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(54) **Title:** ANTIVIRAL, ANTI SARS-COV-2, ANTI H1N3, ANTIBACTERIAL AND ANTIMICROBIAL COMPOSITIONS AND METHODS OF PREPARATION THEREOF

(57) **Abstract:** The present invention relates to an antiviral, anti-SARS-CoV-2, anti H1N3, antibacterial and antimicrobial compositions and methods of preparation thereof. The compositions comprise squaric acid ester or croconic acid ester, amino polymer, ethanol, polypropylene glycol, glycerol and a binder solution, wherein the amino polymer is coupled with squaric acid ester or croconic acid ester. The composition of the present invention has wide ranging application, including, but not limited to a coating for non-metallic surface, metallic surface for air filters, paint, packaging and methods of preparing the composition.

TITLE: ANTIVIRAL, ANTI SARS-CoV-2, ANTI H1N3, ANTIBACTERIAL AND ANTIMICROBIAL COMPOSITIONS AND METHODS OF PREPARATION THEREOF

FIELD OF INVENTION

The present invention relates to an antiviral composition. In particular, the invention pertains to anti-SARS-CoV-2, anti H1N3, antibacterial and antimicrobial compositions which can be used for a number of purposes, including, but not limited to a coating for non-metallic surface, metallic surface for air filters, paint, packaging and methods of preparing the composition.

BACKGROUND

Viral infections such as the coronavirus disease 19 (COVID-19) has raised a global emergency.

The major concern regarding the COVID-19 pandemic is the unprecedented rate of spread that is fast enough to overburden and bend the present healthcare system all over the world to its knees. The novelty of the virus along with the absence of preventive and therapeutic agents against it has led to the unchecked spread of the disease.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV2) is the causative agent of COVID-19 diseases. It belongs to Coronaviridae family and is caused by a novel coronavirus.

Even though prophylactic and therapeutic treatment modalities are being developed, finding newer modalities of prevention the disease is indeed the need of the hour.

There is a substantial unmet need for the development of new preventive modalities for the spread of COVID-19.

The inventors have identified the above challenges and have addressed the same by developing an anti-viral composition for preventing the spread of viral agents such as SARS CoV2.

Thus, the present invention overcomes the problems of the prior art to solve the problem of providing anti-viral composition for preventing the spread of viral agents such as SARS CoV2 and influenza.

SUMMARY OF THE INVENTION

Technical Problem

The technical problem to be solved in this invention is the development of effective and safe anti-viral composition for preventing the spread of viral agents such as SARS CoV2 and influenza.

Solution to the problem

The problem has been solved by a multi-dimensional approach involving developing an antiviral composition comprising squaric acid ester or croconic acid ester, amino polymer, ethanol, polypropylene glycol, glycerol and a binder solution, wherein the amino polymer is coupled with squaric acid ester or croconic acid ester.

The present invention represents a new prevention modality in which an antiviral composition can be used as a coating agent to stop the viral infections from spreading through contact. Specifically, the invention provides a method for coating a range of substrates with an anti-viral composition such that the composition acts as a barrier to block the progress of viral agents such as SARS-CoV2.

The coating agents can also be used in as a coating agent in applications such as filters in air conditioners and air purifiers.

The present invention can also act as excellent coating material on metallic and non-metallic surfaces used in packaging industries. The composition is also useful in paint industries as organic non-toxic material to act as virucidal for longer duration.

Overview of the invention

In one aspect, the invention provides an antiviral composition comprising a product of a coupling reaction between amino polymer with squaric acid ester or croconic acid ester.

The composition may further comprises a binder, preferably organosilicon polymer such as poly dimethyl siloxane (PDMS). In one aspect the binder is polydimethyl siloxane or epoxy resins. In certain embodiments, the binder is an organosilicon polymer or an epoxy resin.

In another aspect the squaric acid ester is squaric acid monoester or squaric acid diester.

In another aspect, the squaric acid monoester ester is an alkyl squarate, an aryl squarate or a combination thereof; and squaric acid diester is a dialkyl squarate, a diaryl squarate, an alkyl aryl squarate or any combination thereof. In preferred embodiment, the squaric acid ester is selected from diethyl squarate and dibutyl squarate.

In another aspect, the amino polymer is selected from a group comprising deacetylated chitosan, folate-polyethylene glycol-amine, ortho-pyridyl disulfide functionalized polyethylene glycol-amine, polyethylenimine-graft-poly(ethylene glycol), polyethylenimine-graft-poly(ethylene glycol)-biotin, polyethylenimine-graft-poly(ethylene glycol)-melamide, polyethylenimine-graft-poly(ethylene glycol)-azide, polyethylenimine-graft-poly(ethylene

glycol)-thiol, poly(D,L-lactide-co-glycolide)-diamine, thiol- polyethylene glycol-thiol-polyethylene glycol-amine and amine-polyethylene glycol-amine.

In another aspect, the chitosan is deacetylated chitosan having a degree of deacetylation of at least 80%. In another aspect, the composition is formulated as a nano composition. The invention also provides methods for preparing the composition.

The composition of the present invention has wide ranging application such as being used as a coating material, as a deposit and as an active ingredient in industrially used chemicals or formulations. One specific use of the composition is as a coating material for air filters.

The invention also provides an article, a composition or formulation comprising the composition of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the FTIR for the partially quantitatively coupled product of chitosan and dibutyl squarate reaction.

Figure 2, 3 and 4 depicts the Mass Spectroscopic studies for the partially quantitatively coupled product of chitosan and dibutyl squarate reaction.

Figure 5, 6 and 7 depicts the PXRD Analysis for the partially quantitatively coupled product of chitosan and dibutyl squarate reaction.

Figure 8 depicts the Scanning Electron Microscope analysis for the antiviral composition.

Figure 9 depicts the measurement of activity of antiviral formulations against SARS-CoV2 virus.

Figure 10 depicts the results of the invitro cytotoxicity.

DEFINITIONS

Embodiments described herein can be understood more readily by reference to the following detailed description, examples, and drawings. Elements, apparatus and methods described herein are merely illustrative of the principles of the present invention and are not limited to the specific embodiments presented in the detailed description, examples, and drawings. Numerous modifications and adaptations will be readily apparent to those of skill in the art without departing from the spirit and scope of the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods belong. Although any antiviral compositions or methods similar or equivalent to those described herein can also be used in the practice or testing of the embodiments of the present invention, representative illustrative methods and compositions are now described.

Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within by the methods and compositions. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within by the methods and compositions, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the methods and compositions.

It is appreciated that certain features of the methods, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the methods and compositions, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. It is noted that, as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other embodiments without departing from the scope or spirit of the present methods. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

The term “amino polymer” refers to a macromolecule containing one or more primary or secondary amine functional groups. In certain embodiments, amino polymers may also comprise thiol functional groups. As used herein, amino polymer refers to a macromolecule which has the capability to couple with a squaric acid ester or croconic acid ester. Preferred amino polymers are selected from a group comprising deacetylated chitosan, folate-polyethylene glycol-amine, ortho-pyridyl disulfide functionalized polyethylene glycol-amine, polyethylenimine-graft-poly(ethylene glycol), polyethylenimine-graft-poly(ethylene glycol)-biotin, polyethylenimine-graft-poly(ethylene glycol)-melamide, polyethylenimine-graft-poly(ethylene glycol)-azide, polyethylenimine-graft-poly(ethylene glycol)-thiol, poly(D,L-lactide-co-glycolide)-diamine, thiol- polyethylene glycol-thiol-polyethylene glycol-amine and amine-polyethylene glycol-amine

The term "binder solution" as used herein refers to a solution comprising one or more components (binders) that facilitates the creation of uniform consistency, solidification and/or cohesion. The binder solution may contain epoxyresins and/or organosilicon polymers or combination thereof. In certain embodiments, the binder solution comprises polydimethylsiloxane, isopropyl alcohol and glycerol.

The term "deacetylated chitosan", as used herein means that the chitosan's degree of N-deacetylation of at least 80%. In certain embodiments, deacetylated chitosan have a degree of N-deacetylation of at least 80%, at least 85%, at least 90%, at least 95% or 100%.

The term "organosilicon polymers" means organometallic polymers or compounds containing carbon–silicon bonds and can be suitably used in a binder solution.

The term "article of manufacture" as used herein refers to a product that is made and sold and that includes a container and packaging, and optionally instructions for use of the product. For purposes herein, articles of manufacture encompass packaged antiviral compositions and products containing the antiviral compositions as disclosed herein.

DETAILED DESCRIPTION OF THE INVENTION

As those in the art will appreciate, the following detailed description describes certain preferred embodiments of the invention in detail and is thus only representative and does not depict the actual scope of the invention. Before describing the present invention in detail, it is understood that the invention is not limited to the particular aspects and embodiments described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the invention defined by the appended claims.

The present invention discloses anti-viral compositions which are useful for controlling a wide variety of viral as well as microbial agents, including SARS CoV2.

For the first time, the inventors have been able to develop anti-viral compositions which represents an advancement over the existing methods for preventing viral agents such as SARS CoV2 from spreading. The advances are characterized by the following features:

(a) Affordable: The anti-viral compositions developed are affordable, safe and can be adopted for use in developing and least-developed nations. All the components used are Generally Recognized as Safe (GRAS) and FDA-approved.

(b) Efficacious: The anti-viral compositions are highly efficacious in controlling highly infectious viral agents such as bacteriophages (Example 7), SARS CoV2 (Example 9) and influenza (Example 8) even in the dried state at 75°C. Hence, the compositions are extremely useful as a coating material, as a deposit and as an active ingredient in industrially used chemicals or formulations.

(c) Anti-static properties: In addition to the anti-viral efficacy, the compositions can prevent the build-up of static electrical charge, and are highly effective in increasing the surface conductance. The compositions are suitable for application over a wide range of surfaces. (Example 11)

(d) Safe: The compositions are non-toxic and safe. (Example 10)

Before the compositions and methods of the present disclosure are described in greater detail, it is to be understood that the invention is not limited to particular embodiments and may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

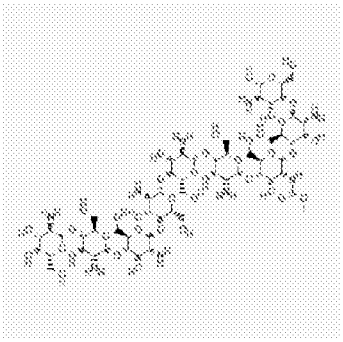
The present invention is based on quantitative nucleophilic substitution of alkoxy groups of dialkyl squarate by free amino groups of chitosan. Mono squaramide with half alkyl squarate can allow the S(-) of cysteine, N-of asparagine (side chain amino group) of Receptor Binding Domain of SARS-CoV-2 or N-terminal amino acids of protein of (S/E/M/N/RNA-bases/DNA bases) of any other RNA and DNA viruses. The free amino groups of chitosan are positive due to proton abstraction from water at pH 7 thus attracting the negatively surface charged viruses namely SARS-CoV-2, Hepatitis -B, MMR etc.

The present investigation relates to the capture of anionic SARS-CoV-2 at neutral pH 7 by positively charged ammonium ions in an amino polymer such as chitosan which is a polysaccharide molecule having free amine groups in large number (approximately 7.6 moles of free Nitrogen in primary amine form per mole of Chitosan sample, which is 85% deacetylated).

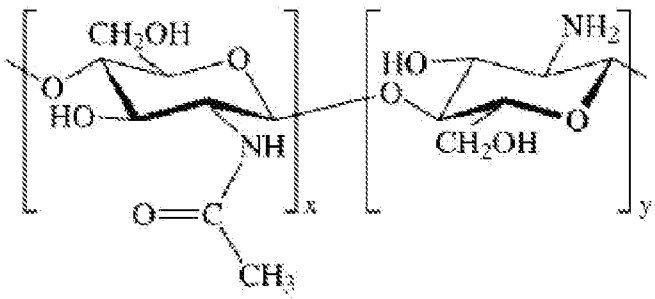
Dialkyl squarates esters including croconic acid mono/di alkyl or aryl esters and 2 or 2,3 or 2,3,5 or 2,3,5,6 mono/di/tri/tetra alkoxy or aryl oxy cyclohexa-2,5-diene-1,4 dione having active electrophilic sites to be attacked by nucleophilic sites N (for Asparagine) or S-(of Cysteine) and other nucleotide bases have been found to work nicely on SARS-CoV-2 in suitable composite liquid mixture with 99.99% efficacy and dialkyl squarates themselves show 40%-50% inhibition to cell entry for pseudo virion while experimented at cell line pH.

The present invention relates to the capture of anionic SARS-CoV-2 at pH 3-5.75 by positively charged ammonium ions in an amino polymer such as chitosan which is a polysaccharide molecule having free amine groups in large number (approximately 7.6 moles of free Nitrogen in primary amine form per mole of Chitosan sample, which is 85% deacetylated).

Chitosan (Molecular formula $C_{56}H_{103}N_9O_{39}$, molar mass 1526.5 grams/mol) contains 7.6 moles of Nitrogen as free amino groups. This show coupling reaction with dialkyl squarate (1%-15% by weight) esters including croconic acid mono/di alkyl or aryl esters and 2 or 2,3 or 2,3,5 or 2,3,5,6 mono/di/tri/tetra alkoxy or aryl oxy cyclohexa-2,5-diene-1,4 dione having active electrophilic sites to be attacked by nucleophilic sites N (for Asparagine) or S-(of Cysteine) and other nucleotide bases quantitatively in one or both the sites of dialkyl squarates in reaction mixture upon heating at 55°C with continuous stirring in ethanol and little glycerol composite solvent medium. Later to dissolve all chitosan for further coupling reactions and to create positively charged ammonium ion in the coupled product pH 3-5.75 was maintained, using acetic acid or Squaric acid. Good amount of nucleophilic substitution reaction occurred at 55°C as confirmed by FTIR and mass spectra.



Chitosan with 100% deacetylation



Chitosan (85% deacetylated) Here x= 0.15 and y=0.85

The deposit is on a matrix of PDMS (poly dimethyl siloxane) in a range from 15% to 40% wt. an organosilicon, which is hydrophobic due to low surface energy, having unusual rheological

properties, nontoxic, non-flammable and a good binder to glass through H bond Si-OH bonds of glass, ion dipole attraction, induced dipole attraction and other van der Waal's forces. It acts as elastic solid with unusual high viscosity. It is a common surfactant and is a common reagent as defoamer, It has use in skin treatment, skin moisturizing lotion with purpose of skin protection. It is nonbiodegradable but absorbed in waste water treatment. It is one of the high-performance polymers, with unique physical and chemical properties like flexible, thermotolerant, resistant to oxidation, ease of fabrication.

PDMS with isopropyl alcohol [20%-40% by weight], glycerol [1%-4% by weight] and PPG [15%-35% by weight] were mixed as binder of active component primary ammonium ion of chitosan and the product dialkyl squarate substituted by chitosan amino groups. It was homogeneously mixed with continuous stirring.

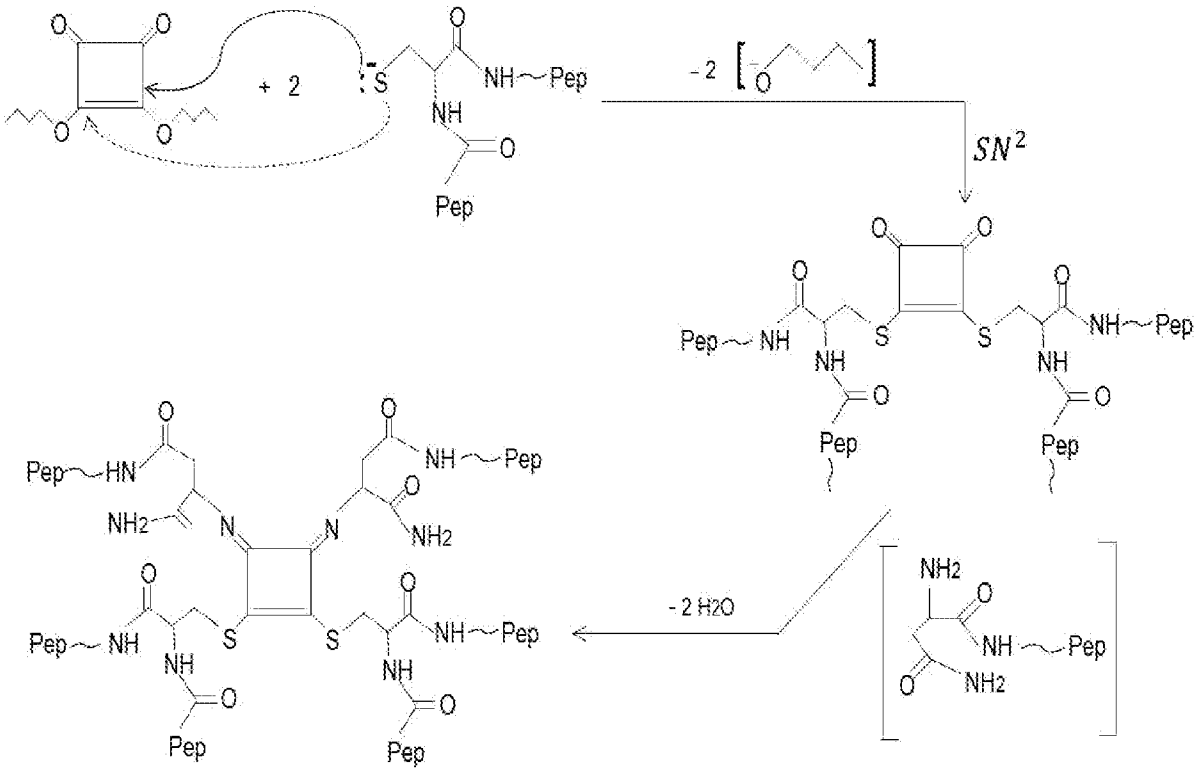
Finally, two compositions added in the suitable proportion with continuous stirring and heating at 35°C. The glass slides and glass wool layer were then given dip coating and one set cured at 45°C and other set kept as such for drying in air at 25°C. The tests were then carried out for characteristic studies of the nano deposit on glass and glass wool along with antiviral efficacy against MS2 bacteriophage with *E.Coli* as host and SARS-CoV-2 at BSL-3 facility.

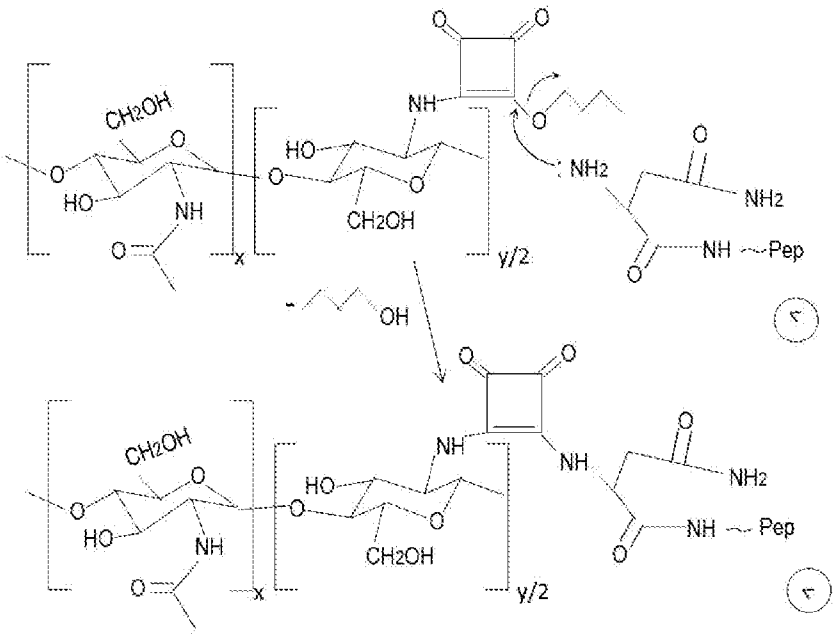
The reactions involved in the conjugate formation and deactivation reactions can take place in two ways:

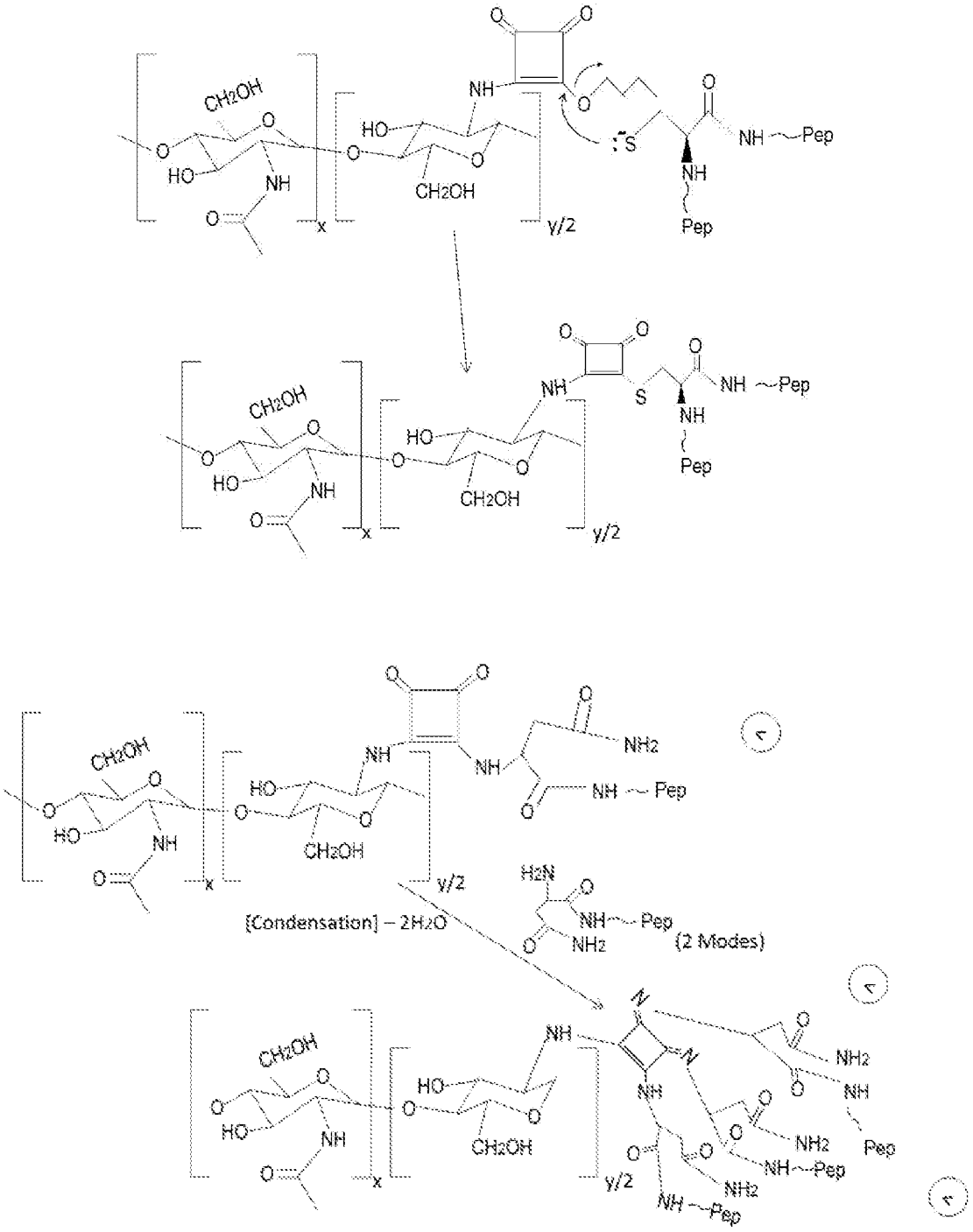
1. Nucleophilic substitution reaction by N of asparagine (side chain amino group), S(-) of cysteine found in Receptor binding domain in plenty of SARS-CoV2
2. RNA bases attack at squarate
3. Condensation of amino groups of free amine side chains with 1, 2 diketo groups
4. the cationic surface charge traps the negatively surface charged SARS-CoV2

The 98% deactivation proves that RNA is killed by the virucidal composition used here.

Deactivation process of SARS-CoV-2 by SN2 reaction by N of amino group of asparagine and S- of cysteine and condensation reactions are described below:


+ 2
- 2
SN2
NH~Pep
Pep
- 2 H2O
NH2
NH~Pep
NH2


CH2OH
HO
NH
NH2
NH~Pep
CH2OH
x
y/2
OH



Deactivation reaction of SARS CoV2

Squaric acid ester or ester of croconic acid

All types of squarate esters like mono/dimethyl squarate, mono/diethyl squarate, mono/diisopropyl squarate, mono/dibutyl squarate or any squaric acid mono/di esters including

croconic acid mono/di alkyl or aryl esters and 2 or 2,3 or 2,3,5 or 2,3,5,6 mono/di/tri/tetra alkoxy or aryloxy cyclohexa-2,5-diene-1,4 dione can be used in the present invention.

In certain embodiments, the squaric acid ester is a mono ester or a diester in which two ester groups may be the same or different.

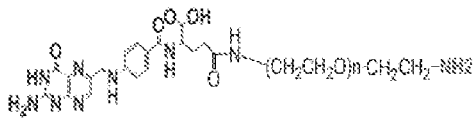
In certain embodiments, the squaric acid ester is squaric acid monoester (also known as squarate monoester). In certain embodiments, the squaric acid monoester ester is an alkyl squarate, an aryl squarate or a combination thereof. Examples of alkyl squarate include, but are not limited to, methyl squarate, propyl squarate, ethyl squarate, butyl squarate, pentyl squarate, hexyl squarate, heptyl squarate, octyl squarate, and the like.

In certain embodiments, the squaric acid ester is squaric acid diester (also known as squarate diester). In certain embodiments, squaric acid diester is a dialkyl squarate, a diaryl squarate, an alkyl aryl squarate or any combination thereof. Examples of dialkyl squarate include, but are not limited to, squaric acid dibutyl ester (SADBE), squaric acid diethyl ester (SADEE), squaric acid monobutyl ester (SAMBE), or squaric acid monoethyl ester (SAMEE). In certain embodiments, dialkyl squarate is squaric acid diethyl ester (SADEE). In some instances, dialkyl squarate is squaric acid dibutyl ester (SADBE).

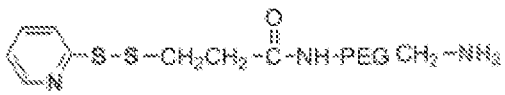
Amino Polymer

Any amino polymer which provides an amino functionality and reacts with squaric acid ester or ester of croconic acid to form a corresponding coupled product may be used in the composition. The amino polymer may also comprise thiol functionality. In certain embodiments, amino polymer may comprise both the amino functionality and thiol functionality.

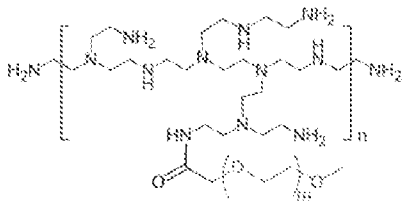
In certain embodiments, the following types of linear or branched polymers having polyamino or amino, SH functional groups may be used in the composition. These polymers together can act as the virucidal deposit in combination with mono/dialkyl squarates (ester) series when used in required proportion.



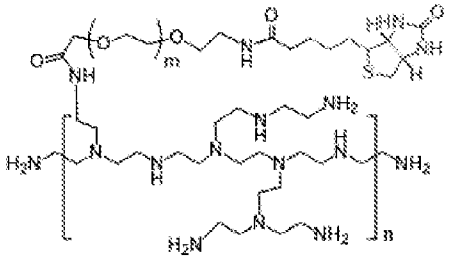
Folate-PEG-Amine [folate- polyethylene glycol-amine]



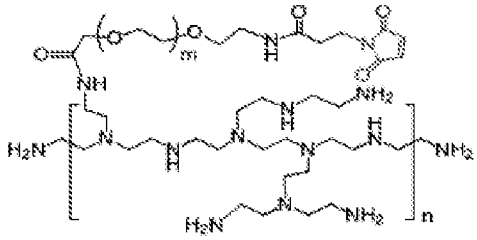
OPSS-PEG-NH2 [Ortho-pyridyl disulfide functionalized polyethylene glycol-amine]



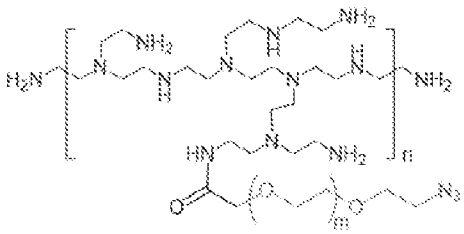
PEI-g-PEG [polyethylenimine-graft-poly(ethylene glycol)]



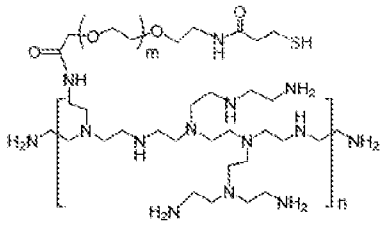
PEI-g-PEG-Biotin [polyethylenimine-graft-poly(ethylene glycol)-biotin]



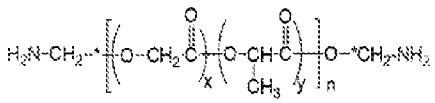
PEI-g-PEG-Melamide [polyethylenimine-graft-poly(ethylene glycol)-melamide]



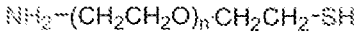
PEI-g-PEG-N3 [polyethylenimine-graft-poly(ethylene glycol)-azide]



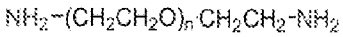
PEI-g-PEG-SH [polyethylenimine-graft-poly(ethylene glycol)-thiol]



PLGA-Diamine (50:50) [Poly(D,L-lactide-co-glycolide)-diamine]



Thiol-PEG-Amine-HS-PEG-NH2 [Thiol- polyethylene glycol-thiol-polyethylene glycol-amine]



NH2-PEG-NH2 [amine-polyethylene glycol-amine]

Compositions

In one embodiment, the invention provides a composition comprising squaric acid ester or croconic acid ester, amino polymer, ethanol, polypropylene glycol, glycerol and a binder solution, wherein the amino polymer is coupled with squaric acid ester or croconic acid ester.

In another embodiment, the squaric acid ester is squaric acid monoester or squaric acid diester.

In another embodiment, the squaric acid monoester ester is an alkyl squarate, an aryl squarate or a combination thereof; and squaric acid diester is a dialkyl squarate, a diaryl squarate, an alkyl aryl squarate or any combination thereof.

In another embodiment, the squaric acid ester is selected from diethyl squarate and dibutyl squarate.

In another embodiment, the amino polymer is selected from a group comprising deacetylated chitosan, folate-polyethylene glycol-amine, ortho-pyridyl disulfide functionalized polyethylene glycol-amine, polyethylenimine-graft-poly(ethylene glycol), polyethylenimine-graft-poly(ethylene glycol)-biotin, polyethylenimine-graft-poly(ethylene glycol)-melamide, polyethylenimine-graft-poly(ethylene glycol)-azide, polyethylenimine-graft-poly(ethylene glycol)-thiol, poly(D,L-lactide-co-glycolide)-diamine, thiol- polyethylene glycol-thiol-polyethylene glycol-amine and amine-polyethylene glycol-amine.

In another embodiment, the deacetylated chitosan has a degree of N-deacetylation of at least 80%.

In another embodiment, the binder solution comprises epoxyresins, organosilicon polymers or combination thereof.

In another embodiment, the binder solution comprises polydimethylsiloxane, isopropyl alcohol and glycerol.

In another embodiment, the concentration of squaric acid ester or croconic acid esterin the composition is in a range from 0.1% to 25% by weight.

In another embodiment, the concentration of amino polymer in the composition is in a range from 1% to 50% by weight.

In another embodiment, the concentration of ethanol in the composition is in a range from 5% to 25% by weight.

In another embodiment, the concentration of polypropylene glycol in the composition is in a range from 15% to 35% by weight.

In another embodiment, the concentration of glycerol in the composition is in a range from 1% to 4% by weight.

In another embodiment, the concentration of polydimethylsiloxane in the composition is in a range from 15% to 40% by weight.

In another embodiment, the concentration of isopropyl alcohol in the composition is in a range from 20% to 40% by weight.

In another embodiment, the pH of the composition is in the range from 4.0 to 5.75.

In another embodiment, the invention provides a composition comprising dibutyl squarate at a concentration of 1.4% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w and polypropylene glycol at a concentration of 22.4% w/w.

In another embodiment, the invention provides a composition comprising diethyl squarate at a concentration of 0.3% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w and polypropylene glycol at a concentration of 23.9% w/w.

In another embodiment, the invention provides an article of manufacture comprising the composition of the present invention.

In another embodiment, the composition of the present invention can be used in wide ranging application such as being used as a coating material, as a deposit and as an active ingredient in industrially used chemicals or formulations. One specific use of the composition is as a coating material for air filters.

In another embodiment, the invention also provides an article, a composition or formulation comprising the composition of the present invention as an active ingredient.

The composition of the present invention and deposition of nanocomposite would add one extra layer to air filters and make air virus free, especially against the most infectious virus SARS-CoV-2.

In one embodiment, the composition of the present invention can be coated as a nano-deposit on glass or glass wool, paint, coat on packaging materials for various delivery system which can show excellent efficacy against SARS-CoV-2 and other viruses, can be used in AC, Air purifiers and HEPA filters, Paint, coat for packaging materials for various product delivery systems to purify air and the air turns out to be clean and pure and the painting inside room, packaging materials to be safe with this current formulation with 98% efficacy against SARS-CoV-2.

Any ammonium/primary/secondary/tertiary ammonium/ phosphonium ion linked with polymeric chain coupled with active substituent can deactivate any viruses namely SARS-CoV-2 or other DNA/RNA viruses/bacteria effectively.

Methods of preparation

In one embodiment, the invention provides a method of preparing the antiviral composition, comprising the steps of:

a. preparing a reaction mixture comprising squaric acid ester or croconic acid ester present at a concentration in a range from 0.1% to 25% by weight, amino polymer present at a concentration in a range from 1% to 50% by weight, ethanol present at a concentration in a range from 5% to 25% by weight, polypropylene glycol present at a concentration in a range from 15% to 35% by weight and glycerol present at a concentration in a range from 1% to 4% by weight;
b. preparing a binder solution; and
c. mixing the reaction mixture of step (a) with the binder solution in presence of propylene glycol to obtain the composition.

In another embodiment, the step of preparing the reaction mixture comprises the steps of:

a. adding squaric acid ester or croconic acid ester, amino polymer and ethanol to form a reaction mixture;
b. adding an acid at a concentration in the range from 0.0005 M to 0.0015 M to maintain the pH of the reaction mixture at 5.25;
c. adding polypropylene glycol and glycerol to the reaction mixture; and
d. stirring the reaction mixture at a temperature in the range from 45-65°C to obtain the reaction mixture of step (a).

In another embodiment, the invention provides a method for preparing the binder solution comprising the steps of:

a. mixing polydimethylsiloxane at a concentration in a range from 15% to 40% by weight, isopropyl alcohol at a concentration in a range from 20% to 40% by weight and glycerol at a concentration in a range from 1% to 4% by weight; and
b. stirring the reaction mixture to obtain the binder solution.

In certain embodiments of the method, acetic acid is used to maintain the pH of the reaction mixture.

In certain embodiments of the method, squaric acid ester, ester of crococnic acid, amino polymer are same as defined above.

In certain embodiments, the squaric acid ester is squaric acid monoester or squaric acid diester.

In certain embodiments, the squaric acid monoester ester is an alkyl squarate, an aryl squarate or a combination thereof; and squaric acid diester is a dialkyl squarate, a diaryl squarate, an alkyl aryl squarate or any combination thereof.

In certain embodiments, the squaric acid ester is selected from diethyl squarate and dibutyl squarate.

In another embodiment, the amino polymer is selected from a group comprising deacetylated chitosan, folate-polyethylene glycol-amine, ortho-pyridyl disulfide functionalized polyethylene glycol-amine, polyethylenimine-graft-poly(ethylene glycol), polyethylenimine-graft-poly(ethylene glycol)-biotin, polyethylenimine-graft-poly(ethylene glycol)-melamide, polyethylenimine-graft-poly(ethylene glycol)-azide, polyethylenimine-graft-poly(ethylene glycol)-thiol, poly(D,L-lactide-co-glycolide)-diamine, thiol- polyethylene glycol-thiol-polyethylene glycol-amine and amine-polyethylene glycol-amine.

EXAMPLES

The invention will now be further illustrated by the following non-limiting examples. The specific examples below are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. The components and/or reagents of the present disclosure are commercially available and/or can be prepared according to methods readily available to a skilled person.

Example 1: Preparation of the anti-viral composition comprising dibutyl squarate (Formulation I)

A first reaction mixture (Reaction Mixture 1) was prepared using dibutyl squarate, 85% deacetylated chitosan, ethanol, acetic acid, polypropylene glycol (PPG) and glycerol. Briefly, 2 gm (0.01 moles) of dibutyl squarate, 4 gm (0.025 moles) 85% deacetylated chitosan and 17 gms ethanol was added to the reaction mixture. 0.001 M acetic acid was added to maintain pH at 5.25. Thereafter, 1 gm of polypropylene glycol and 2 gm of glycerol was added. This mixture was stirred at 55°C for 15 minutes.

A second reaction mixture (Reaction Mixture 2) as binder solution was prepared with 40 grams of polydimethylsiloxane (PDMS) and 60 grams isopropyl alcohol (IPA) and 3 grams glycerol. The reaction mixture was stirred at room temperature for 15 mins.

28 grams of the Reaction Mixture 1, 80 grams of Reaction Mixture 2 and 30 grams polypropylene glycol were mixed together and stirred at 35°C for 15 minutes to obtain the anti-viral composition.

The final anti-viral composition had dibutyl squarate at a concentration of 1.4% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w, polypropylene glycol at a concentration of 22.4% w/w. The anti-viral formulation had a pH of 5.25 and is in the form of a nanodeposit.

Example 2: Preparation of the anti-viral composition comprising diethyl squarate (Formulation II)

A first reaction mixture (Reaction Mixture 1) was prepared using diethyl squarate, 85% deacetylated chitosan, ethanol, acetic acid, polypropylene glycol (PPG) and glycerol. Briefly, 0.5 gm of diethyl squarate, 4 gm (0.025 moles) 85% deacetylated chitosan and 17 gm ethanol was added to the reaction mixture. 0.001 M acetic acid was added to maintain pH at 5.25. Thereafter,

3 gm of polypropylene glycol and 2 gm of glycerol was added. This mixture was stirred at 55°C for 15 minutes.

A binder solution was prepared with 40 grams of polydimethylsiloxane (PDMS) and 60 grams isopropyl alcohol (IPA) and 3 grams glycerol. The reaction mixture was stirred at room temperature for 15 mins.

28 grams of the Reaction Mixture 1, 80 grams of Reaction Mixture 2 and 30 grams polypropylene glycol were mixed together and stirred at 35°C for 15 minutes to obtain the anti-viral composition.

The final anti-viral composition had diethyl squarate at a concentration of 0.3% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w, polypropylene glycol at a concentration of 23.9% w/w. The anti-viral composition had a pH of 5.25.

Example 3: FTIR Studies

FTIR studies were conducted to for characterizing the coupled product. Figure 1 depicts the FTIR for the partially quantitatively Coupled product of Chitosan and Dibutyl squarate reaction (Formulation I of Example 1). The observations were made during FTIR studies with 85% Chitosan and FTIR studies with squaric acid dibutyl ester were compared.

Analysis of FTIR of coupling product of Chitosan and Dibutyl squarate reaction:

- 3465.97 cm-1 due to free NH bond that is primary amine (Amide A band) and OH
- 2922 cm-1 due to C-H stretch
- 1437cm-1 NH bending Amide II band
- 1651.02 Squaric acid amide linkage
- 1651.02cm-1 C=O of amide of squaramide (Amide I band)
- 1261.53 cm-1 Squaramide (Amide-III) in aromatic ether
- 1061.92 cm-1 due to C1-H bending vibration in sugar
- 1033.01cm-1, the intensity of primary alcohol due to C=O stretching becomes much smaller than pure chitosan.
- 1000-1200 cm-1 due to C-N bond
- 663.63 cm-1, 895.42 cm-1 due to NH2, NH wagging (shifts on H bonding)

The absence of peak at 1583.12 cm-1 and presence of new peak at 1651 cm-1 are due to loss of primary amino group of chitosan confirming the C-N coupling of chitosan with dibutyl squarate

List of peak area (height)	
Peak number	**X cm-1**
1	3734.03
2	3465.97
3	2922.32
4	1807.20
5	1651.02
6	1437.0
7	1376.03
8	1261.53
9	1156.05
10	1061.92
11	1033.01
12	895.42
13	663.63
14	560.55

Example 4: Mass Spectroscopic Studies

Mass Spectroscopic studies were performed on chitosan and partially quantitatively coupled product of chitosan and squaric acid dibutyl ester. The results are depicted in Figure 2 (- mode mass spectra of chitosan), Figure 3 (- mode mass spectra of coupled product) and Figure 4 (+ mode mass spectra of coupled product). This was concluded after comparing the mass spectrometric findings of pure Chitosan.

Chitosan (-) Mode Mass Spectra	Coupled Product (-) Mode Mass Spectra
322.8	139.9
325.5 (very large)	163.2
326.1 (large)	169.5 (very large)

337.0	251.3
341.2	255.1
349.2	265.3
350.5 (large)	297.1 (large)
353.0	366.9
381.5	373.4
395.2	427.5 (Additional peak)
396.9	454.5 (Additional peak)
	511.9 (Additional peak)
	567.0 (Additional peak)
	653.2 (Additional peak)

Table 1: Additional peaks of coupled product depicted in (-) mode mass spectra

Chitosan (+) Mode Mass Spectra	Coupled Product (+) Mode Mass Spectra
180	108
188	133.1
203.9	151
208	172.5
214	173.1 (large peak)
229.8	177.1
223	217.0 (Very large peak)
239	235.0
249.2 (large peak)	255.7
276.4	275.6
303.0 (Very large peak)	277.6
304.9	290.5
306.2	305.0 (Very very large peak)
334.9	340.0 (large peak)
391.3	344.4
412	349.2
468.6	365.3
	413.1

	413.3
	437.1
	471.4 (Additional peak)
	567.6 (Additional peak)
	615.9 (Additional peak)

Table 2: Additional peaks of coupled product depicted in (+) mode mass spectra

Other than the fragment form in mass spectra the higher peaks at higher m/z value clearly indicates the N of primary amine substituted squarates quantitatively and cross linkage of Chitosan through squarate coupling. The higher peaks are absent in mass spectrometry of Chitosan while compared with the mass spectrometry with squaramide derivative both in positive and negative mode of mass spectroscopy.

Example 5: PXRD Analysis

PXRD Analysis was performed on the coupled product as depicted in Figure 5, Figure 6 and Figure 7. The PXRD result shows the peaks at 2 Theta of 10 degree, 20 degree (sharp peak), 30 degree, 35 degree and 40 degree. This is due to the particular plane in chitosan.

The N (primary amine of chitosan) substituted shows shift of sharp peak at 23 degree with other peaks at 18degree and 35degree. This shift indicate that the distortion of the diffracting plane due to bond formation of chitosan and dibutyl squarate confirmed by FTIR.

From Figure 7, it is understood that the diffracting atoms of chitosan of same plane have interacted with dibutyl squarate and thus smaller peak compared to that of chitosan at 2 Theta of 20 degree was found. Another reason of smaller nature of the peak might be due to amorphous nature achieved due to glass wool matrix interaction. Other small maxima were found at 32° and at other angles many smaller peaks arose.

Example 6: Scanning Electron Microscopy Analysis

Scanning electron microscopic analysis was done as shown in Figure 8. The SEM studies show the cross chain polymeric fiber layer with active ingredient (Composition) deposited on Glass surface.

Example 7: Measurement of activity against MS2 Bacteriophage on plastics and other non-porous surfaces and coating materials

A study was conducted for measuring antiviral activity of the compositions on plastic and other non-porous surface of antiviral-treated products against MS2 bacteriophage. The studies were conducted at Biotech Testing Services, Mumbai, India.

MS2 Bacteriophage (MS2) is an RNA virus of the family Leviviridae. *Escherichia coli* ATCC 15597 are the hosts for bacteriophages. Due to its environmental resistance, MS2 bacteriophages are used as a surrogate virus (particularly in place of Picornaviruses such as Poliovirus and human Norovirus) in water quality and antimicrobial studies.

Pre-sterilized samples were loaded with diluted viral suspension to 10^6 PFU/ ml. Virus suspension at an inoculation volume of 0.4 ml was added to 50 mm x 50 mm of Test substrate. lt was covered with 40 mm x 40 mm low density polyethylene (LDPE). Following exposure time, virus was eluted and neutralized by serial tenfold dilution and assayed to determined surviving viruses in comparison with Control without test product in sq. cms.

Assay with antiviral formulation comprising dibutyl squarate

The assay was done with antiviral formulation comprising dibutyl squarate. The results after 2 hrs and 24 hrs are depicted in the following table:

After 2 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 7.00 x 10^4 PFU/sq cm				Log=4.84
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Antiviral formulation with dibutyl squarate (Formulation I)	10	1.00	3.84	99.98

After 24 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample identification	**Average No. of Plaques recovered from**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**

	treated samples (A_t)			
Antiviral formulation with dibutyl squarate (Formulation I)	550	2.74	2.20	99.38

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 2/24 hrs after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen after 2/24 hrs after inoculation, in PFU/ cm^2.

Assay with antiviral formulation comprising dibutyl squarate at 20 minutes

Virus assay was quantitative as Plaque forming unit (PFU) visible as area of clearance. The results are depicted in the following table:

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 5.80 x 10^4 PFU/sq cm				Log=4.76
Untreated: Average no. of Plaques recovered at 20 min (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentaqe**
Antiviral formulation with dibutyl squarate (Formulation I)	2200	3.34	1.60	97.52

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 20 mins after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen atler 20 mins after inoculation, in PFU/ cm^2

The formulation has a very high efficacy of 97.52% reduction of bacteriophage MS2 within 20 minutes.

Assay with antiviral formulation comprising dibutyl squarate dried at 75°C

The assay was done with antiviral formulation comprising dibutyl squarate dried at 75°C. The results after 2 hrs and 24 hrs are depicted in the following table:

After 2 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 7.00 x 10^4 PFU/sq cm				Log=4.84
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Antiviral formulation with dibutyl squarate (Formulation I)	200	2.30	2.54	99.71

After 24 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Antiviral formulation with dibutyl squarate (Formulation I)	1040	3.01	1.93	98.83

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 2/24 hrs after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen after 2/24 hrs after inoculation, in PFU/ cm^2.

Assay with antiviral formulation comprising diethyl squarate

The assay was done with antiviral formulation comprising diethyl squarate. The results after 2 hrs and 24 hrs are depicted in the following table:

After 2 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 7.00 x 10^4 PFU/sq cm				Log=4.84
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Antiviral formulation with diethyl squarate (Formulation II)	780	2.89	1.95	98.88%

After 24 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Antiviral formulation with diethyl squarate (Formulation II)	1000	3.00	1.94	98.87%

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 2/24 hrs after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen after 2/24 hrs after inoculation, in PFU/ cm^2.

Assay with binder solution

The assay was repeated with only the binder formulation. The results after 2 hrs and 24 hrs are depicted in the following table:

After 2 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 7.00 x 10^4 PFU/sq cm				Log=4.84
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Binder solution	8100	3.90	0.94	88.42%

After 24 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Binder solution	9500	3.97	0.97	89.32%

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 2/24 hrs after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen after 2/24 hrs after inoculation, in PFU/ cm^2.

Assay with blank glass

The assay was repeated with only the blank glass as control. The results after 2 hrs and 24 hrs are depicted in the following table:

After 2 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019

Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 7.00 x 10^4 PFU/sq cm				Log=4.84
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Binder solution	59000	4.77	0.07	15.71%

After 24 hrs

Quantitative Assessment of Antiviral Activity - ISO 21702: 2019				
Untreated: Average no. of Plaques recovered at 0 hours (U_0): 6.80 x 10^4 PFU/sq cm				Log=4.83
Untreated: Average no. of Plaques recovered at 24 hours (U_t): 8.90 x 10^4 PFU/sq cm				Log=4.94
Sample identification	**Average No. of Plaques recovered from treated samples (A_t)**	**Log of Plaques recovered from treated samples (A_t)**	**Antiviral Activity (R) (Log U_t – A_t)**	**Virus Reduction Percentage**
Binder solution	10500	4.02	0.92	88.20

Wherein,

R= Antiviral activity

U_0= Log of PFU recovered from Untreated specimen immediately after inoculation, in PFU/ cm^2

U_t= Log of PFU recovered from Untreated specimen after 2/24 hrs after inoculation, in PFU/ cm^2

A_t= Log of PFU recovered from Treated specimen after 2/24 hrs after inoculation, in PFU/ cm^2.

Example 8: Measurement of activity against Influzena virus on plastics and other non-porous surfaces and coating materials

A study was conducted for measuring antiviral activity of the compositions on plastic and other non-porous surface of antiviral-treated products against Influenza A virus (H3N2): A/Hong Kong/8/68: ATCC VR-1679 using Formulation I of Example 1. The studies were conducted at Biotech Testing Services, Mumbai, India

MDCK cell ATCC CCL-34 were used as the hosts for the virus. TCID50 infectivity titer method was used.

Pre-sterilized samples were loaded with diluted viral suspension to 10^6 PFU/ ml. Virus suspension at an inoculation volume of 0.4 ml was added to 50 mm x 50 mm of Test substrate. It

was covered with 40 mm x 40 mm low density polyethylene (LDPE). Following exposure time, virus was eluted and neutralized by serial tenfold dilution and assayed to determined surviving viruses in comparison with Control without test product in sq. cms. Virus assay was quantitative as Plaque forming unit (PFU) visible as area of clearance. The results are depicted in the following table:

Virus	**Contact Duration**	**Group**	**Logarithm of infectivity of virus ($IgTCID_{50}/cm^2$)**	**Average titre infectivity of virus ($IgTCID_{50}/cm^2$)**
Influenza virus suspension (2.00 X 10^8 PFU/ml)	0 hours	Control (U_0)	5.50	5.40
			5.40	
			5.30	
	2 hours	Control (U_t)	5.00	4.96
			5.00	
			4.90	
	2 hours	Test (A_t)	1.20	1.56
			1.80	
			1.70	
Antiviral activity R= U_t - A_t			-	3.40 **(99.95%)**

Wherein,

R= Antiviral activity

U_0= Average of common logarithm from three control/ untreated specimen immediately after inoculation

U_t= Average of common logarithm from three control/ untreated specimen after 2 hours

A_t= Average of common logarithm from three treated specimen after 2 hours

The formulation has a very high anti-viral activity of 99.95% against Influenza A virus (H3N2) within 2 hours.

Example 9: Measurement of activity of antiviral formulations against SARS-CoV2 virus

A study was conducted for measuring antiviral activity of the Formulation I against SARS CoV2 virus. The study was conducted at Centre for Cellular and Molecular Biology, Hyderabad, India.

A viral RNA extraction assay and qRT-PCR assay was performed on the composition and glass slide coated with the composition. Viral RNA Extraction assay was performed using a MagMAX™ Viral/Pathogen Extraction Kit (manufactured by Applied Biosystems, Thermo Fisher). Further, the qRT-PCR assay was performed using a Meril Covid-19 One- Step RT-PCR Kit (manufactured by Meril Diagnostics Pvt. Ltd.)

Further, in the glass slide coated with the composition, a 98% reduction in viral load was observed. The viral particles reduced from $10^{5.8}$ to $10^{4.0}$. The results are shown in Figure 9.

Example 10: Invitro Cytotoxicity Assay

A study was conducted for measuring invitro cytotoxicity of the compositions on animal cell lines. The studies were conducted at Biotech Testing Services, Mumbai, India.

Test for cytotoxicity are designed to determine the biological response of mammalian cells to the test material/ Extract of test material. At the end of the exposure time, the evaluation of the presence and the extent of Cytotoxic effect is assessed. Cytotoxicity tests signifies biological compatibility of the test material and its potential to cause cell damage.

The test standards used were:

- ISO 10993-5:2009 (E) - Biological evaluation of medical devices; Tests for in vitro cytotoxicity
- EN ISO 10993-12:2004 (E) - Biological evaluation of medical devices; Sample preparation and reference materials

L929 cells (Mouse connective tissue) from Passage no. 90 (PN 90) were seeded in 96 well plates at a concentration of 10,000 cells per 100 µl of MEM culture medium with 10% FBS per well were maintained in culture for 24 hours to form a semi confluent layer and were exposed to the test material over a range of concentration from 1% to 10%. The positive control used was 0.001% SDS (Sodium Dodecyl sulphate) solution and negative control was Complete MEM medium with 10% FBS. The cell lines were incubated at 37°C with with 5% Carbon dioxide atmosphere.

After 24 hours exposure, Formazan formation is determined for each treatment concentration and compared to that determined in growth control. For each treatment the percentage inhibition of growth is calculated by Viability of cells as per formula –

$$\text{Viability Percentage} = \frac{100 \text{ X O. D. 570 nm for extract}}{\text{O. D. 570 nm for blank}}$$

Evaluation criteria was set as:

- The lower the viability percentage value, the higher the cytotoxic potential
- The percentage viability of 100% test sample is < 70%, it has cytotoxic potential
- The percentage viability of 100% test sample is ≥ 70%, it is non cytotoxic

The results are depicted in the following table and in Figure 10.

	Neg. control	**Pos. control**	**Growth control**	**1%**	**2%**	**3%**	**4%**	**5%**	**6%**	**7%**	**8%**	**9%**	**10%**
% cell viability	-	0.980	100	115.39	110.71	107.8	104.27	101.76	97.97	86.37	80.14	87.24	77.69
P value	-	-	-	0.47	0.44	0.34	0.36	0.34	0.26	0.26	0.20	0.20	0.17

The values obtained were statistically significant with a p-value <0.05. The sample was found to be non-toxic to the cells at all the concentration ranges.

Example 11: Measurement of Surface Resistivity using antiviral coating material on different surfaces

The surface resistivity of the antiviral composition was measured. The results are provided in the below table:

Material	**Actual resistivity base values (Control) Ohm/Square**	**Initial (Just after coat) Ohm/Square**	**Final After time interval mentioned in hours Ohm/Square**
Coated sample on glass plate, dried three days	10^{10}(Antistatic)	10^{10}(Antistatic)	NA
Coated sample on glass plate, dead dried after 0 washes	10^{10}(Antistatic)	NA	NA
Coated sample on glass plate, dead dried (75 Degree Centigrade, 6 hrs) after 30 washes (Equivalent to 1 year)	NA	NA	10^{11}(Antistatic)
Glass wool freshly uncoated	10^{7}(Electro static dissipative)	NA	NA

Glass wool	10^7(Electro static dissipative)	10^9 Electro Static Dissipative)	10^9(Antistatic) After 4 months
Coat on paper board (front)	10^{10}(Antistatic)	10^8 (Electro Static Dissipative)	10^8 (Electro Static Dissipative) After 12 hrs
Coat on paper board (back)	10^{11}(Antistatic)	10^8 Electro Static Dissipative)	10^9(Antistatic) After 12 hrs
Coat on cloth	10^{12}(Insulator)	10^7(Electro Static Dissipative)	10^8(Electro Static Dissipative) After 12 hours
Coat on plastic	10^{13}(Insulator)	10^{11}(Antistatic)	10^{12}Insulative (After12 hrs)
Coat on steel	10^3 (Conductive)	10^3(Conductive)	NA
Coat on laptop	10^{13}(Insulative)	10^{11} (Antistatic)	10^{12} Insulative (After 12 hrs)
Coat on wood	10^{11}(Antistatic)	10^{10}(Antistatic)	10^{10}(Antistatic) After12 hrs)
Coat on cigarette packet	10^{12} Insulator)	10^9(Electro Static Dissipative)	10^{10}(Antistatic) After 12 hrs.
Coat on blank glass	10^{12} (Insulator)	NA	NA
AC Filter available in Market	10^{13}(Insulative)	10^{11}(Antistatic)	10^{11}(Antistatic) After 12 hrs
Coat on leather chair	10^{10}(Antistatic)	10^9(Electro Static Dissipative)	After 2 hrs. 10^{10}(Antistatic)

Coat on bedsheet	10^9(Electro Static Dissipative)	NA	NA
Coat on mask cloth (Non- woven fabric, 75 GSM)	10^{13}(Insulator)	10^{11}(Antistatic)	10^{12}(Insulative) After 12 hours
Human skin	10^5	-	-
Human Hair	10^8	-	-

Conductive range: 10^3-10^6- Ohm/Square

Electro-static dissipative range: 10^6-10^9 Ohm/Square

Antistatic range: 10^{10}-10^{11} Ohm/Square

Insulator range: 10^{12}-10^{13} Ohm/Square

The results indicate that the compositions can be used as anti-static agents which prevents the build-up of static electrical charge. They are effective in increasing the surface conductance or reduces surface resistivity and are suitable for use over wide range of applications.

The claims:

1. A composition comprising squaric acid ester or croconic acid ester, amino polymer, ethanol, polypropylene glycol, glycerol and a binder solution, wherein the amino polymer is coupled with squaric acid ester or croconic acid ester.
2. The composition as claimed in claim 1, wherein squaric acid ester is squaric acid monoester or squaric acid diester.
3. The composition as claimed in claim 2, wherein squaric acid monoester ester is an alkyl squarate, an aryl squarate or a combination thereof; and squaric acid diester is a dialkyl squarate, a diaryl squarate, an alkyl aryl squarate or any combination thereof.
4. The composition as claimed in claim 1, wherein the squaric acid ester is selected from diethyl squarate and dibutyl squarate.
5. The composition as claimed in claim 1, wherein the amino polymer is selected from a group comprising deacetylated chitosan, folate-polyethylene glycol-amine, ortho-pyridyl disulfide functionalized polyethylene glycol-amine, polyethylenimine-graft-poly(ethylene glycol), polyethylenimine-graft-poly(ethylene glycol)-biotin, polyethylenimine-graft-poly(ethylene glycol)-melamide, polyethylenimine-graft-poly(ethylene glycol)-azide, polyethylenimine-graft-poly(ethylene glycol)-thiol, poly(D,L-lactide-co-glycolide)-diamine, thiol- polyethylene glycol-thiol-polyethylene glycol-amine and amine-polyethylene glycol-amine.
6. The composition as claimed in claim 5, wherein the deacetylated chitosan has a degree of N-deacetylation of at least 80%.
7. The composition as claimed in claim 1, wherein the binder solution comprises epoxyresins, organosilicon polymers or combination thereof.
8. The composition as claimed in claim 1, wherein the binder solution comprises polydimethylsiloxane, isopropyl alcohol and glycerol.
9. The composition as claimed in claim 1, wherein the concentration of squaric acid ester or croconic acid esterin the composition is in a range from 0.1% to 25% by weight.
10. The composition as claimed in claim 1, wherein the concentration of amino polymer in the composition is in a range from 1% to 50% by weight.
11. The composition as claimed in claim 1, wherein the concentration of ethanol in the composition is in a range from 5% to 25% by weight.
12. The composition as claimed in claim 1, wherein the concentration of polypropylene glycol in the composition is in a range from 15% to 35% by weight.

13. The composition as claimed in claim 8, wherein the concentration of glycerol in the composition is in a range from 1% to 4% by weight.
14. The composition as claimed in claim 8, wherein the concentration of polydimethylsiloxane in the composition is in a range from 15% to 40% by weight.
15. The composition as claimed in claim 8, wherein the concentration of isopropyl alcohol in the composition is in a range from 20% to 40% by weight.
16. The composition as claimed in claim 1, wherein the pH of the composition is in the range from 4.0 to 5.75.
17. A composition comprising dibutyl squarate at a concentration of 1.4% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w and polypropylene glycol at a concentration of 22.4% w/w.
18. A composition comprising diethyl squarate at a concentration of 0.3% w/w, chitosan at a concentration of 2.8 % w/w, ethyl alcohol at a concentration of 12.3% w/w, glycerol at a concentration of 1.4% w/w, polydimethylsiloxane at a concentration of 22.51% w/w, isopropyl alcohol at a concentration of 33.7% w/w and polypropylene glycol at a concentration of 23.9% w/w.
19. A method of preparing the composition as claimed in claim 1, comprising the steps of:
    a. preparing a reaction mixture comprising squaric acid ester or croconic acid ester present at a concentration in a range from 0.1% to 25% by weight, amino polymer present at a concentration in a range from 1% to 50% by weight, ethanol present at a concentration in a range from 5% to 25% by weight, polypropylene glycol present at a concentration in a range from 15% to 35% by weight and glycerol present at a concentration in a range from 1% to 4% by weight;
    b. preparing a binder solution; and
    c. mixing the reaction mixture of step (a) with the binder solution in presence of propylene glycol to obtain the composition.
20. The method as claimed in claim 19, wherein the step of preparing the reaction mixture of step (a) comprises the steps of:
    a. adding squaric acid ester or croconic acid ester, amino polymer and ethanol to form a reaction mixture;
    b. adding an acid to maintain the pH of the reaction mixture at 5.25;

c. adding polypropylene glycol and glycerol to the reaction mixture; and
d. stirring the reaction mixture at a temperature in the range from 45-65°C to obtain the reaction mixture of step (a).

21. The method as claimed in claim 19, wherein the step of preparing the binder solution comprises the steps of:
    a. mixing polydimethylsiloxane at a concentration in a range from 15% to 40% by weight, isopropyl alcohol at a concentration in a range from 20% to 40% by weight and glycerol at a concentration in a range from 1% to 4% by weight; and
    b. stirring the reaction mixture to obtain the binder solution.
22. An article of manufacture comprising the composition as claimed in claim 1.
23. The composition as claimed in any of the claims 1 to 18, wherein the composition has antiviral, antibacterial, antifungal, antimicrobial and anti-SARS-CoV-2 activity.

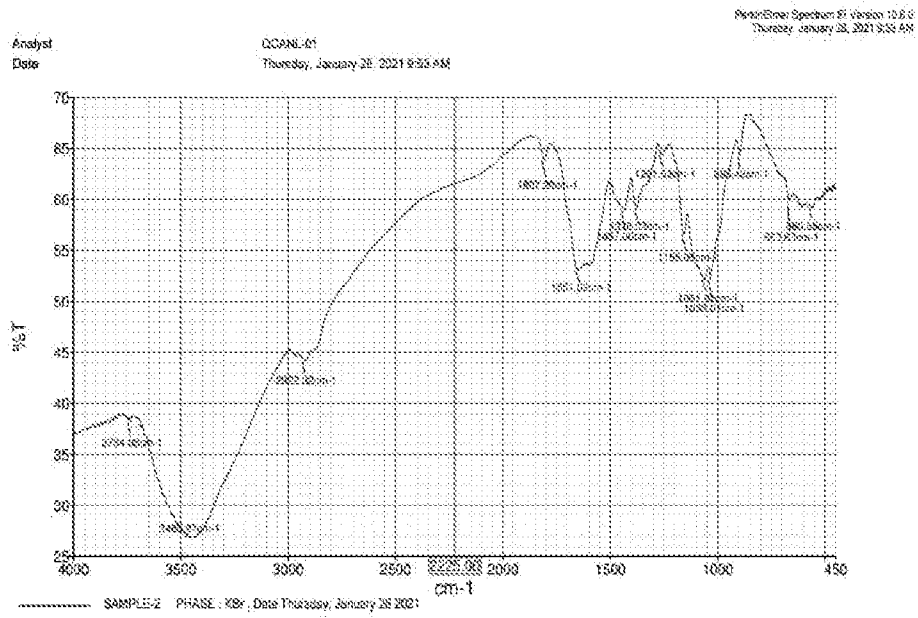


FIGURE 1

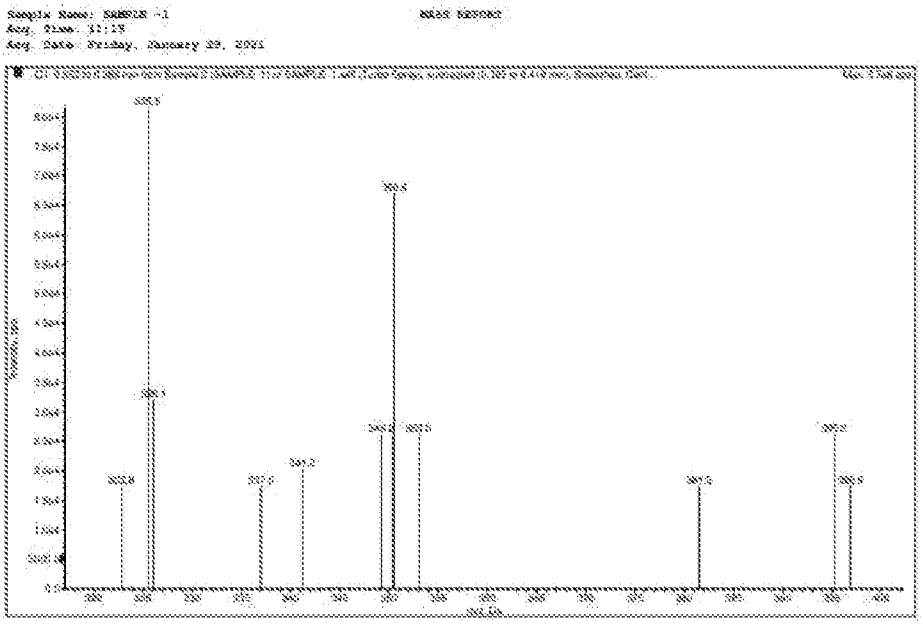


FIGURE 2

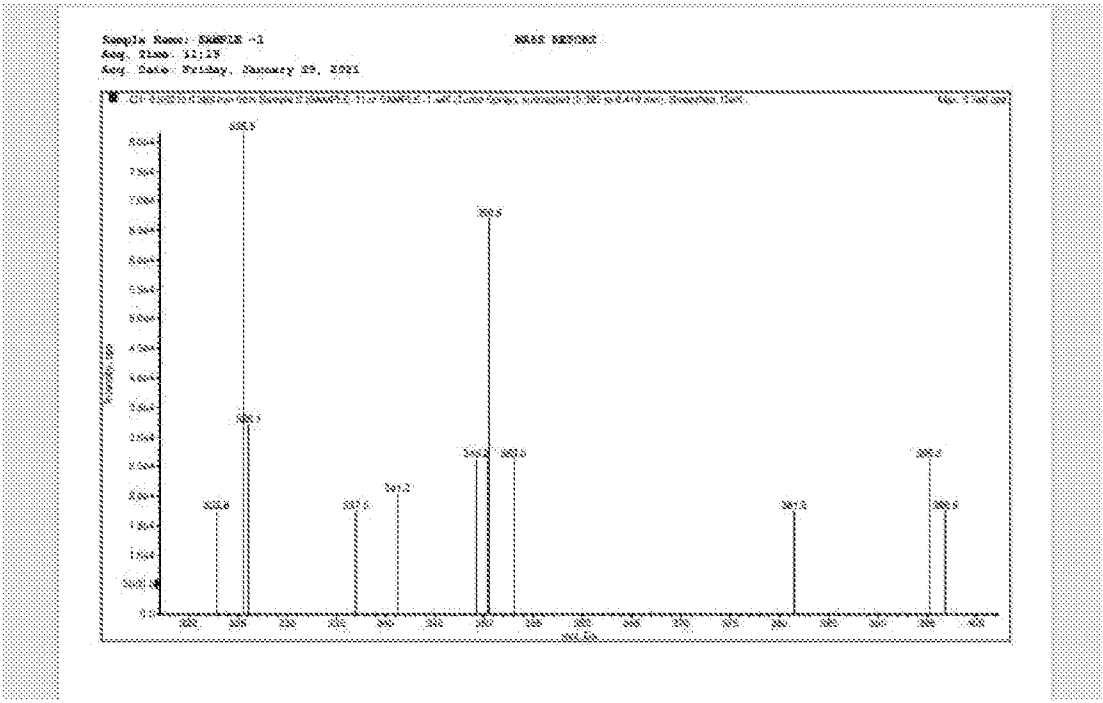


FIGURE 3

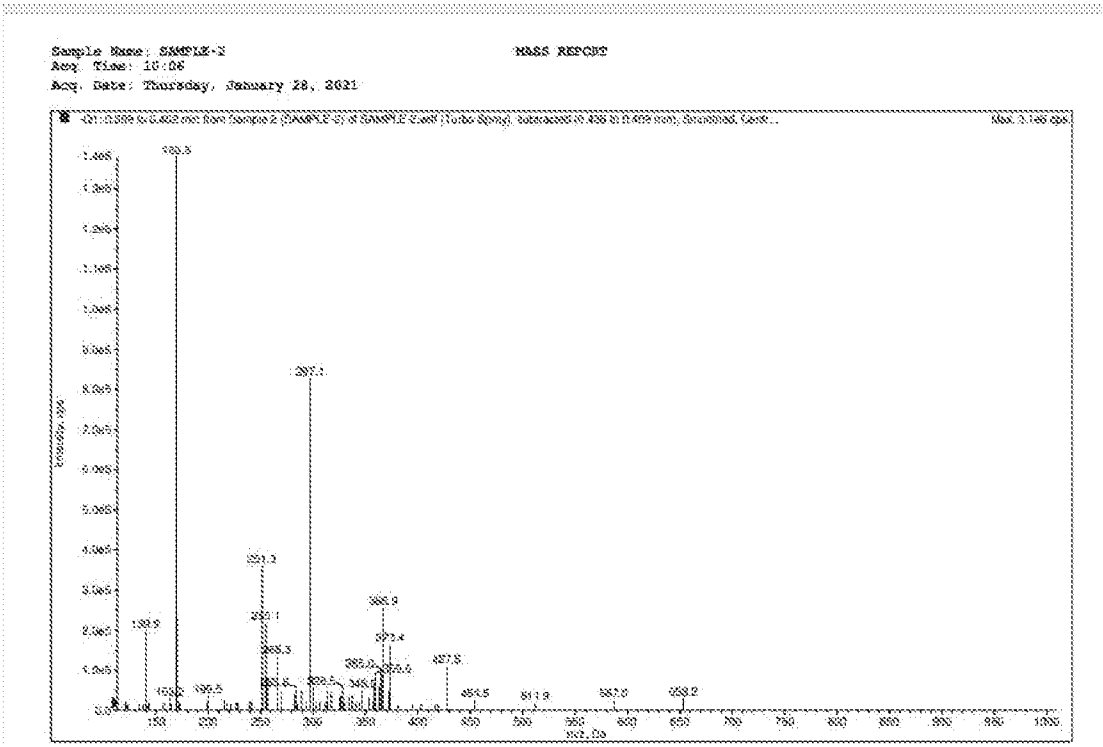


FIGURE 4

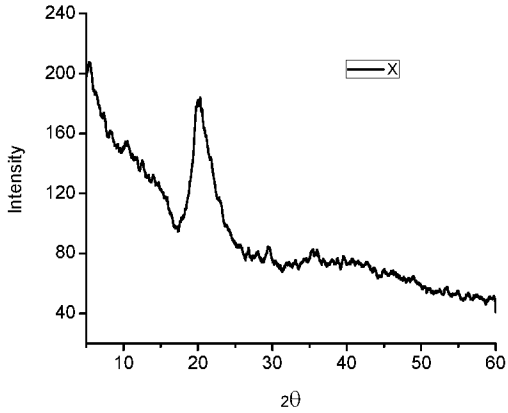


FIGURE 5

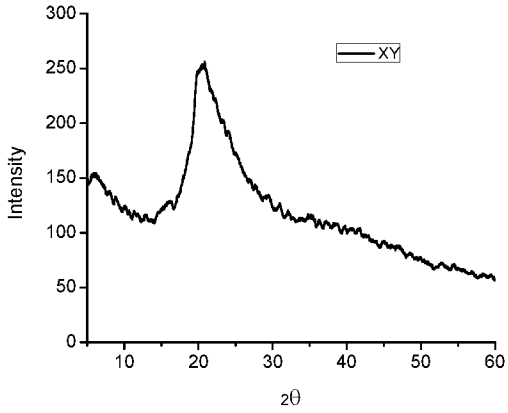


FIGURE 6

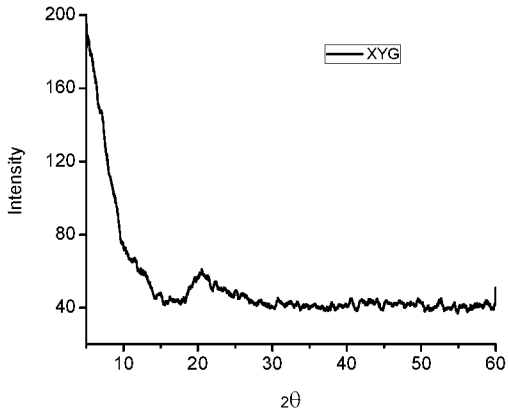


FIGURE 7

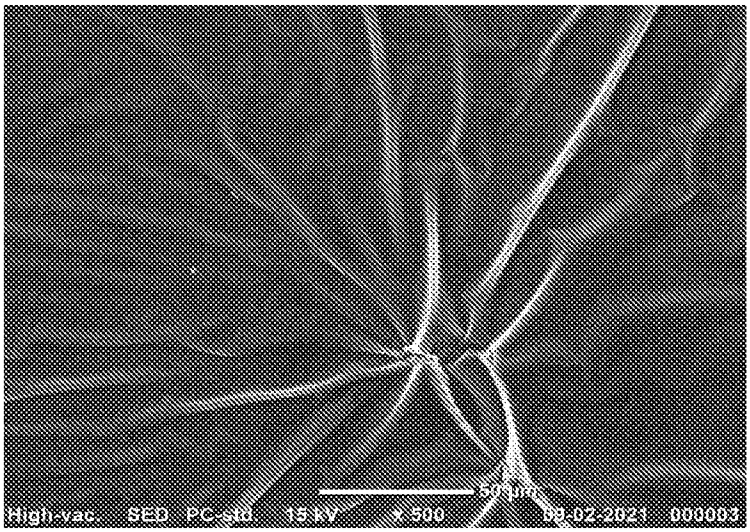


FIGURE 8

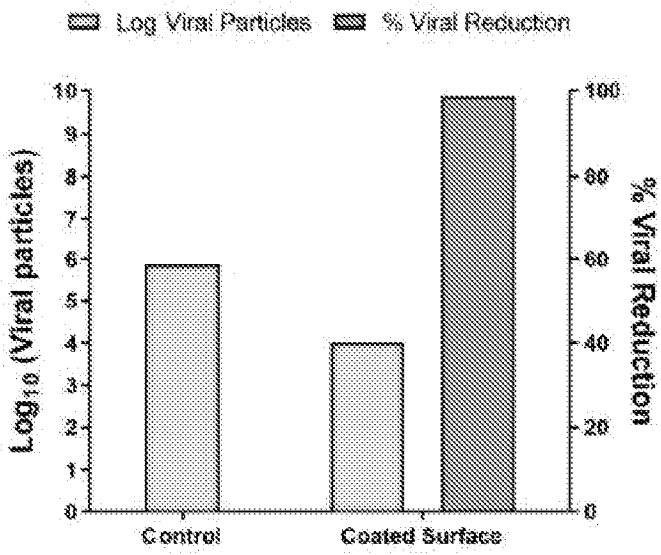


FIGURE 9

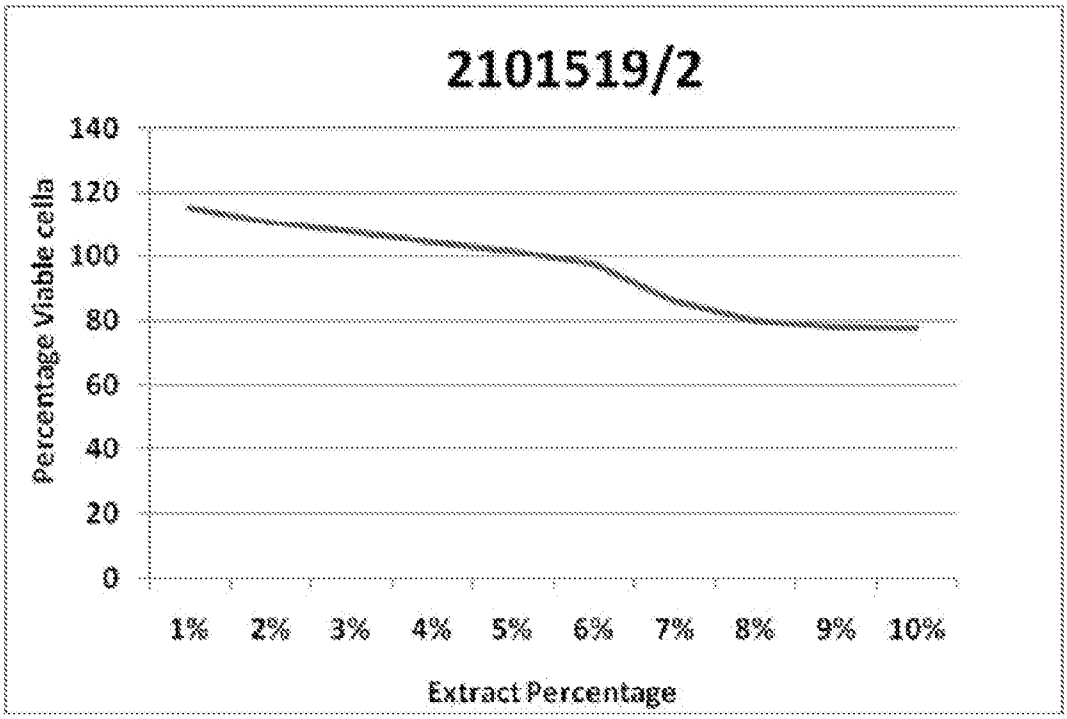

2101519/2
Percentage Viable cells
140
120
100
80
60
40
20
0
1%
2%
3%
4%
5%
6%
7%
8%
9%
10%
Extract Percentage

FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2021/050498

A. CLASSIFICATION OF SUBJECT MATTER
A61K31/00,C07D405/12,C09D7/00,A61P31/12 Version=2021.01

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)

A61K,C07D,C09D,A61P

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)

PatSeer, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005074947 A2 CHEN ANDREW XIAN[US] 18 August 2005(18/08/2005) Abstract ; claims 1-6	1-23
A	WO 2008105934 A2 OREGON BIOMEDICAL ENGINEERING [US] 4 September 2008 (04/09/2008) Abstract ; paragraph [0033];claims 1-6	1-23
A	WO 2012159215 A1 POLYVALOR SEC[CA] 29 November 2012 (29/11/2012) Abstract; claim 1	1-23

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

* Special categories of cited documents:
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"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
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"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	Date of mailing of the international search report
13-08-2021	13-08-2021
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Donga Naga Raveendra Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2021/050498

Citation	Pub.Date	Family	Pub.Date
WO 2005074947 A2	18-08-2005	US 2011223259 A1	15-09-2011
		KR 20060118573 A	23-11-2006
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