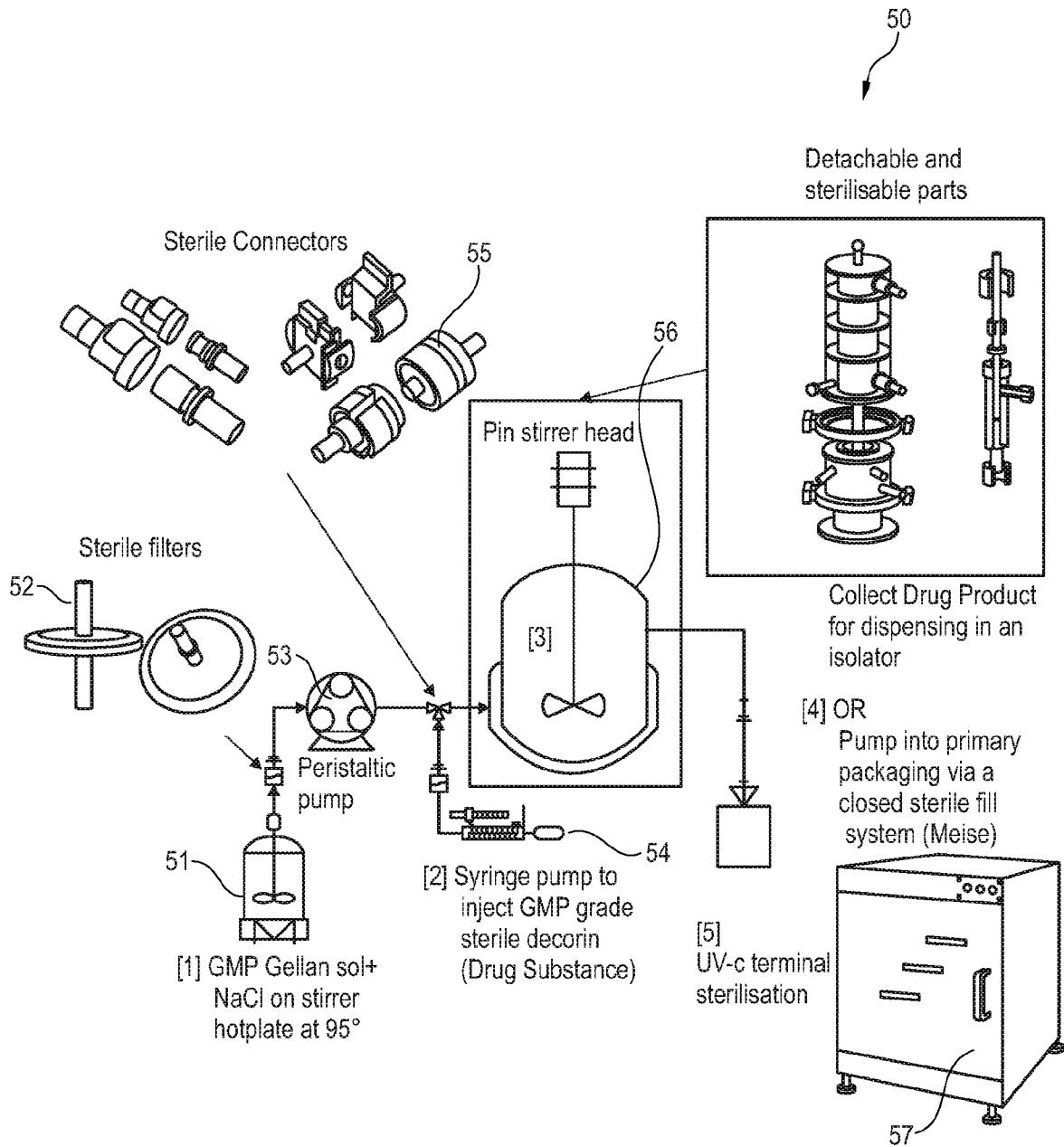


Fig. 5

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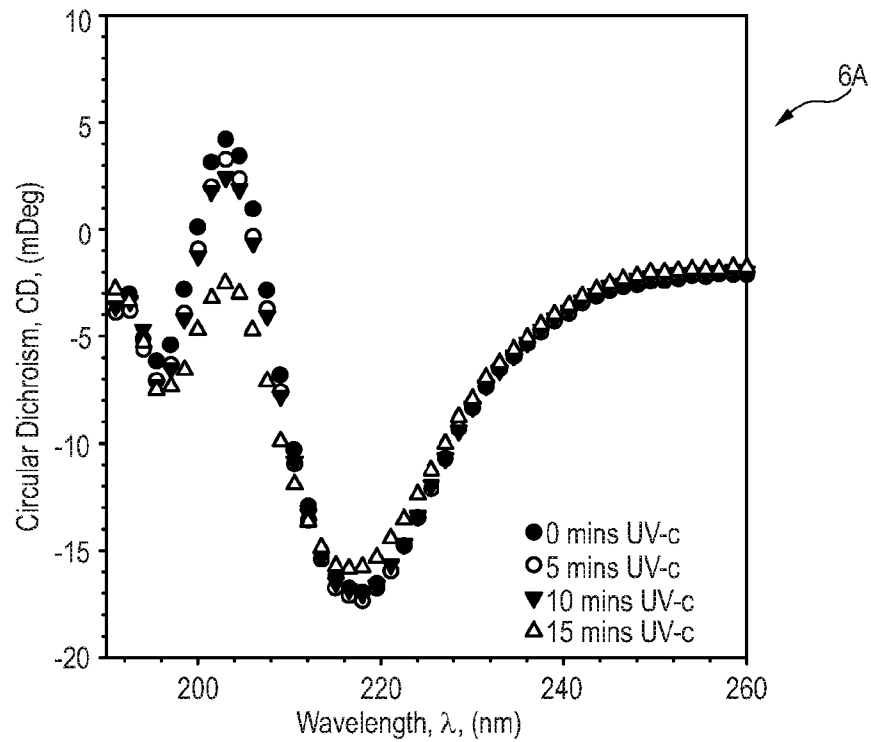


Fig. 6A

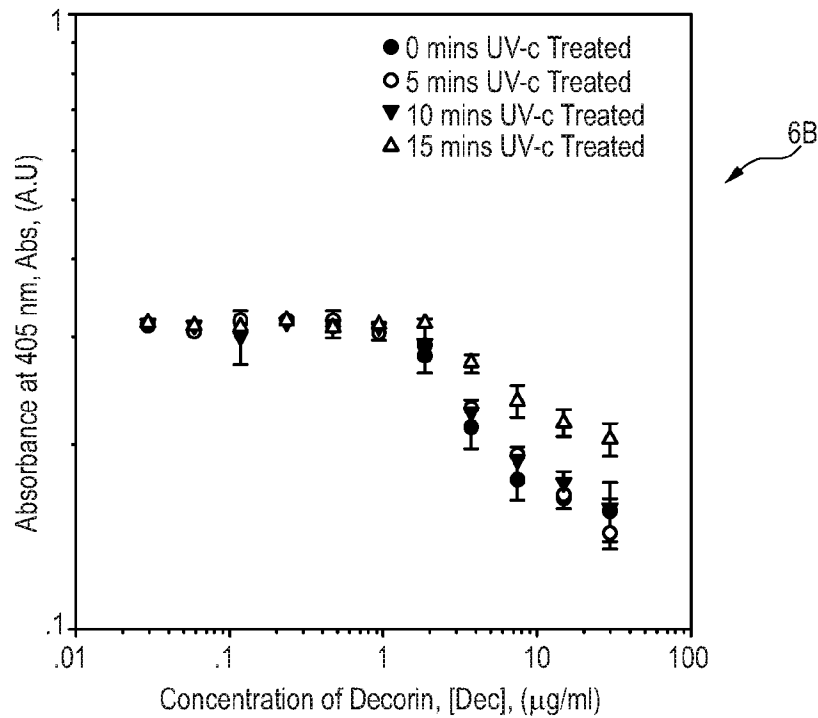


Fig. 6B

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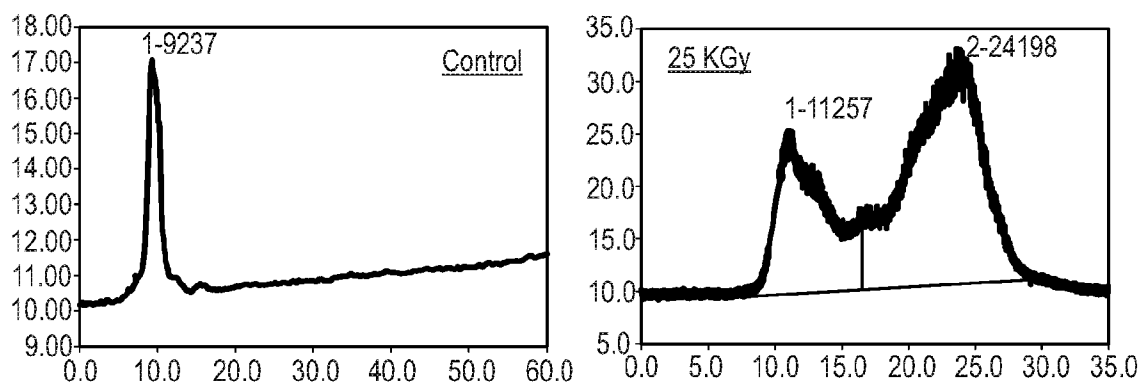


Fig. 7A

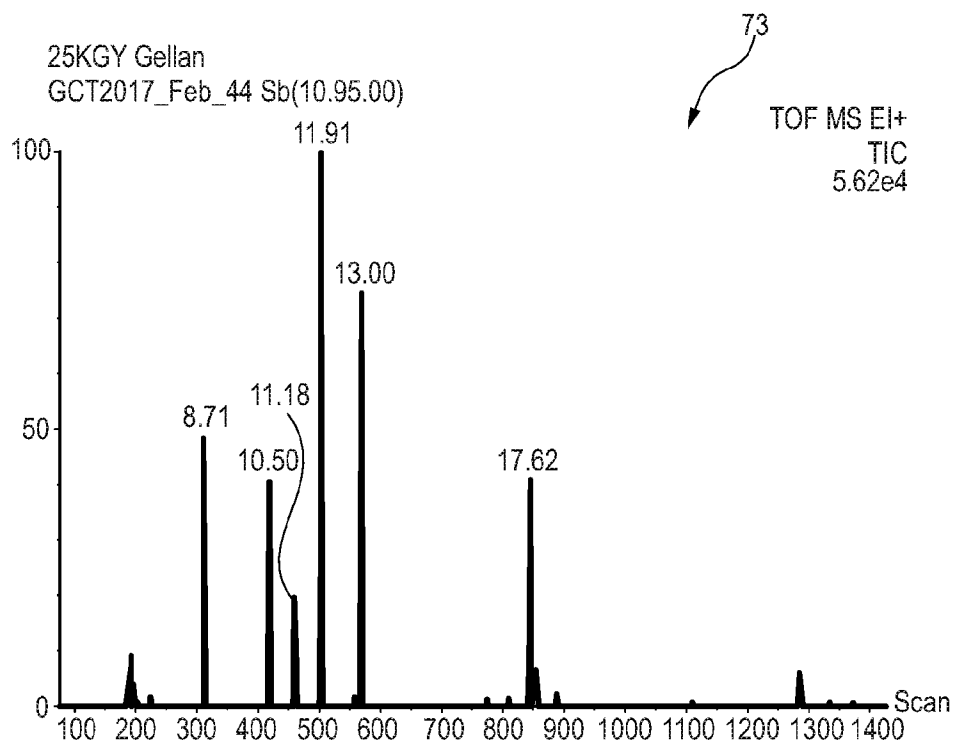


Fig. 7B

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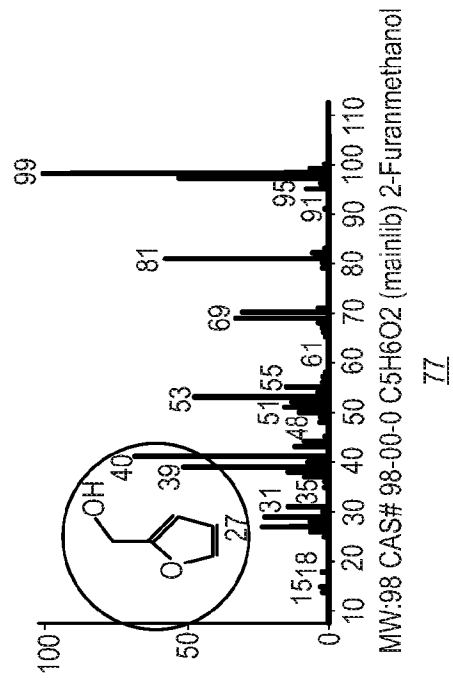
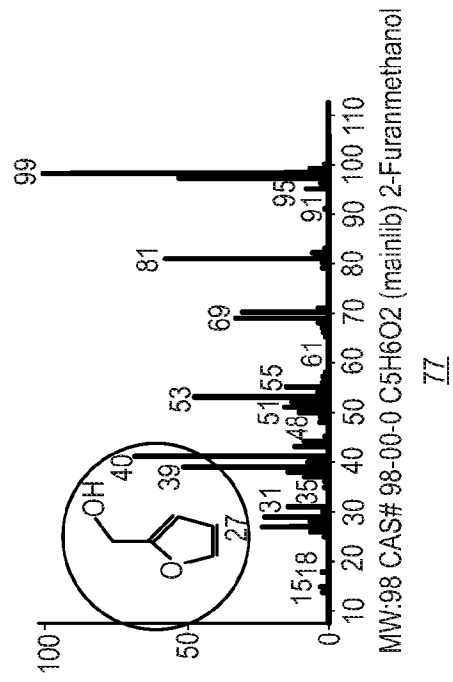
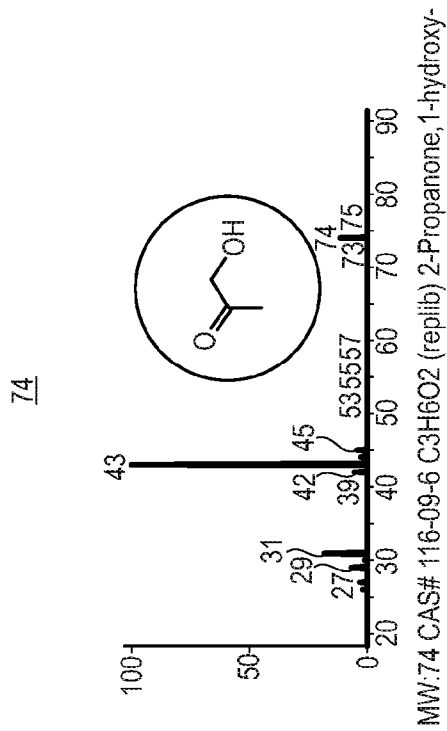
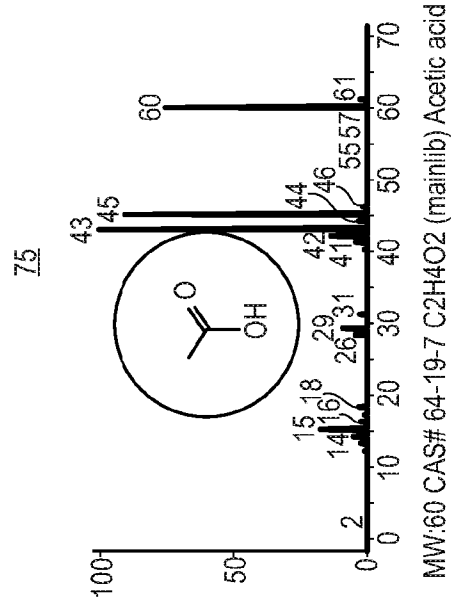


Fig. 7C

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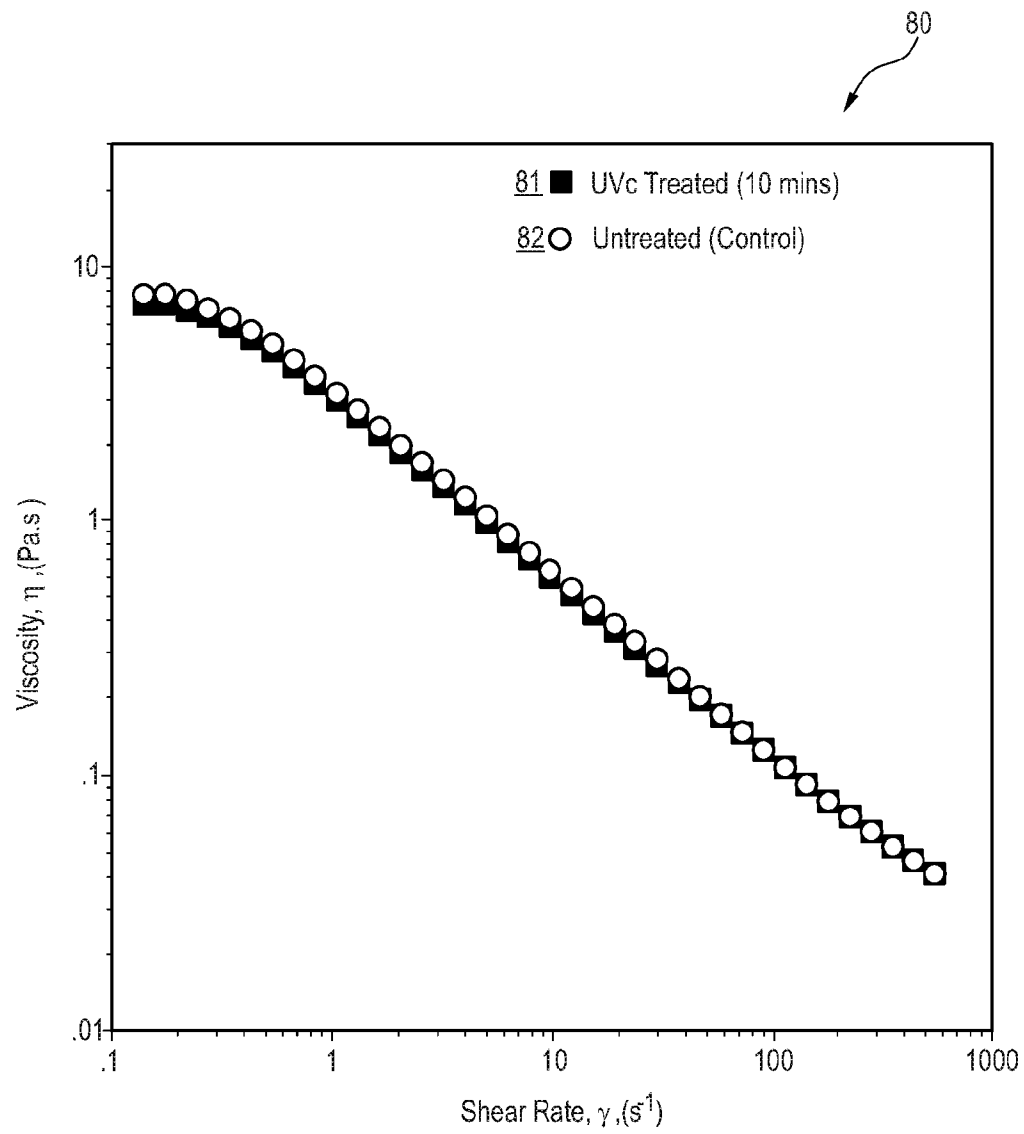


Fig. 8

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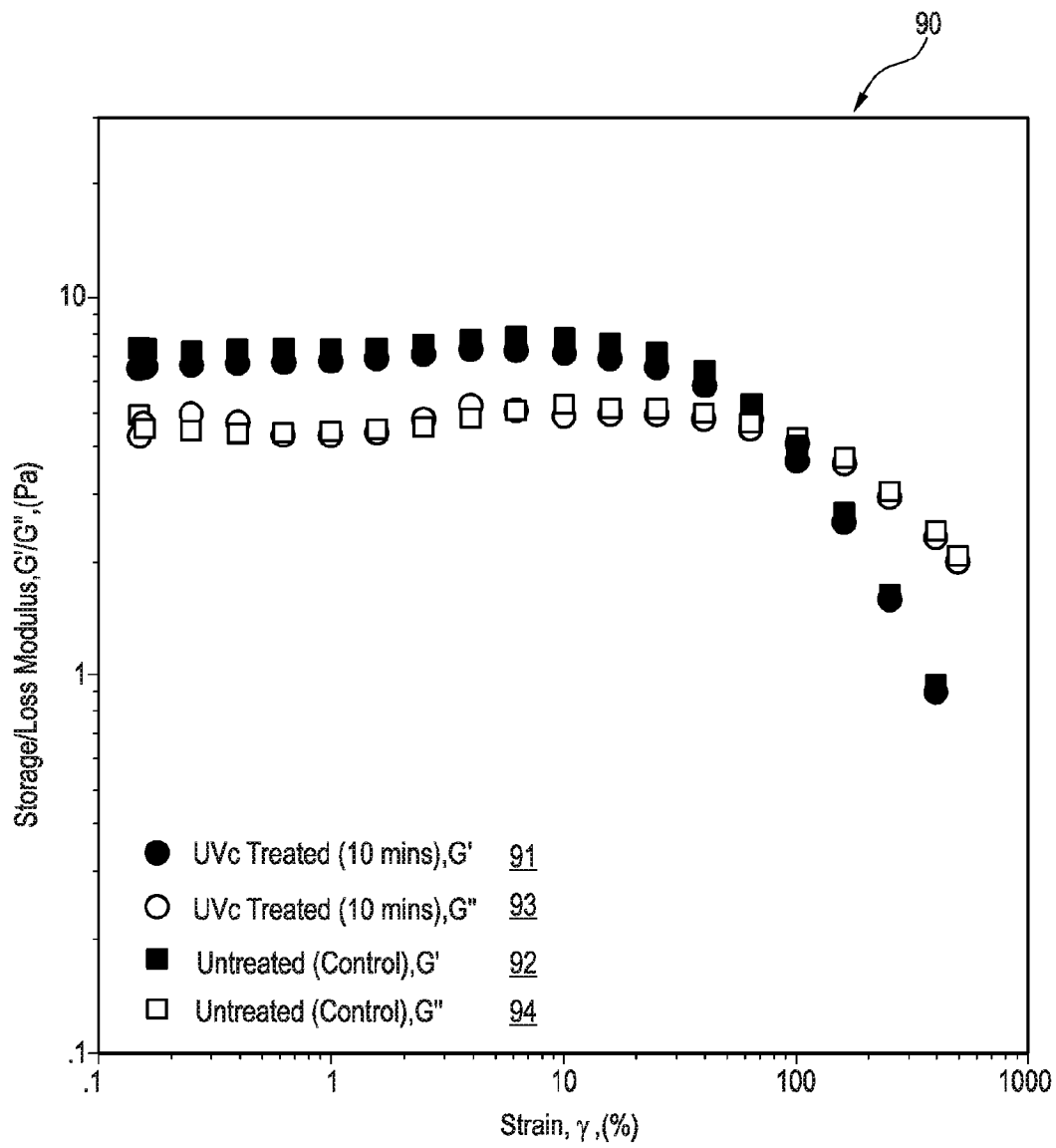


Fig. 9

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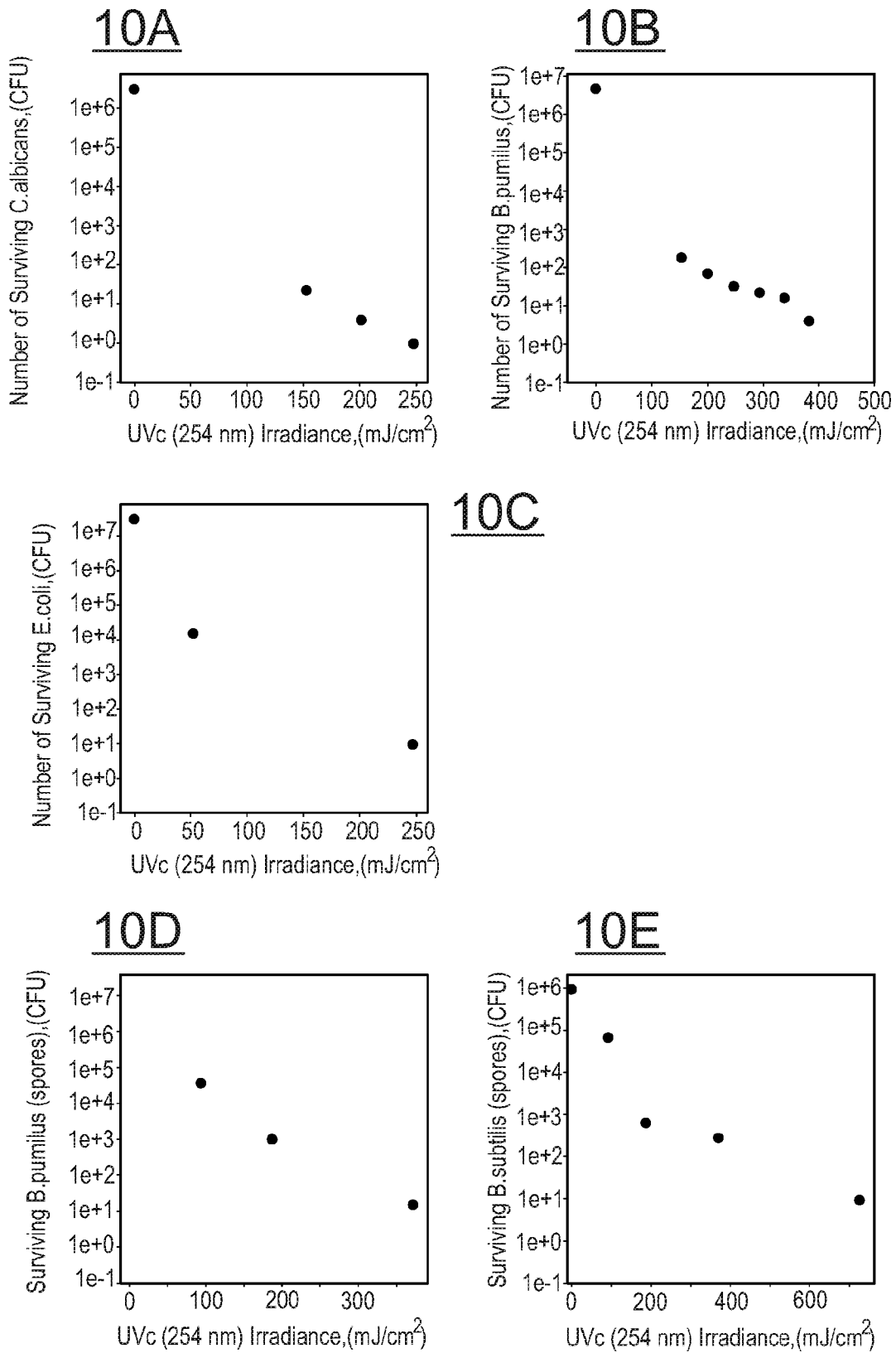


Fig. 10

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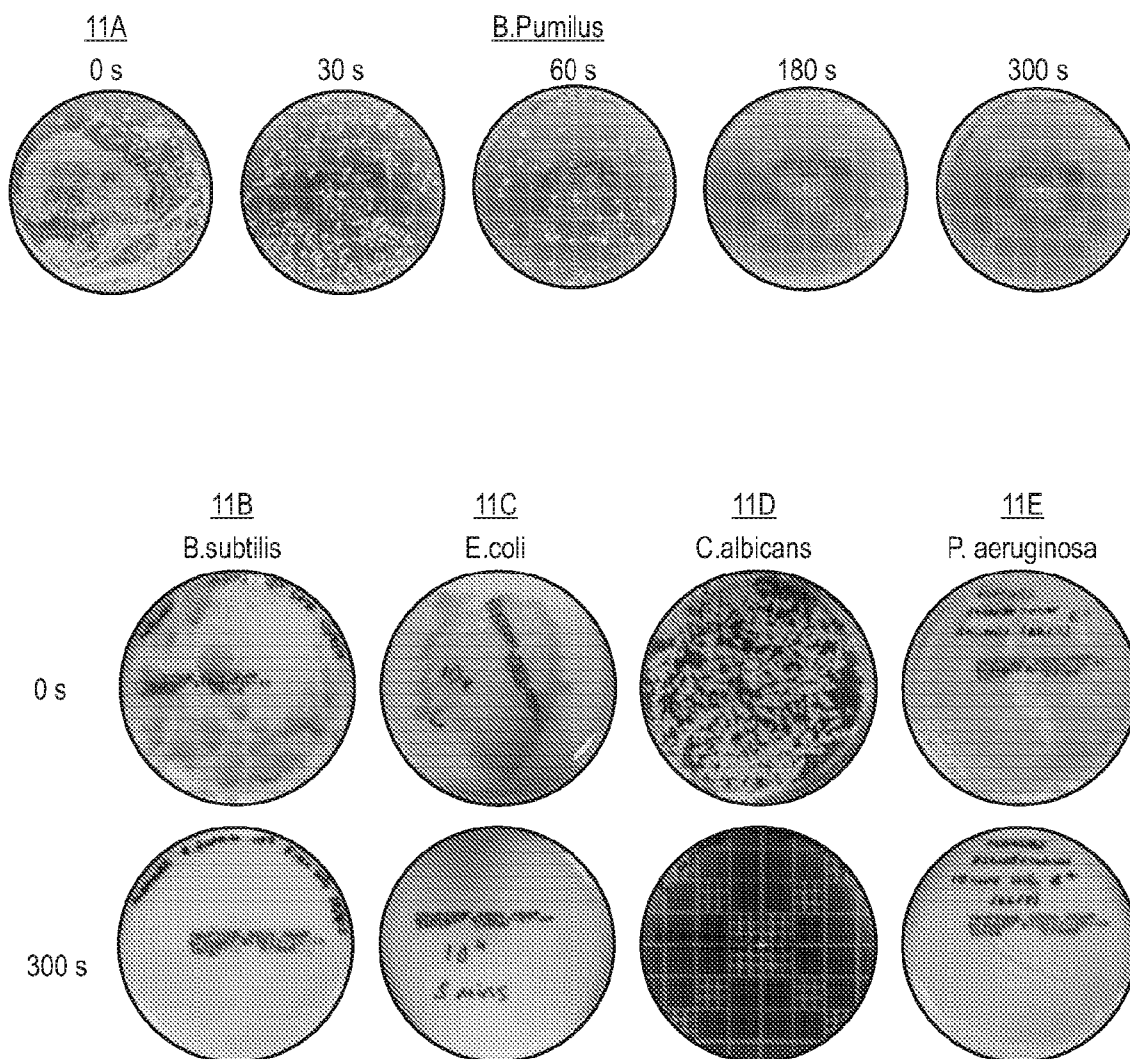


Fig. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/053465

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61L2/00 A61K41/17 A61K47/50 A61L2/10
ADD.

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)

A61L A61K

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

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

EP0-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2005/079096 A1 (BROWN-SKROBOT SUSAN K [US] ET AL) 14 April 2005 (2005-04-14) paragraph: [0048], [0049], [0050], [0052], [0053] -----	1-7,11, 13-15, 17,19-21
X	US 7 217 936 B2 (RESSLER BARRY [US]) 15 May 2007 (2007-05-15) column 3: line 66-67; column 4: line 1-10; column 7: line 44-65 -----	1,2,4,5, 7,15-17, 20-22
X	US 2018/179703 A1 (KAJANTO ISKO [FI] ET AL) 28 June 2018 (2018-06-28)	1,4, 7-10,12, 15,17, 18,20,21
Y	paragraph: [0074], [0092] ----- -/--	1,9-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

26 February 2020

Date of mailing of the international search report

13/03/2020

Name and mailing address of the ISA/

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Authorized officer

Nania, Manuela

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/053465

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MINNA H. CHEN ET AL: "Methods To Assess Shear-Thinning Hydrogels for Application As Injectable Biomaterials", ACS BIOMATERIALS SCIENCE & ENGINEERING, vol. 3, no. 12, 11 December 2017 (2017-12-11), pages 3146-3160, XP055671653, US ISSN: 2373-9878, DOI: 10.1021/acsbiomaterials.7b00734	1,9-11, 15,17-21
Y	page 3153, column 2: line 33-38; page 3147, column 1: line 8-11; figure: 2	12
X	----- US 2014/011980 A1 (CHITRE KAUSTUBH S [US] ET AL) 9 January 2014 (2014-01-09)	1,9-11, 17-21
Y	paragraph: [0002], [0043], [0044], [0048] -----	1,9-11

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Information on patent family members

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