CHARGE CONTROLLED BIOCIDE APPARATUS AND METHOD OF USE THEREOF

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to and contains subject matter related to: U.S. patent application no. 18/980,530 filed December 13, 2024, U.S. patent application no. 18/973,785 filed December 9, 2024, U.S. patent application no. 18/964,384 filed November 30, 2024, U.S. patent application no. 18/953,361 filed November 20, 2024, U.S. patent application no. 18/950,493 filed November 18, 2024, U.S. patent application no. 18/940,362 filed November 7, 2024, U.S. patent application no. 18/937,301 filed November 5, 2024, U.S. patent application no. 18/914,614 filed October 14, 2024, U.S. patent application no. 18/914,124 filed October 12, 2024, U.S. patent application no. 18/913,127 filed October 11, 2024, U.S. patent application no. 18/908,004 filed October 7, 2024, U.S. patent application no. 18/907,511 filed October 5, 2024, U.S. patent application no. 18/907,318 filed October 4, 2024, U.S. patent application no. 18/905,986 filed October 3, 2024, U.S. patent application no. 18/904,506 filed October 2, 2024, U.S. patent application no. 18/900,787 filed September 29, 2024, U.S. patent application no. 18/896,333 filed September 25, 2024, U.S. patent application no. 18/889,131 filed September 18, 2024, U.S. patent application no. 18/887,620 filed September 17, 2024, U.S. patent application no. 18/886,364 filed September 16, 2024, U.S. patent application no. 18/885,695 filed September 15, 2024, U.S. patent application no. 18/827,773 filed September 8, 2024, U.S. patent application no. 18/826,607 filed September 6, 2024, U.S. patent application no. 18/742,592 filed June 13, 2024, U.S. patent application no. 18/663,414 filed May 14, 2024, U.S. patent application no. 18/645,880 filed April 25, 2024, U.S. patent application no. 18/641,922 filed April 22, 2024, U.S. patent application no. 18/638,981 filed April 18, 2024, U.S. patent application no. 18/637,668 filed April 17, 2024, July U.S. provisional patent application no. 63/567,553 filed March 20, 2024, U.S. provisional patent application no. 63/569,165 filed March 24, 2024, U.S. provisional patent application no. 63/633,967 filed April 15, 2024. All of which are incorporated by reference herein it their entirety.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention relates generally to antibacterial / antimicrobial / antiviral / antifungal formulations, solutions, films, coatings, and/or compositions and/or the method of manufacture/use thereof.

DISCUSSION OF THE PRIOR ART

Bacteria / Virus / Fungus Treatment

A number of compositions acting as biocides have been reported. Patents / publications related to the current invention are summarized here.

Purschwitz, *et. al.*, "Enhancing the Antimicrobial Activity of Biocides with Polymers", U.S. patent no. 11,666,050 (June 6, 2023), and corresponding U.S. patent application publication no. 2014/0369953, each describe biocide compositions comprising halogen atoms and/or phenolic moieties, formic acid, chlorine dioxide, dialdehydes, and antimicrobial silver in combination with polyethylenimine.

Purschwitz, *et. al.*, "Enhancing the Antimicrobial Activity of Biocides with Polymers", EP 2 817 353 describe an antimicrobial agent, 4,4'-dichloro-2'-hydroxy-diphenylether, in combination with polyethylenimine.

Cheng, W., *et. al.*, "Antimicrobial Cationic Polymers", U.S. patent no. 9,399,044 and corresponding U.S. patent application publication no. 2015/0342984 describe antimicrobial, non-hemolytic cationic polyamines.

Hedrick, J. L., *et. al.*, "Cationic Polyamines for Treatment of Viruses", U.S. patent no. 10,485,824 and corresponding U.S. patent application publication no. 2017/0266224 describe antiviral cationic polyamines.

Hedrick, J. L., *et. al.*, "Cationic Polyamines for Treatment of Viruses", U.S. patent no. 9,682,100 and corresponding U.S. patent application publication no. 2016/0213707 each describe antiviral cationic polyamines, such as cationic polyamines comprising pendant monosaccharide groups.

Hedrick, J. L., *et. al.*, "Therapeutic Compositions Comprising n-alkyl-hydroxy Polymers", U.S. patent application publication no. 2017/0056513 describe polymers modified with n-alkyl-hydroxy groups comprising one or more carbon atoms for antiviral activity.

Asgari, P., *et. al.*, "Antimicrobial Coating Compositions and Residual Antimicrobial Coatings Resulting Therefrom", U.S. patent application publication no. 2023/0108533 describe aqueous coating compositions capable of forming residual antimicrobial coatings comprising non-silane quaternary compounds and quaternary silanes.

Gibney, K., "Polymeric Antibacterial Agents: Cytotoxicity and Antimicrobial Properties of Amphiphilic Polymers", Honors Thesis, Department of Chemistry, University of Michigan, April 28, 2009 describes modifying synthesized polymer structures and observing the effect on antimicrobial activity.

Fitchmun, M. I., "Surface Sanitizer", U.S. patent application publication no. 2008/0045491 describes an antimicrobial surface sanitizer composition comprising a water-miscible alcohol, water, a weak acid, and a multivalent cation.

Snyder, M., *et. al.*, "Antiviral Method" U.S. patent no. 8,450,378, May 28, 2013 and corresponding U.S. patent application publication no. 2009/0018213

describe inactivating human noroviruses with a composition comprising: an alcohol, cationic oligomers and polymers, and chaotropic agents.

Snyder, M., *et. al.*, "Antiviral Method" U.S. patent application publication no. 2007/0185216 describe inactivating non-enveloped virus particles with a composition comprising: an alcohol, cationic oligomers and polymers, proton donors, and chaotropic agents.

Niemela, E. J., "Virus-Like Particles for Preventing the Spreading and Lowering the Infection Rate of Viruses", U.S. patent no. 11,564,892 describes nanometer particles with a functionalized surface capable of binding target areas of pathogens.

Farha, S., *et. al.*, "Antiviral and Antibacterial Composition", U.S. patent application publication no. 2023/0157297, May 25, 2023 describe an antiviral / antimicrobial agent comprising: a positively charged amino acid, an organic acid, a cationic polymer, and a zwitterionic surfactant.

Djenadi, F., *et. al.*, "Coating Composition Comprising Polyethylenimine and Poly(meth)acrylic Acid", U.S. patent application publication no. 2010/0143632 described polyethylenimine and poly(meth)acrylic acid for coating steel for corrosion control.

Lan, Tian, *et. al.*, "Surface Disinfectant with Residual Biocidal Property", U.S. patent no. 11,026,418, June 8, 2021 published as US 2016/0143275 on May 26, 2016 describe a disinfectant formulation imparting a residual biocidal property.

Ricke, S. C., "Formic Acid as an Antimicrobial for Poultry Production: A Review", Frontiers in Veterinary Science, Sept. 3, 2020, doi: 10.3389/fvets.2020.00563 describe formic acid as a means to limit Salmonella.

Problem

However, there exists in the art a need for a forming and implementing antimicrobial / antiviral / antifungal formulations, solutions, films, and/or coatings that are relatively non-toxic and effective.

SUMMARY OF THE INVENTION

The invention comprises antimicrobial/antiviral/antifungal formulations, solutions, films, and coatings and apparatus and methods of use/formation thereof.

DESCRIPTION OF THE FIGURES

A more complete understanding of the present invention is derived by referring to the detailed description and claims when considered in connection with the Figures, wherein like reference numbers refer to similar items throughout the Figures.

Figure 1 illustrates a method of tuning a polymer as an antimicrobial agent;

Figure 2 illustrates tuned charged polymers and tuned counterions;

Figure 3A and Figure 3B each illustrate tuned cationic polymers;

Figure 4 illustrates polymer properties/states;

Figure 5A illustrates a set of polymers, Figure 5B illustrates polyethylenimine and polyDADMAC, Figure 5C illustrates a copolymer, and Figure 5D provides an example of a copolymer;

Figure 6 illustrates polymer protonation tuning;

Figure 7 illustrates polymer counterion tuning;

Figure 8 illustrates polymer acidification/alkalization processes;

Figure 9A illustrates acidification of a polymer and Figure 9B illustrates unprotonated and protonated monomers;

Figure 10 illustrates alkalization of a polymer;

Figure 11 illustrates a first alkalization polymer tuning process;

Figure 12 illustrates a second alkalization polymer tuning process;

Figure 13 illustrates a third alkalization polymer tuning process;

Figure 14 illustrates a first acidification polymer tuning process;

Figure 15 illustrates a conjugate base polymer tuning process;

Figure 16 illustrates a polymer slurry acid reduction process;

Figure 17 illustrates a polymer - salt pair salt reduction process;

Figure 18 illustrates polymer tuning with a weak acid;

Figure 19A and Figure 19B each illustrate polymer tuning with a weak acid and salt/counterion production;

Figure 20, illustrates relative concentration of conjugate base tuning;

Figure 21 illustrates first conjugate base availability determination;

Figure 22 illustrates second conjugate base availability determination;

Figure 23 illustrates adjusting conjugate base concentration;

Figure 24 illustrates relative tuning of two conjugate bases;

Figure 25A and Figure 25B illustrate two counterion ratios;

Figure 26 illustrates relative concentration of conjugate base tuning with three counterion types;

Figure 27 illustrates relative concentration of conjugate base tuning with polyanionic compounds;

Figure 28 illustrates relative tuning of multiple conjugate bases/counterions;

Figure 29 illustrates charged chemical backbone structures;

Figure 30A illustrates a primary amine, Figure 30B illustrates a secondary amine, Figure 30C illustrates a tertiary amine, and Figure 30D illustrates a quaternary amine;

Figure 31A and Figure 31B illustrate deprotonated and protonated repeating units, respectively, Figure 31C illustrates a polypropylenimine, Figure 31D illustrates a generalized amine polymer, and Figure 31E illustrates a nitrogen containing repeating backbone monomer of a polymer;

Figure 32 illustrates conjugate acid formation from a base;

Figure 33A, Figure 33B, Figure 33C, and Figure 33D illustrate progressively basic to acidic forms of polyethylenimine;

Figure 34A and Figure 34B illustrate branched polyethylenimine in unprotonated and protonated forms, respectively;

Figure 35 illustrates a backbone using amino acids;

Figure 36 illustrates a generic backbone structure;

Figure 37 illustrates tuning a polymer state with controlled parameters;

Figure 38 illustrates tuning cationic charge / charge effectiveness;

Figure 39 illustrates protonating a polymer;

Figure 40A and Figure 40B illustrate two polymer protonation states, respectively;

Figure 41 illustrates first and second levels of protonation at a fixed pH;

Figure 42 illustrates effective charge of the polymer as a function of activity;

Figure 43A illustrates a non-enveloped virus and Figure 43B illustrates an enveloped virus;

Figure 44A and Figure 44B illustrate a wrapped and partially wrapped virus;

Figure 45 illustrates polymer conglomerations of viruses;

Figure 46A illustrates high activity attraction between polyethylenimine and a virus and Figure 46B illustrates attraction between polyethylenimine and a virus at lower activity;

Figure 47 illustrates charge control of a liquid biocide and/or a biocidal film;

Figure 48A, Figure 48B, and Figure 48C illustrate delivery of a biocide with a spray bottle, a spray canister, and a wipe, respectively;

Figure 49 illustrates treating a substrate with a biocide;

Figure 50A and Figure 50B illustrates liquid and solid films, respectively; Figures 50C and Figure 50D illustrate an adhesion promoter in the film and settled out of the film, respectively;

Figure 51 illustrates a process of forming polyethylenimine;

Figure 52 illustrates a process 5200 of forming a poly(2-alkyl-2-oxazoline);

Figure 53A illustrates oxazolines and cationic ring opening polymerization and Figure 53B illustrates a copolymer derived from removal of an acyl group;

Figure 54A and Figure 54B illustrate deacylation of poly(2-alkyl-2-oxazoline) and poly(2-ethyl-2-oxazoline), respectively;

Figure 55 illustrates purification of a polyalkylenimine;

Figure 56 illustrates generation of a formulation;

Figure 57 illustrates additive inactivation of bacteria;

Figure 58 illustrates preparation/use of a formulation; and

Figure 59 illustrates adhesion of a film on a substrate.

Elements and steps in the figures are illustrated for simplicity and clarity and have not necessarily been rendered according to any particular sequence. For example, steps that are performed concurrently or in different order are

illustrated in the figures to help improve understanding of embodiments of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A method and apparatus for dispensing a biocide is described, comprising: a container and a biocide formulation in the container, the biocide formulation comprising: (1) the biocide and a solvent, the biocide comprising a protonated polymer comprising repeating charged units; (2) a total cationic charge density in a range of 0.005 to 0.95 meq/g, at least ten percent of the total cationic charge density from charges of non-quaternary amines of the protonated polymer; (3) an activity coefficient, γ, of a protonated site of the protonated polymer, of greater than 0.7; and (4) a counterion chloride concentration of less than 900 ppm.

Generally, the method optionally and preferably controls salts/anions of the solution, to enhance the inactivating step, such as measured by conductivity, activity, sodium concentration, organic acid/base types, and/or counterion concentrations/types. The formulation, optionally sprayed to form a film, has antivirion, antiviral, antibacterial, and/or antifungal properties that persist through time. The copolymer has monomers in equilibrium with other chemical forms, where the equilibrium is a function of pH of the formulation/process of making the formulation. Any additive to the formulation is optionally used, such as an addition of a quaternary ammonium, a polydiallyldimethylammonium salt, and/or a partially protonated polyethylenimine. The formulation is generally an antipathogen, such as finding applications in a washing machine additive, a hand sanitizer, and/or in at least partial coating of a fabric/garment. In this method, the deacylation step optionally and preferably forms an at least partially protonated polyethylenimine.

Optionally and preferably, the method comprises the steps of: partially deacylating a poly(2-alkyl-2-oxazoline) to yield a copolymer, an alkyl in the

poly(2-alkyl-2-oxazoline) comprising a hydrocarbon chain, the copolymer comprising: first monomers, the first monomers comprising a $-NH_2^+CH_2CH_2-$ group; and second monomers, the second monomers comprising an acyl group, -C(=O)-R', attached to a nitrogen in a polymer backbone group comprising $-NCH_2CH_2-$, wherein R' comprises the hydrocarbon chain, the first monomers and the second monomers arranged in any order in the copolymer; preparing a formulation, the formulation comprising the copolymer and a total cationic charge in a range of 0.2 to 10 C/cm^3, the total cationic charge at least 95% offset by counteranions in the formulation; contacting the pathogens with the formulation; and inactivating at least ninety percent of the pathogens within ninety minutes of the step of contacting.

Overview

A method for inactivating pathogens is described, comprising the steps of: (A) partially deacylating a poly(2-alkyl-2-oxazoline) to yield a first copolymer and/or providing the first polymer, an alkyl in the poly(2-alkyl-2-oxazoline) comprising a hydrocarbon chain, the copolymer comprising: first monomers, the first monomers comprising a $-NH_2^+CH_2CH_2-$ group; and second monomers, the second monomers comprising an acyl group, -C(=O)-R', attached to a nitrogen in a polymer backbone group comprising $-NCH_2CH_2-$, wherein R' comprises the hydrocarbon chain and/or (B) preparing/providing a second copolymer, the second copolymer comprising: first monomers, the first monomers comprising a $-NH_2^+CH_2CH_2-$ group and second monomers, the second monomers comprising a $-NHCH_2CH_2-$ group, the first monomers and the second monomers arranged in any order in the copolymer; preparing a formulation, the formulation comprising the copolymer and a total cationic charge in a range of 0.2 to 10 C/cm^3, the total cationic charge at least 95% offset by counteranions in the formulation; contacting the pathogens with the formulation; and inactivating at least ninety percent of the pathogens within ninety minutes of the step of contacting. Generally, the method optionally and preferably controls salts/anions of the solution, to enhance the inactivating step, such as measured by conductivity, activity, sodium concentration, organic acid/base types, and/or counterion concentrations/types. The formulation, optionally sprayed to form a

film, has antivirion, antiviral, antibacterial, and/or antifungal properties that persist through time. The copolymer has monomers in equilibrium with other chemical forms, where the equilibrium is a function of pH of the formulation/process of making the formulation. Any additive to the formulation is optionally used, such as an addition of a quaternary ammonium, a polydiallyldimethylammonium salt, and/or a partially protonated polyethylenimine. The formulation is generally an antipathogen, such as finding applications in a washing machine additive, a hand sanitizer, and/or in at least partial coating of a fabric/garment. In this method, the deacylation step optionally and preferably forms an at least partially protonated polyethylenimine.

Herein, EPA refers to the United States Environmental Protection Agency and ISO stands for the International Organization for Standardization.

Overview

Microbes exist everywhere. While many are beneficial, many have negative consequences. Industries and environments where microbes are optionally and preferably controlled include at least: hospitals, food service areas, public areas, and in the home. It would be beneficial if surfaces could be treated to kill/inactivate bacteria, viruses, and/or fungi and/or to have a residual effect that treats a surface against future bacteria, viruses, and/or fungi.

Generally, methods and processes that comprise the implementation of a new polymer-based antimicrobial technology that provides safe, durable, and/or readily removable antimicrobial films capable of inactivating bacteria, viruses, and/or fungi, in real-time with protection into the future, and are durable enough to pass the EPA (01-1A) and/or PAS2424 international durability tests are described herein. The PAS stands for a publicly available specification and the PAS2424 is from the British Standards Institution.

PAS2424

The PAS2424 specification is briefly summarized herein for clarity of presentation; however, the entirety of the PAS2424 specification is incorporated in its entirety herein by this reference thereto. The PAS2424:2014 standard quantifies surface testing for the evaluation of residual antimicrobial efficacy of liquid chemical disinfectants on hard/non-porous surfaces. The PAS standard specifies a test method for residual bactericidal and/or yeasticidal activity of liquid, chemical disinfectant products that are applied to hard, non-porous surfaces which are likely to undergo abrasive action. The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used in accordance with BS EN 13697:2001. In the United States, the Environmental Protection Agency (EPA) approved method for residual claims uses EPA 01-1A "Residual self-sanitizing activity of dried chemical residues on hard, non-porous surfaces". In Europe, the PAS 2424 test method (PAS 2424:2014) "Quantitative surface test for the evaluation of residual antimicrobial (bactericidal and/or yeasticidal) efficacy of liquid chemical disinfectants on hard non-porous surfaces" is the only method available for residual disinfection claims, but so far this method has not been accepted as an official standardized method by the European Standards Organization.

EPA 01-1A

The EPA 01-1A requirements are summarized herein. However, the entirety of the EPA 01-1A requirements and guidelines are incorporated herein by this reference thereto. Generally, the EPA01-1A residual sanitization test method guidelines are:

- the product or products (test substance) being evaluated for efficacy along with the method-specified control substance, are tested in quadruple replicate on 1 x 1 inch glass carriers or stainless-steel carriers. *EPA currently allows for liquid formulations or spray products;*
- method-specified test microorganisms for the residual sanitization test method include *Staphylococcus aureus* (ATCC6538) and *Klebsiella aerogenes* (ATCC 13048), or *Klebsiella pneumoniae* (ATCC 4352);

- a 48 – 54 hour culture of the test microorganism, with a minimum of 3 consecutive transfers and supplemented with "organic soil load" is dried onto test and control carriers;
- the dry, inoculated carriers are treated with the test substance and control substance and allowed to dry for at least 3 hours or until completely dry at room temperature and ~45-55% relative humidity;
- after the minimum 3-hour dry time, each set of test and control replicates undergo a wear and re-inoculation regimen, which takes place over 24 hours at room temperature and 45-55% relative humidity, with alternating dry and wet abrasions. Abrasion of the test surface is done using a standardized Gardco abrasion machine;
- a residual sanitization test requires a total of 12 alternating wet-dry abrasion cycles and a total of 5 re-inoculations, with a minimum of 15-minute rest times after each abrasion and a minimum of 30-minute dry times after each re-inoculation;
- after the abrasion and re-inoculation wear regimen is complete, the carriers undergo a sanitization (RSS) test (performed at least 24 hours after application of the test product);
- the sanitization test includes inoculating each treated and abraded carrier with test culture, allowing it to sit for the Study Sponsor's requested contact time, and then neutralizing it in the appropriate neutralizer. The parallel control carriers are treated in the same manner as in the test carriers; and/or
- To obtain a bacterial residual sanitizer claim, the product(s) should achieve ≥ 3-log reduction in ≤5 minutes when compared to the control-treated test surfaces.

Herein, a testing time is optionally less than or equal to 5, 10, 20, 50, 90, 100, 120, 300, 600, or 720 minutes. Optionally and preferably, a testing time is an elapsed time from inoculating a substrate, coated with an antipathogen, and/or from a time of contacting a pathogen with the antipathogen, until the testing period is terminated, such as with a wash off

of the inoculum, a filtering step that separates the antipathogen polymers from the pathogens, and/or neutralization of the antipathogen, such as by altering/neutralizing charged site of the antipathogen polymer and/or by neutralizing the pH, such as by bringing the pH toward neutral by greater than 0.5, 1, 1.5, 2, 2.5 or 3 pH units.

The EPA 01-1A procedures for testing against bacteria is modified herein for testing against viruses.

Further, the ISO 21702 durability test against viruses in summarized here. However, the entirety of the ISO 21702 standard is incorporated herein by this reference thereto. Generally, the International Standard (ISO 21702 : 2019) is a method for measuring antiviral activity on plastics and other non-porous surfaces of antiviral-treated products against viruses. Generally, the ISO 21702 Test Method guidelines are:

- control and treated samples are placed separately in sterile plates;
- 400 µL of test inoculum is spread over the surface of the samples;
- a 40 mm X 40 mm cover slip is placed on the inoculum and pressed gently such that the inoculum spreads to the edges but does not leak outside the edges
- immediately after inoculation, 10 mL of either the SCDLP broth or other neutralizer is added to the petri dishes comprising 3 untreated test specimens; and/or
- after a contact period of 24 hours, 10 ml of either the SCDLP broth or other neutralizer is added to treated and untreated samples to recover the remaining virus. Further, this neutralizing solution is serially diluted up to 10 dilutions and the infectious titre of the recovered virus is determined either by Plaque assay or by TCID50 assay. Other assays can also be used based on the virus strain.

The measuring test method of antiviral activity on porous product, such as textiles, is described in ISO 18184, which is incorporated herein in its entirety by this reference thereto.

Further, the JIS : 1922 test method is summarized here. However, the entirety of the JIS 1922 test method is incorporated herein by this reference thereto. Generally, the JIS : 1922 test is an antiviral textile test, where:

- control (untreated) and test (treated) fabric samples are placed in the vial containers;
- all vial containers are further kept in the autoclave for sterilization for 15 minutes at a temperature of 121 °C and pressure of 15 psi;
- immediately after the virus inoculation, 20 ml of Soya Casein Digest Lecithin Polysorbate Broth (SCDLP) is added to 3 control samples; to verify the cell sensitivity and cytotoxic activity, 3 control and 3 treated samples are put in sterilized vial containers. Then, 20 mL of SCDLP is added into these vials and agitated for 5 seconds and 5 times. 5 mL of washout solution from each vial is now transferred into new test tubes. 50 µL of virus suspension of known titer is added to these test tubes and incubated for 30 minutes at 25°C. After the incubation, if cell damage is observed then washout solution needs to be modified or increased carefully;
- after the contacting period of 2 hours, 20 mL of SCDLP is added to 3 control and 3 test samples. Vials are agitated using a vortex mixer to extract the remaining virus particles from the sample; and/or
- wash-out viral suspension is further used to make serial dilutions and the infectivity titre of virus is determined either using Plaque assay or TCID50 assay.

Parameters for forming and implementing various polymers, such as cationic polymers; techniques for protonating the cationic polymers to achieving

maximum charge; and formulation with various counter ions are provided herein.

The high performance and safety of products described herein are derived from the inventive use of charged polymers, such as cationic polymers, and their concomitant counter ions, which is starkly different than the current use of toxic small molecule antimicrobial chemicals in the art.

Technology Attributes

Key attributes of the technology are:

1. Use of formulations/dispersions of polymers and/or small polymer particle dispersions are used as disinfectants, such as when at least partially suspended in the liquid state.

2. In a solid state, cationic polymers create a contact-active durable antimicrobial film that yields future protection against disease-causing pathogens, which is starkly different than legacy disinfectants.

3. Because the cationic polymer technology is not based on toxic germicidal chemicals, new products arise from the tuned polymers, described *infra*, that are safe for humans, animals, and/or the environment.

4. The cationic polymer technology is optionally implemented in antimicrobial films capable of inactivating: bacteria, viruses, and/or fungi as demonstrated in ISO 22196 testing resulting in reduction in microbial population by a factor of 10,000 or more (referred to herein as " > log-4 inactivation") against a wide range of bacteria and fungus in ten minutes.

5. Antimicrobial films incorporating the cationic polymer technology kill both enveloped and non-enveloped viruses.

6. The cationic polymers, such as described herein, show a continuous (additive) killing action against a large population of bacteria, with inactivating > log 7 population, on a 5-day old film, as tested with ISO 22196.

7. Antimicrobial films created with the cationic polymers described herein pass both the EPA 01-1A durability test and the PAS2424 international durability test against, bacteria, virus, and fungi. The protocol of EPA 01-1A is incorporated in its entirety herein as published at: https://www.epa.gov /sites/default/files/2015-09/documents/cloroxpcol_final.pdf.

8. Unlike germicidal chemical disinfectants that quickly dissipate, the polymeric antimicrobial films reported herein provide an ongoing continuous killing action, such as for days and/or weeks.

9. The methods and processes taught herein are flexible and adaptable allowing for the creative implementation of new products, all to the benefit of humankind.

Generally, ISO 22196 is a standard that pertains to the measurement of antibacterial activity on plastics and other non-porous surfaces, which specifies a test method to evaluate the antibacterial activity of such surfaces by measuring the ability of bacteria to survive and multiply on them. Stated again, if a material undergoes ISO 22196 testing and achieves log-4 inactivation, it means that it has demonstrated a strong ability to inhibit or kill bacteria, making it suitable for applications where maintaining a hygienic environment is essential, such as in healthcare facilities, food processing areas, or public spaces.

Antimicrobial Action

The antimicrobial cationic polymers films described herein provide antimicrobial action in at least one and optionally in all of several ways:

1. the polymer at least partially sterically blocks action of the bacteria/virus/fungus;

2. the cationic polymer attracts and then disrupts the anionic charge of a pathogens biological membrane;

3. counter ions chemically denature the membrane and can enter the pathogen's interior; and

4. the acidic nature of the disinfectant disrupts the natural pH of the pathogens' chemistry, such as the pathogen's exterior and/or interior chemistry.

Herein, a virion comprises a virus. A virion includes the virus elements of an outer protein shell called a capsid and an inner core of nucleic acid.

Background

There is an urgent worldwide need for a new antimicrobial technology that aids in mitigation of the transmission of infectious diseases.

There are two main reasons for not winning the war against disease-causing pathogens. First, despite the development of vaccines and antibiotics, the war on infectious diseases is being lost because the focus has been primarily on using medical interventions to combat disease-causing pathogens inside the body rather than helping to eradicate germs outside the body using disinfectants. This is in large part because current disinfectant technologies are limited. The disinfectants of today only kill pathogens while wet. Thus, they do not offer continuous, long-term protection. Further, to truly combat the transmission of infectious diseases, there is a need for residual coating disinfectants that can continuously and effectively inactivate disease-causing pathogens, as described herein, on surfaces, textiles, and skin/hands over an extended period, and do so with little or no toxicity. Second, presently insufficient emphasis is placed on disrupting the chain of infection. This is largely because existing technologies cannot break the chain. They only "disinfect". They do not "protect".

The “chain of infection” describes how diseases are transmitted from surfaces to hands, to the face, and then into the body. It is estimated that 80% of human infections occur from microbe-contaminated surfaces and that hands are the main pathway to pathogens to enter the body. One study showed that humans,

on average, touch their face twenty-three times per hour. Today's hand sanitizers do not break the "chain of infection" and/or mode of transmission.

Legacy hand sanitizers are only effective while the disinfectant is drying. Once dried, they provide little protection. It is only a matter of time before hands become re-infected with disease-causing pathogens

Understanding the chain of infection narrative is important because it helps prioritize various disinfectant applications, especially in the context of residual antimicrobial films that provide longer term protection.

A residual hand sanitizer, such as using the cationic polymers described herein, is a beneficial application because hands are the major object of transmission. When contaminated surfaces are touched the transmission is two-fold: first to the individual as he or she touches their face and second to other surfaces, which in turn exposes others.

Perhaps the next most important application to offer residual protection is textile clothing. This is because individual's hand touches their clothing and then they continue to carry the disease-causing germs with them, which exposes others.

Protecting surfaces, is important but in general it is limited, because there are so many surfaces to be protected. Still, protecting surfaces is important, especially key surfaces such as in healthcare settings and/or in food preparation.

Since most viruses are transmitted as an aerosol spray, a truly antiviral face mask could significantly mitigate the transmission of viruses. The polymeric antiviral coatings described herein are optionally and preferably used in an aerosol spray and/or a coating to achieve this.

Small molecule germicidal chemical are generally poor at / not capable of creating durable residual antimicrobial films. However, the cationic polymers described herein are applicable to durable applications of residual antimicrobial films.

Regulators require that for a residual claim to be made, antimicrobial films must pass in the USA the EPA (01-1A) and internationally the PAS2424 durability protocols; the cationic polymer films described herein more than adequately meet the standards of these tests. Herein, the antiviral inactivity of a virus exceeds 10, 20, 30, 40, 50, 60, 70, 80, 90, 99, 99.9, and/or 99.99 percent inactivity after a contact time of the virus with the antimicrobial film exceeding five minutes.

Applications

Stakeholders and the EPA are asking for disinfectants that leave a residual antimicrobial coating that "kill" / block disease causing pathogens into the future.

More specifically, the EPA is seeking new disinfectant technologies to offer essentially two things. First, a residual antimicrobial action against a wide spectrum of pathogens, but particularly against viruses; and second, a new technology not based on toxic biocides and that provides residual longer-term protection on surfaces and skin.

The methods and processes taught herein meet these standards as they are based on non-toxic polymeric coatings, are applicable to coating a range of surfaces, and leave residual antimicrobial coatings. These new products significantly reduce the transmission of infectious diseases, particularly in a world where viral pandemics are a reality.

For clarity of presentation and without loss of generality, some examples include of applications of the tuned polymers, described *infra*, are: (1) consumer and health care products, (2) medical applications, (3) industrial applications, and/or (4) agricultural applications, as further described herein.

Consumer and health care products:

1. A residual kill hand sanitizer is a preferred application for this new technology. Until now, the EPA and other regulators have not approved residual "killing" hand sanitizers due to safety and effectiveness.

Based on methods described herein, an extremely low toxicity, durable, and high killing action residual, polymer-based hand sanitizer has been produced. The viability of such a residual hand sanitizer was demonstrated with a > log-3 kill of bacteria and enveloped virus on artificial skin exposed to a 24 hour durability test as per the PAS2424 international durability protocol.

2. The tuned polymer is optionally used as a textile residual protective film on rinse additives, band-aids, and/or dryer sheets and/or is applied as an aerosol spray.

3. The tuned polymer is optionally used as a spray or wipe disinfectant that inactivates pathogens faster than traditional disinfectants and then forms a self-sanitizing antimicrobial coating on nonporous surfaces.

Medical Applications:

Medical applications of the tuned polymer include, but are not limited to, antiviral face masks, medical device sterilant, medical device hoses, surgical handwash, wound dressings, antimicrobial ointments, and/or reducing biofilms on implants. The highly durable and remarkably non-toxic coatings offered by

this technology reduce biofilm accumulation on various implanted medical devices.

Industrial Applications:

Industrial applications of the tuned polymer includes replacing/supplementing any existing toxic biocides, such as used in packaging and industrial fluids.

Agricultural Applications:

Agricultural examples of applications of the tuned polymer optionally include animal skin care or antimicrobial protection in large feeding operations, which reduces healthcare acquired infections (HAI) that stem largely from bacteria and or individual illnesses due to viruses.

Generally, the continuous killing action of the antimicrobial polymeric films/compositions produced by the processes taught herein are a novel approach to acting on bacteria and also, over time, a surface can remain virtually bacteria-free, which reduces the potential for residual (self-generating) bacteria to mutate and become antibiotic-resistant.

Tuned Polymer

Generally, the polymers described herein are "tuned" in one or more ways as illustrated and described in Figures 1-20.

Referring now to Figure 1, polymer tuning 100 is described. Generally, in a first process, a polymer is provided 110. In a second process, the polymer is tuned 120. The process of tuning a polymer is detailed *infra*; however, generally, repeating units of the polymer are charged 121 and/or repeating unit counter ions are incorporated 122 to, separately and/or in combination, yield an antimicrobial component 123, an antibacterial component 124, an antiviral component 125, and/or an antifungal component 126. In an optional third

process, the physical state of the tuned polymer is changed 130, such as from a liquid, to a gel, to a solid or *vice versa*. In a fourth process, the resulting tuned polymer is optionally used to treat a substance 140 and/or to treat a material or condition. Optional and preferably methods of tuning the polymer are further described, *infra*. Optionally, a protein is substituted for a polymer in any embodiment herein. Optionally, any backbone chain is substituted for the polymer in any embodiment herein. For instance, any organic molecule having a chain of carbons at least 3, 5, 10, 15, 20, 100, 500, or 1000 carbons long is optionally used in place of the polymer as described herein. Optionally, a backbone molecule is used in place of the polymer 200. For instance, a chain of any combination of carbons, oxygens, and nitrogens of any length greater than 2, 5, 10, 20, or 50 atoms is used as a backbone where the backbone is optionally protonated, has counterions, and/or is tuned according to any of the approaches described herein for tuning a polymer.

The tuned polymer is optionally and preferably used in a formulation with other agents, as further described *infra*. Notably, the formulation alters physical and/or chemical properties of the tuned polymer, such as tackiness, film thickness, total charge, total cationic charge, viscosity, porosity, charge density, pH, stability, and the like.

Referring now to Figure 2, a polymer 200, such as provided in the polymer tuning 100 process, is further described.

Still referring to Figure 2, in a first process, the polymer 200 is optionally tuned to form a tuned polymer 210 having a state of cationic charge, a tuned pH, tuned charge, tuned salts/counterions, tuned combinations of polymers, tuned molecular weight, tuned solubility, tuned viscosity, tuned durability, and/or a tuned surface tension, which are each further described, *infra*. Generally, any one or more of the tuning steps are optionally performed in any order and/or are performed simultaneously, as further described *infra*. For clarity of presentation and without loss of generality, a polymer with repeating cationic

units is used as an example herein to describe the chemistry, apparatus, methods, and processes and in particular a specific polymer of polyethylenimine is used to illustrate any polymer with repeating units/monomers that are charged and/or are chargeable. The polymer 200 is optionally a copolymer, where a copolymer is a polymer formed by linking two or more different types of monomers into a single polymeric chain. The process of creating a copolymer is called copolymerization. An example of a copolymer comprising two monomer types is a copolymer comprising: acylated monomers comprising a -COR_1 group attached to a nitrogen of a -N-CH_2-CH_2- element, wherein R_1 comprises an alkyl group, and protonated monomers comprising a -NH_2^+-CH_2-CH_2- group, the acylated monomers and the protonated monomers comprising any order in the copolymer.

Another example of a copolymer is a polymer comprising first monomers comprising a -$NH_2^+CH_2CH_2$- group and a second monomers, the second monomers comprising an acyl group, -C(=O)-R', attached to a nitrogen in a polymer backbone group comprising -NCH_2CH_2-, wherein R' comprises a hydrocarbon chain, the first monomers and the second monomers arranged in any order in the copolymer. If the first monomer is partially deprotonated to form a -$NHCH_2CH_2$- group in equilibrium with the -$NH_2^+CH_2CH_2$- group, the polymer is still a copolymer, as the copolymer can optionally have more than two monomer types by definition. In some applications the copolymer is a cationic polyamine.

Polyethylenimine is also referred to as polyethyleneimine and/or polyaziridine. Polyethylenimine is a polymer with repeating units composed of the amine group and two carbon aliphatic CH_2CH_2 spacers. Optionally and preferably, any number of the nitrogens in polyethylenimine are protonated to yield a localized positive cationic charge, which is typically associated with an anionic counterion. State another way, polyethylenimine is optionally protonated at any number of sites.

Still referring to Figure 2, the tuned polymer 210 is optionally and preferably cationic 211 and/or has repeating cationic polymer units; however, the polymer 200 is optionally anionic and/or has repeating anionic polymer units; and/or has a functional group that is optionally subjected to a chemical reaction to form a cationic, anionic, and/or zwitterionic repeating group on repeating sections of the polymer 200. An anionic polymer comprises a long chain of carbon atoms with negatively charged groups of ions attached along the chain. The negatively charged spots act like hooks that attract positively charged particles.

Notably, while the polymer 200 is optionally and preferably tuned; optionally and preferably the formulation that contains the polymer is optionally tuned to any of the below described levels of the tuned polymer 210, such as in terms of viscosity, durability, surface tension, and/or in terms of effectiveness against any microbe, bacteria, virus, and/or fungus.

Still referring to Figure 2, the polymer 200 is optionally and preferably pH tuned 212 to a desired pH, such as: in a range with a pH greater than 2, 3, 4, or 5; to a pH less than 14, 12, 10, 8, 7, or 6; in a pH range of 2-10, 3-9, 3-8, 3-7, and/or 4-6; to a pH within any of ± 0.25, 0.5, 0.75, 1.0, or 2 of any of 3, 4, 5, 6, or 7; and/or any combination of these pH ranges.

Still referring to Figure 2, the polymer 200 is optionally and preferably tuned to a charge, such as in a range of $1*10^{-20}$ to $1*10^{-12}$ coulombs/molecule and more preferably in a range of $1*10^{-18}$ to $1*10^{-14}$ coulombs/molecule; coulombs abbreviated as C. Optionally and preferably, between 10 and 90 percent of the secondary amines of polyethylenimine 250 are positively charged or the same percentage of any protonatable group of the polymer 200. More preferably, greater than 15, 20, 25, or 30 percent of the nitrogens, or protonatable groups, are positively charged and/or less than 80, 70, 60, 50, or 40 percent of the protonatable groups are charged. Herein, a total cationic charge, such as calculated from the cationic sites, is a charge of all of the positively charged groups, such as cationic sites, which does not include anionic charge from any

counterion and/or any anion on the polymer. For instance, cationic sites in a dose of dispensed contents from a container comprise a total cationic charge and anionic sites in the dose of dispensed contents comprise a total anionic charge, which are optionally equal or nearly equal in terms of absolute charge. Optionally and preferably, a total cationic charge, such as a total cationic charge from present cationic sites, is used in a biocide treatment, such as in a unit of liquid dispensed and/or in a film formed is greater than 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, or 1.8 coulombs and/or is less than 100, 75, 50, 25, 10, or 5 coulombs, such as where a unit of fluid delivered is within 10, 25, or 50% of any of 0.1, 0.2, 0.5, 1, 2, 3, 5, or 10 mL. The total cationic charge per spray is optionally and preferably greater than 0.1, 0.2, 0.3, or 0.5 C and/or less than 8, 7, 6, 5, or 4, 3, or 2 C.

A preferred total cationic charge per polymer is optionally tuned, such as in a range of 1 to 5000, 5 to 3000, and/or 1000 to 2500 positively charged sites per polymer chain. Optionally, any of the charges per molecule are optionally reduced by 10, 20, 30, 40, 50, 60, 70, 80, 90, or more percent as the total chain lengths of polymer decreases from thousands to hundreds to tens. A percentage of a total cationic charge from cationic sites in an aliquot of sample/product in a composition and/or on/in a film from a cationic polymer is optionally greater than 1, 2, 5, 10, 25, 50, or 75 percent and/or is less than 100, 99, 95, 90, or 80 percent. A concentration of salt in the composition is optionally greater than or less than 0, 10, 100, 250, 500, 1000, 2000, 5000, or 10,000 ppm. Charges, total charges, and effective charges are further described, *infra*.

Still referring to Figure 2, the polymer 200 is optionally salt/counterion tuned 214 with one or more salt anions in solution; salt cations in solution, and/or counterions, which are optionally anionic or cationic; electrostatically and/or attracted via charge to charges, such as protons on monomers of the polymer 200, as further described *infra*.

Still referring to Figure 2, the polymer 200 optionally has a tuned combination of polymers 215, such as a mix of molecular weights, polymer types, and/or types of counterions associated with each individual polymer or set of polymers. Further, the tuned combination of polymers 215 optionally contains a polymer or a group of polymers where one or both are antimicrobial, antibacterial, antiviral, a biocide, and/or antifungal.

Still referring to Figure 2, the polymer 200 optionally has a tuned molecular weight 216. Longer chains have advantages like higher charge and/or complexing more than one object, such as a virus. Shorter chains have advantages, such as more precise charge. In general, the molecular weight of the polymer is greater than 100, 1000, 10,000, or 100,000 g/mol; less than 5,000,000, 1,000,000, or 500,000 g/mol; and/or within 5, 10, 25, or 50 percent of 100,000, 200,000, 300,000, 400,000, or 500,000 g/mol. Optionally, viscosity of a formulation is tuned with molecular weight of the polymer with or without a traditional viscosity/thickening tuning substance.

Still referring to Figure 2, the polymer 200 is optionally solubility tuned 217, such as by adjusting pH of the solution, monomer size, monomer functional groups, length of the polymer 200, size of counterions, degree of salt, activity of the solution, and/or through use/application of any agent designed to complex the polymer 200 and/or attract the polymer 200 to a surface, such as the surface of a treated product, any of which may be expressed at solubility of the polymer 200 in g/L. Herein, the solution optionally and preferably contains greater than or less than 0, 1, 2, 3, 5, 10, 15, 20, 25, 50, 75, or 95 percent water and/or any solvent such as ethanol and/or isopropanol.

Still referring to Figure 2, the polymer 200 and/or polymer formulation is optionally viscosity tuned 218, such as in a range of centipoise units. Optionally, centipoise is expressed in units of poise, m^2/s, Newton-second per square meter, and equivalents thereof. Optional ranges of viscosities of the polymer 200 and/or a formulation containing the polymer in centipoise is near

water in a range of 1-5, that of blood or 15 centipoise, that of corn syrup or 50-100 centipoise, that of motor oil 150 to 500 centipoise, than of syrup or 1000-3000 centipoise, that of molasses to peanut butter or 5000 to 200,000 centipoise, or higher in a more solid state, such as lard to window putty or 1,000,000 to 100,000,000 centipoise, and/or within 10, 25, 50, 75, or 100% of any of these levels. An additional component in a formulation is optionally used to tune viscosity, such as a polyDADMAC.

Still referring to Figure 2, the polymer 200 and/or polymer formulation is optionally durability tuned 219 according to any metric, such as durability metrics in PAS2424 and/or in the more rigorous U.S. 01-1A tests, referenced *supra*. For instance, a durability test is to whether or not a treated substance still passes an EPA test, such as effectiveness against a microbe, bacteria, virus, and/or fungus after a series of web rub then dry cycles, such as 6 to 12 cycles, at a testing pressure, such as about 5, 10, 15, or 20 pounds of pressure plus or minus 25 or 50 percent.

Optional tests for determining effectiveness of antimicrobial and/or antiviral agents include:

(i) a germicidal spray test according to American Society for Testing and Materials (ASTM) international method E1153 that meets the Environmental Protection Agency (EPA) requirement of log 3 reduction for viruses and log 5 reduction for bacteria,

(ii) a spray or film killing at least one of:

(ii-a) at least 95% of log 5 population, in 30 minutes, of at least one of a gram positive bacteria and a gram negative bacteria;

(ii-b) at least 95% of log 4 population of an enveloped virus within 30 minutes of contact;

(ii-c) at least 95% of a non-enveloped virus within 30 minutes of contact; and

(ii-d) at least 94% of a log 4 population of *Clostridium difficile* bacteria within 24 hours of contact, in accordance with Japanese Industrial Standard (JIS) Z 2801 (2006) test for antimicrobial activity, or a modified version of such test, and

(iii) a durability test comprising at least one of:

(iii-a) a film formed from the dispensed contents kills at least 99.9% of gram-positive bacteria and gram-negative bacteria according to EPA Protocol #01-1A residual self-sanitizing activity test; and

(iii-b) waiting seven days after film formation, a film formed from the composition kills at least one of: (a) 95% of gram-positive bacteria and gram-negative bacteria and (b) enveloped and non-enveloped viruses according to a modified version of Protocol #01-1A residual self-sanitizing activity test.

Test (i) refers to ASTM E1153, the entire contents of which are incorporated herein by this reference thereto, which is a germicidal spray test that is used to evaluate the antimicrobial efficacy of one-step cleaner-sanitizer formulations recommended for use on lightly soiled, inanimate, nonporous, and/or non-food contact surfaces. In ASTM E1153, the antimicrobial efficacy of sanitizers is tested on pre-cleaned, inanimate, hard, nonporous, non-food contact surfaces against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and/or a combination thereof.

Test (ii) is a suspension test in accordance with ASTM E1052-96 (2002) or ASTM E2315 (2016), included in its entirety by this reference thereto, to determine effectiveness of an antimicrobial solution that is in the form of a suspension against specific viruses, such as adenovirus, coronavirus, influenza viruses, rhinovirus, and/or rotavirus.

For test (iii), the ability for a film formed from the antimicrobial composition to kill gram positive and gram negative bacteria, an enveloped virus, a non-enveloped virus, and/or *Clostridium difficile* bacteria can be tested in accordance with the conditions set forth in JIS Z 2801, which is known as the Japanese Industrial Test for Antimicrobial Activity and Efficacy in Antimicrobial Products, and identical ISO 22196, the entire contents of which are incorporated herein by this reference thereto. The JIS Z 2801 test method is used to assess the antibacterial effectiveness on materials such as plastics, ceramics, glass, and other non-porous materials. In particular, in accordance with JIS Z 2801 or a modified version thereof, a film formed from an antimicrobial composition of at least one embodiment of the present invention kills: (iii-a) at least 95% of a log 5 population of a gram positive and/or a gram negative bacteria in 30 minutes, (iii-b) at least 95% of a log 4 population of an enveloped virus within 30 minutes of contact, (iii-c) at least 95% of a non-enveloped virus within 30 minutes of contact, and/or (iii-d) at least 94% of a log 4 population of *Clostridium difficile* bacteria within 24 hours of contact. In a preferred embodiment, a film formed from an antimicrobial composition, as described herein, meets 1, 2, 3, or 4 of the requirements (iii-a)-(iii-d).

Still referring to Figure 2, the polymer 200 and/or polymer formulation is optionally surface tension tuned 220 to a range of 0.001 to 0.3 N/m; to greater than 0.01, 0.02, 0.03, 0.04 N/m; less than 0.3, 0.2, 0.1 N/m; and/or to within 10, 25, 50, 75, or 100% of 0.01, 0.02, 0.03, 0.04, 0.06, 0.08, or 1.0 N/m. For instance, a starch, slurry, paste, and/or binding agent is optionally used to increase the surface tension of the polymer 200.

Still referring to Figure 2, in a second process, the polymer 200 is optionally tuned to form a tuned polymer counterion 230, where each of one or more counterions have antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234 properties. Specific properties found to have antimicrobial 231 effectiveness in association with a charged polymer are further detailed below. Generally, herein, an antimicrobial 231 is one or more of antibacterial 232,

antiviral 233, and/or antifungal 234. Generally, a biocide is antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234.

Still referring to Figure 2, the first process and second process are optionally tuned at the same time; in either order, and/or are tuned in sub-parts in any simultaneous and/or time ordered manner.

Referring now to Figure 3A, a tuned cationic polymer 300 is described, which is an example of a polymer 200. Again, for clarity of presentation and without loss of generality, linear polyethylenimine 250 (PEI) or polyaziridine, is used as an example of a polymer 200, a polyalkylenimine, and/or a protonated polymer 240. Further, polyethylenimine 250 is used as a general case of linear polyethylenimine and/or branched polyethylenimine, which are further described *infra*. Further, polyethylenimine 250 refers to any unprotonated, partially protonate, or fully protonated version of polyethylenimine. Optionally, the polymer 200 includes any derivative of polyethylenimine 250 and/or any derivative of a polyalkylenimine. Herein, an alkyl group, such as in polyalkylenimine, is an alkane missing one hydrogen. Again, polyethylenimine is optionally and preferably protonated at any number of sites, such as at any given or any given set of secondary amine sites. The protonated sites are optionally and preferably associated with anionic counterions; however, the total cationic charge of the protonated sites is determined by looking at only the cationic sites. Thus, a total cationic charge of X coulombs could be offset by a total anionic charge of essential X coulombs, where X is a rational number. The polyalkylenimine is optionally polyethylenimine and/or is a mixture comprising at least 1, 2, 5, or 10 percent polyethylenimine, less than 1, 2, 5, or 10 percent polyethylenimine, and/or no polyethylenimine.

Some specific examples of a suitable cationic polymers include a polydiallyldialkylammonium salt an acryloxyalkyltrialkylammonium salt, (e.g. acryloxyethyltrimethylammonium halide and methacryloxyethyltrimethylammonium halide), a

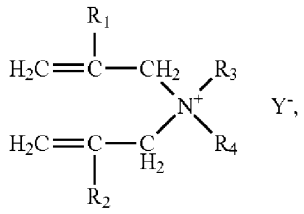
vinylphenalkyltrialkylammonium salt (e.g. vinylbenzyltrimethyl-ammonium halide), an acrylamidoalkyltrialkylammonium salt (e.g., 3-acrylamido-3-methylbutyltrimethylammonium halide), a poly(acrylamide-co-diallyldialkylammonium salt) (e.g., poly(acrylamide-co-diallyldimethylammonium chloride)), a polyethylenimine-based polymer, chitosan, or a combination thereof. In any of the foregoing polymers, each alkyl group is the same or different and is a straight chain c1-6 or branched c3-6 (e.g., methyl, ethyl, t-butyl) group, and the salt is an anion, such as a halide (e.g., chloride, fluoride, bromide), a halide-containing anion (e.g., bis(trifluoro- methane)sulfonimide, trifluoroacetate), a sulfate, and/or a phosphate. Preferably, the cationic polymer is a polydiallyldialkylammonium salt (e.g., polydiallyldimethylammonium halide), a poly(acrylamide-co-diallyldialkylammonium halide) (e.g., poly(acrylamide-co-diallyldimethylammonium chloride)), and/or a polyethylenimine-based polymer (e.g., linear, non-chemically modified PEI). In some embodiments, the composition does not contain a bridged polycyclic compound (e.g., a cavitand structure), including a polymer-bound bridged polycyclic compound (e.g., a polymer-bound cavitand). In some embodiments, the cationic polymer is not a hybrid material that comprises one or more divalent metals and siloxane bridges. Herein, an alkyl group is an alkane missing one hydrogen.

In some instances, a combination of two or more cationic polymers are used in a composition, such as selected from a polydiallyldialkylammonium salt (e.g., polydiallyldimethylammonium halide), an acryloxyalkyltrialkylammonium salt, a vinylphenalkyltrialkylammonium salt, an acrylamidoalkyltrialkylammonium salt, a poly (acrylamide-co-diallyldialkylammonium halide), a polyethylenimine-based polymer, and/or chitosan are used in the composition. In a particular embodiment, a polydiallyldialkylammonium salt, such as a polydiallyl-dimethylammonium halide) is used in combination with a polyethylenimine based polymer (e.g., a linear or branched polyethylenimine (PEI)). In a preferred embodiment, polydiallyl-dimethylammonium chloride or poly(acrylamide-co-diallyldialkylammonium chloride) is used in combination with a

non-chemically modified linear PEI and/or a polyethylenimine only chemically modified to adjust protonation.

In an embodiment, the cationic polymer is a polydiallyldialkylammonium salt, such as a polydiallyldialkylammonium halide (e.g., a halide or halide-containing anion), a polydiallyldialkylammonium sulfate, or polydiallyl-dialkylammonium phosphate. In the polydiallyldialkylammonium halide, the halide can be any suitable compound in which the anion is a halide or includes a halide (e.g., bis(triflouromethane)sulfonimide, trifluoroacetate), such as, polydiallyldimethylammonium fluoride, polydiallyldimethylammonium chloride, polydiallyldimethylammonium bromide, polydiallyldimethylammonium iodide, polydiallyldimethylammonium bis(triflouromethane)sulfonimide or a combination thereof. In preferred embodiments, the polydiallyldimethyl-ammonium halide is polydiallyldimethylammonium fluoride, polydiallyldimethyl-ammonium chloride (polyDADMAC), or a mixture of polydiallyldimethyl-ammonium chloride and polydiallyldimethylammonium fluoride and/or polydiallyldimethylammonium bis(triflouromethane)sulfonimide.

Preferred polydiallyldialkylammonium salts are those polymers made from polymerization of diallyldialkylammonium compounds, which can be represented by the following formula:



in which R_1 and R_2 are the same or different and each is hydrogen or C_1-C_6 alkyl; R_3 and R_4 are, independently, hydrogen or an alkyl, hydroxyalkyl,

carboxyalkyl, carboxyamidalkyl or alkoxyalkyl group with 1 to 12 carbon atoms; and Y^- represents an anion such as a halide, a halide-containing anion (e.g., bis(triflouromethane)sulfonimide), a sulfate, or a phosphate. Examples of the preferred diallydialkylammonium monomer include diallyldimethyl-ammonium chloride (DADMAC), diallyldimethylammonium fluoride, diallyl-dimethylammonium bis(triflouromethane)sulfonimide, diallyldimethyl-ammonium bromide, diallyldimethylammonium sulfate, diallyldimethyl-ammonium phosphate, dimethyallyldimethylammonium chloride, dimethyallyl-dimethylammonium fluoride, dimethyallyldimethylammonium bis(triflouromethane)sulfonimide, diethylallyldimethylammonium chloride, diethylallyldimethylammonium fluoride, diethylallyldimethylammonium bis(triflouromethane)sulfonimide, diallyldi(beta-hydroxyethyl) ammonium chloride, diallyldi(beta-hydroxyethyl)ammonium fluoride, diallyldi(beta-hydroxyethyl)ammonium bis(triflouromethane)sulfonimide, diallyldi(beta-ethoxyethyl) ammonium chloride, diallyldi(beta-ethoxyethyl)ammonium fluoride, diallyldi(beta-ethoxyethyl)ammonium bis(triflouromethane)sulfonimide, diallyldiethylammonium chloride, diallyldiethylammonium fluoride, and diallyldiethylammonium bis(triflouromethane)sulfonimide. In a preferred embodiment, the cationic polymer is polyDADMAC.

Still referring to Figure 3A, the polyethylenimine 250 or protonated polymer 240 is illustrated with repeating monomers, which have a carbon-carbon-nitrogen backbone, which are representative of any repeating background and/or monomer, dimer, or trimer of the polymer 200. The nitrogens are optionally protonated 310, where protonation increases with decreasing pH and decreases with increasing pH. At a pH of 4, protonation of the polyethylenimine 250 is about 60±5 percent; however, protonation is variable with treatment of the polyethylenimine 250, as further described *infra*. Generally, any protonation 310 level of the polymer 200 is optionally used, such as within 1, 2, 5, or 10% of 30, 40, 50, 60, or 65 percent.

Still referring to Figure 3A, the protonation 310 sites of the protonated polymer 240 are associated with counterions 320. For instance, when polyethylenimine 250 is manufactured, polyethylenimine 250 is provided in hydrochloric acid, so the far dominant conjugate base counterion is the chloride ion 322, such as at greater than 98, 99, 99.5, or 99.9 percent of the counterions. Methods of exchanging the chloride counterion 322 with another counterion are described *infra*. Alternatively, polyethylenimine 250 is manufactured in a "free base" form, such as a solid with a pH greater than 9, 10, 11, 12, or 13, where protonation 310 is very low, such as less than 20, 10, 5, 3, 2, 1, or 0.1 percent. Another counterion is optionally formate 324, which has at least antibacterial and/or antiviral properties, such as in combination with the protonated polymer 240, such as polyethylenimine 250. Still another counterion is optionally acetate 326, which has at least antiviral properties, such as with the protonated nitrogen and/or a protonated amine, such as in the repeating monomer of the polymer 200. Formate 324, acetate 326, citrate^{-} and citrate^{2-} 327 are all examples of weak acids, organic acids, and conjugate bases, such as of carboxylic acids 321. Yet another exemplary counterion is sulfonate 328, which is at least antimicrobial in combination with polyethylenimine 250 and/or with a cationic polymer. Generally, any anion conjugate base is optionally used as the counterion 320, such as: iodide, I^-, bromide, Br^-, a form of citrate^{-} or citrate^{2-}, nitrate ion, NO_3^-, sulfate ion, SO_4^{2-}, nitrite, NO_2^-, hydrogen carbonate, HCO_3^-, hydroxide ion, OH^-, sulfide ion, and S^{2-}. Generally, any combination of 1, 2, 3, or more counterions are optionally and preferably associated with the polymer 200, such as at a charged site of the repeating monomers, such as at the protonated sites 310, and/or as functional groups that are chargeable with a reaction, such as a secondary amine in polyethylenimine 250. Further, the ratio of the 1, 2, 3, or more counterions are optionally controlled at any ratio. For instance, two different counterions are optionally controlled at a ratio of greater than 1:0, such as greater than 1:0.01, 1:0.05, 1:0.1, 1:0.2, 1:0.3, 1:0.4, or 1:0.5 and/or at a ratio of less than 0:1. Similarly three counterions are optionally held/produced at any ratio 0:1 to 0:1 to 0:1 for the relative concentrations of the three, or indeed *n*, counterions, where *n* is a positive integer greater than 1, 2, 3, 4, or 5. For instance, the ratio of three counterions is 0.1 to 0.3 to 0.6; 0.33 to 0.33 to 0.33; or any set of ratios. Further, any of the *n* counterions optionally

and preferably are selected for one or more of the properties of being antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234. Referring now to Figure 3B, the additional counterions 320 of iodide 325, I^-, and citrate 327 in an optional basic form of $citrate^{2-}$ are illustrated.

Still referring to Figure 3A, the tuned polymer 210 is optionally dried into a film, is a semi-solid, and/or contains a level of salt, in solid and/or ionic form about the tuned polymer 210. As illustrated, a salt solution 330 about the tuned polymer 210 optionally contains a set of ions 332, a set of one or more cations, represented as X^+ 332, and/or a set of one or more anions, represented as Y^- 334. Tuning the type and concentration of each member of the sets the cations 332 and anions 334 tunes the chemical activity coefficient, γ, such as according to any version of the Debye-Hückel equation. Tuning the activity coefficient to a value less than 0, 0.1, 0.2, 0.3, 0.4, 0.5; to greater than 1, 0.9, 0.8, 0.7, or 0.6; and/or in a range of within 1, 2, 5, 10, 20, or 30% of 0.7, 0.8, 0.9, 0.92, or 0.95 alters the ability of the ability of the charges on the polymer repeating units and/or their associated counterions to kill, bind with, react with, hold onto, block, and/or hinder function of the antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234. Thus, control of salt about the polymer 200 and/or the at least partially protonated repeating units of the polymer 200 tunes the antimicrobial 231 and/or biocide function of the tuned polymer 210. Preferred activity coefficients are greater than 0.7, 0.8, 0.85, 0.9, 0.95, 0.97, 0.98, 0.99 and/or less than 1.000, 0.999, 0.998, or 0.996.

Referring now to Figure 4, the polymer 200 is further described. Optionally and preferably, the polymer 200 is in a state comprising one or more of: a charged repeating unit 402; an uncharged repeating unit 404 and/or section; is cationic 406; is anionic 408; is a zwitterion; has a cationic repeating unit 410, has an anionic repeating unit 412; has a repeating amine 414; has a repeating chargeable monomer or polymer section; has a repeating unit with a pKa in a range of three to six 416; is in a basic form 418, such as at a pH >7; is in a pH neutral form; is in an acidic form 420, such as at a pH < 7; has a fixed charge

422, such as per unit length of the polymer 200; and/or is partially protonated 424, such as on average every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more sets of repeating monomers. A charge of a monomer and by extension a polymer is optionally measured and/or is preferably calculated using the Faraday constant, which is a quotient of the electric charge, such as in Coulombs, per amount, such as per mole. The Faraday constant is often expressed in C/mol and/or is 96,485 C/mol. Herein, charges of the solution, formulation, and/by extension dried films are optionally calculated using the Faraday constant, concentrations of the formulation components, and/or a mass/volume of the formulation elements used in a volume of the formulation. For example, for a constituent of polyethylenimine, a concentration, a volume/mass, and/or a pH (for determination of percent protonation), is used to determine a charge of the formulation element. Charges from formulation elements are summed as appropriate.

Referring now to Figure 5A, the polymer 200 is optionally a group of polymers 500, such as a first polymer 502, a second polymer 504, a third polymer 506, or *n* polymers 508, where each of the *n* polymers differ by any state property, such as any of the polymer states described above. Combined, the polymers 200 in a formulation comprise a set of polymers 510. For example, some have longer chains than others, some have higher charges than others, some are cationic, some have a first pKa while others have a second pKa, and/or others have first counterion types and others second counterion types.

Referring now to Figure 5B, a protonated version of polyethylenimine 552 and polyDADMAC 554, which is protonated, are illustrated, which is an example of two polymers in the set of polymers 510. PolyDADMAC is also referred to as poly(diallyldimethylammonium chloride), a poly(diallyldimethylammonium Y^-), where Y^- is an anion, a poly(diallyldimethylammonium salt), where the salt is a chloride, fluoride, and/or an anion, and/or is referred to as a polydiallyldimethylammonium chloride, which contains a homopolymer of diallyldimethylammonium chloride. Molecular weights of polyDADMAC are

optionally hundreds to millions, but are preferably in a range of 1, 2, 3, 4, 5, 6, 7, 8, or 9 hundreds of thousands of grams/mol. Herein, anywhere a single polymer 200, a cationic polymer, and/or an anionic polymer is used, the set of polymers 510 is optionally used.

Referring now to Figure 5C, a copolymer 520 is described. Generally, a copolymer has two or more different types of monomers linked into a single polymeric chain. As illustrated, the copolymer 520 has a first copolymer monomer 522, a second copolymer monomer 524, and optionally a third copolymer monomer 526. Generally, any number, *n*, of monomers 528 are optionally present in the copolymer 520, where *n* is a positive integer of two or more. The monomers are optionally arranged in any repeating and/or random order in the copolymer. Herein, any polymer, such as the first polymer 502 is optionally a copolymer.

Referring now to Figure 5D, an illustrative example of a copolymer 580 is provided. In this example, the copolymer 520 comprises a first copolymer monomer 5260 that includes an acyl group with an ethyl hydrocarbon chain 5261 linked/attached to a nitrogen in a polymer backbone group comprising -NCH_2CH_2-. The acyl group, -C(=O)-R', optionally comprises any hydrocarbon chain, R', in place of the ethyl group. In this example the copolymer 520 also comprises a second copolymer monomer comprising a -$NHCH_2CH_2$- group 5270 and a third copolymer monomer comprising a -$NH_2^+CH_2CH_2$- group 5280. Notably, the second and third monomers in this example are acid base pairs, and as such are pH dependent in terms of relative concentration.

Processes

Referring now to Figures 6-17, exemplary process for forming the tuned polymer 210 are described. The exemplary processes are optionally combined. Further, any one or more elements/steps of one of the processes is optionally used in combination with any one or more elements/steps of another one of the processes to form the tuned polymer 210.

Referring now to Figure 6, in a first general process 600, the tuned polymer 210 is formed, such as by controlling/adjusting pH 610. As illustrated, in a first step the polymer is provided 110. Again, for clarity of presentation and without loss of generality, polyethylenimine 250 also referred to as polyethyleneimine and/or polyaziridine, is used to illustrate any polymer 200. In another step, which is optionally performed in parallel with the first step, the pH is adjusted 610. For instance, the pH of the polymer 200 is adjusted, the pH of an aqueous solution containing the polymer is adjusted, and/or a pH of the polymer in any physical state, such as in a gel state, is pH adjusted. Generally, in one case a base is added 620, such as by addition of sodium hydroxide 622 in any form or concentration. Any base is optionally used in this case to reduce protonation of the polyethylenimine 250. In a second case, an acid is added 630, such as by addition of hydrochloric acid 632 in any form or concentration. Any acid is optionally used in this case to increase protonation of the polyethylenimine 250. In a third case, an acid/conjugated base 640 and/or linked pair of acid and conjugate base is used to adjust the pH 610. For instance, addition of formic acid, also referred to as methanoic acid, to an acidic solution below the pKa of formic acid will increase the pH of the solution, deprotonate the polyethylenimine 250, and add a counterion of formate. Conversely, addition of formic acid to a basic solution above the pKa of formic acid will decrease the pH of the solution, protonate the polyethylenimine 250 and/or add a counterion of formate. Adjustment of pH to tune protonation of the polyethylenimine 250 is further described, *infra*. In a third step, resultant from the second step, protonation is tuned 650. For example, protonation of the polyethylenimine 250 is tuned to less than one hundred percent 662, to a protonation range 664, and/or to greater than ten percent protonation 666. Preferred protonation ranges are: greater than 0.1, 1, 5, 10, 15, 20, or 30 percent; less than 100, 99, 90, 80, 70, 60, or 50 percent; and/or in a range of ± 0, 1, 5, 10, 15, 20, 25, or 50 percent of any of 30, 35, 40, 45, 50, 55, 60, 65, or 70 percent, where any combination of ranges described herein are optionally used, such as 35-45 percent and/or 30-40 ± 5 percent on either end. A protonation range of 20-65 percent is the preferred state of the tuned protonation 650.

Still referring to Figure 6, before, during, and/or after the steps of providing the polymer 110, adjusting pH 610, and/or tuning protonation 650, a step of drying 660 is optionally used. The drying step 660 forms an at least partially dried substance/product 670, such as a where a film is formed 672, a form or semi-crystalline solid is formed 674, a semi-solid 676 / gel is formed, a wet film, a dry film, and/or an antimicrobial substance 678 is formed/concentrated. Generally, the drying step 660 increases the concentration of the polyethylenimine 250, the counterions 320, and/or the salt solution 330. the drying step 660 is optionally used to remove a product, such as propionic acid, as further described *infra*. Optionally greater than 1, 2, 3 and/or less than 99, 98, or 97 percent of the propionic acid is removed.

Referring now to Figure 7, in a second general process, the tuned polymer 210 is formed by controlling and/or adjusting pH 610 to yield a tuned counterion solution 700 / formulation. Generally, the steps of tuning the protonation 650, illustrated in Figure 6, are optionally and preferably performed in combination with the steps of tuning the counterion solution 710. A process of tuning the counterion 710 optionally includes any process of controlling one or more counterion types, counterion concentrations, ions, and/or molecules associated with cations or charges on the repeating units of the polymer 200. Exemplary methods of tuning the counterion 710 comprise: (1) adding sodium hydroxide 622, in any concentration, for replacing chloride counterions with hydroxide counterions 623 on the polyethylenimine 250; (2) adding hydrochloric acid 632, in any concentration, to protonate the polyethylenimine 250, a secondary amine, and/or any protonatable functional group of the polymer 200; (3) adding a conjugate base of an organic acid 642 to replace the chloride counterion with the conjugate base anion 643, where more generally the conjugate base anion optionally displaces any counter anion associated with the polyethylenimine 250; (4) adding salt, such as where the chloride ion, the anion 334, Y^-, or indeed any counter anion is replaced with one or more added salt anions; (5) adding an anion 740 where the counterion associated with polyethylenimine 250 is replaced by the anion 334, Y^-, and/or (6) using any substitution method(s) of

anions, such as via an anion exchange column/method. Again, the resulting solution / compound / formulation is optionally dried 660 to form at least a partially dried product 670, as described *supra*. For clarity, in an example of adding sodium formate and/or formic acid, the added formate anion is used to replace a counterion associated with polyethylenimine 250, such as the chloride ion, such as when starting with a protonated form of polyethylenimine 250 in hydrochloric acid, as further described *infra*. Similarly, addition of sodium acetate and/or acetic acid adds the counterion of acetate to the polyethylenimine 250 and displaces counterions associated with polyethylenimine 250, where the overall process of tuning the counterion solution 700 tunes/controls the mean percentage of any 1, 2, 3, or more counterions associated with the polyethylenimine 250. Formate ion, HCO_2^-, is a conjugate base of formic acid, HCOOH.

Still referring to Figure 7 and referring again to Figure 3, a carboxylic acid and/or a conjugate base of a carboxylic acid 321 is optionally used to displace the chloride ion, such as in step three of the previous paragraph, and/or more generally to supply a counterion. For instance, formic acid, a carboxylic acid, is used to provide formate (a conjugate base of formic acid) and/or a proton to any formulation herein. Similarly, acetic acid, also a carboxylic acid, is used to provide acetate (a conjugate base of acetic acid) and/or a proton to any formulation herein. Additional carboxylic acids that are optionally used to supply counterions and/or other counterions isolated from carboxylic acid include any of: acetic acid (ethanoic acid); formic acid (methanoic acid); citric acid; propionic acid (propanoic acid); butyric acid (butanoic acid); benzoic acid; lactic acid; tartaric acid; malic acid; oxalic acid; succinic acid; palmitic acid; stearic acid; oleic acid; and/or linoleic acid. The propionic acid is optionally generated in-situ in the formation of the polypropylenimine, such as from a oxazoline.

Referring now to Figures 8-11, for clarity of presentation and without loss of generality, four exemplary processes of tuning protonation of the polymer 650,

tuning counterions 710 of the polymer 200, and/or tuning the salt solution 330 about the polymer 200 are described. Referring now to Figure 8, a flowchart of the four exemplary process is provided, where an initial polymer 110, such as polyethylenimine 250, is pH adjusted 610 along a first path to form an acidic pH form 216 of the polyethylenimine 250 and/or along a second path to form a basic pH form 218 of the polyethylenimine 250. Three processes using the acidic pH form 216 are illustrated: (1) a first process of alkalization polymer tuning 1100 that raises pH of the polyethylenimine 250, forms a salt, removes the formed salt, and lowers the pH to tune the polyethylenimine 250; (2) a second process of alkalization polymer tuning 1200 that uses ion exchange to raise the pH about the polyethylenimine 250 to tune the polyethylenimine 250; and/or (3) a third process of alkalization polymer tuning 1300 that raises the pH about the polyethylenimine 250 through linear and/or sequential addition of a base and ion exchange, in either order of addition, to tune the polyethylenimine 250 and associated counterions. One process using the basic form 218 is illustrated: (4) a fourth acidification polymer tuning process 1400 of lowering the pH of the polyethylenimine 250 through a combination of adding acid and/or use of ion exchange to yield a tuned polyethylenimine 250 in terms of protonation, counterions, and/or salt solution. The fourth process 1400 optionally has sub-parts associated with each of processes I-III, albeit in reverse direction of pH adjustment. Each of the four processes are further described in the following examples. However, first generic processes of tuning with a conjugate base are illustrated in two subprocesses.

Referring now to Figure 9A, a first subprocess of using a conjugate base to acidify 900 the polymer 200, such as with repeating monomers 253, such as polyethylenimine 250, is illustrated. As illustrated, a basic form of polyethylenimine 252 is interacted/reacted with an acid 910 – conjugate base 920 pair or indeed with just the acid 910 or with just the conjugate base 920, where an equilibrium between the acid 910 and the conjugate base 920 suffices to continue to provide each of the acid 910 and/or the conjugate base in the reaction / counterion supply. Generally, the acid or H^+ protonates the polyethylenimine 250 to form a tuned protonation level of the polyethylenimine

254 and the salt anions, Y^-, provide the counterions 320. Notably, when a weak acid, such as formate, acetate, citrate, any monoprotic acid, and/or any dibasic acid, is used to acidify the polyethylenimine 250 to form a tuned protonation level of the polyethylenimine 254, little to no salt is formed. In the strict case of a pure weak acid with no impurities, no salt is formed. However, salt concentrations are typically held to less than 20, 10, 5, 4, 3, 2, 1, 0.5 or 0.1 percent with this approach, such as due to presence of impurities in the reagents/solvent(s), such as the presence of sodium carbonate in the water.

Referring now to Figure 9B, unprotonated monomers 255 and protonated monomers 257 of polyethylenimine 250 are illustrated. Herein, polyethylenimine 250 generally is at least partially protonated. The unprotonated monomers 255 and protonated monomers 257 are attached together in any sequence and in any length to form the polyethylenimine polymer.

Referring now to Figure 10, a second subprocess of using a conjugate base to alkalize 1000 the polymer 200, such as polyethylenimine 250, is illustrated. As illustrated, an acidic form of polyethylenimine 256 interacts with an acid 910 – conjugate base 920 pair or indeed with just the acid 910 and/or with just the conjugate base 920, where an equilibrium between the acid 910 and the conjugate base 920 suffices to continue to provide each of the acid 910 and the conjugate base in the reaction / counterion supply. Generally, the conjugate base 920 deprotonates the acidic form of the polyethylenimine 256 to form a tuned protonation level of the polyethylenimine 254 and the salt anions, Y^-, provide the counterions 320. Notably, when a weak acid, such as formate, acetate, or citrate, is used alkalize the acidic form of the polyethylenimine 256 to form a tuned protonation level of the polyethylenimine 254, salt is formed. For example, when the acidic form of the polyethylenimine 256, such as formed with hydrochloric acid, has the counterion of chloride 322, is used/supplied, the original counterion (Cl^-) associated with the acidic form of the polyethylenimine 256 is displaced to form part of the salt solution 330. Thus, the first process

has an advantage of limiting to eliminating production of the salt solution 330 about the tuned protonation level of the polyethylenimine 254 and/or the second process has an advantage of yielding salt and optionally a controlled amount of salt in the tuned protonation level of the polyethylenimine 254, which affects the antimicrobial 231 properties of the composition. Yet, it is noted that the resulting tuned cationic polymer 300 differs using the first process compared to using the second process without use of an additional optional salt control step, such as further described *infra*. Notably, different processes, such as forming or not forming a salt, yield different formulations, where efficacy, such as killing bacteria, differs from formulation to formulation. Similarly, changes in temperature, pressure, or reaction times yield differing formulations, which again differ in efficacy.

Example I

(Process I)

Referring now to Figure 11, the first alkalization polymer tuning process 1100 is further described. As described, *supra*, generally the first alkalization polymer tuning process 1100 provides a polymer 110, such as polyethylenimine 250, such as in an acidic pH form 216; adds a base 620 to raise the pH, which yields a salt; in a subsequent and/or concurrent step removes at least a portion of the formed salt 1140; and adds an acid to bring the pH back down. Generally, the process of raising the pH, such as with sodium hydroxide 622, in any form or concentration, and/or adding any base 624, such as even a conjugate base, raises the pH of the formulation containing polyethylenimine 250 to a level where salt is formed, such as to a raised pH level of 4.5 1110, which yields a salty product 1120 containing polyethylenimine 250 or raises the pH even further 1130, such as to a pH greater than 4, 5, 6, 7, 8, or 9, which generates even more salt. Essentially, forming the salt, which precipitates, allows for an easy removal of the salt, whether in a precipitate form or in solution, through the process of removing the salt 1140. Generally, the salt removal step 1140 optionally includes one or more of the steps of drying 1150, filtering 1160, and/or separating 1170 the salt with any salt removal technique, such as

centrifugation, washing, rinsing, decanting, and the like. Once the salt is removed, the polyethylenimine 250 is brought back down in pH 1180, such as to a target pH of less than 6, 5.5, 5, 4.5, 4, 3.5, or 3 and/or to greater than 2, 3, or 4, which reprotonates the polyethylenimine 250 – yielding the antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234 properties, described *supra*, associated with the more protonated form of polyethylenimine 250 at the lower pH and with a reduced salt concentration 1190 / chemical activity allowing better interaction with bacteria and especially viruses, in any form. Again, formulation methods alter efficacy, such as antibacterial, antifungal, and/or antiviral efficacy.

Example II

(Process II)

Referring now to Figure 12, the second alkalization polymer tuning process 1200 is further described. In the second alkalization polymer tuning process 1200, the polymer is provided 110, such as the polyethylenimine 250, in a solution, such as in acidic pH form 216 and ion exchange 1210 is used to increase the pH of the polyethylenimine 250 formulation. For example, an ion exchange material 1220, such as ion exchange beads 1222 and/or an in exchange resin 1224 is used in an ion exchange column 1226, a container, and/or in ion exchange chemistry 1228 to exchange ions/counterions in/on the polyethylenimine 250 for anions on the anion exchange material. Optionally and preferably, anions on the ion exchange material 1220 are exchanged for counterions associated with positively charged sites on the polymer 200, such as for chloride anions functioning as counterions to protonated secondary amines of the polyethylenimine 250. Optionally and preferably, greater than 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 95, or 99 percent of counterions / counter-anions, such as chloride anions functioning as counterions to the polymer 200 are adsorbed onto and/or are bound to the ion exchange material 1220. Optionally and preferably, the percentage of counterions to protonated sites on the polymer, such as the percentage of chloride ions, is reduced to less than 99, 95, 90, 80, 70, 60, 50, 40, 30, 20, 10, or 5 percent of a total number of

counterions, such as at a fixed point in time. The ion exchange material 1220 is optionally used to provide any anion to the polymer solution, such formate, acetate, iodide, and/or any anion described herein. In one optional and preferable case, the ion exchange material 1220 exchanges hydroxide anion, OH^-, for the chloride anions. The hydroxide anions and protons of the acidic solution combine to form water, which raises the pH of the solution, such as to a pH greater than 2, 3, 4, 5, or any pH described herein. Essentially, the ion exchange process 1210 achieves a task of raising the pH of the polyethylenimine 250 to a tuned pH level. During the process of raising the pH, the salt concentration of the polymer and/or a concentration of anions/counterions in the solution containing the polymer 200 is reduced, is not increased, and/or is raised by less than 10, 5, 2, 1, or 0.1 percent. Optionally, the ion exchange process 1210 yields a target pH based on concentration and pKas and/or binding/exchange coefficients of the exchange material. For instance, a formate exchange, a formic acid/formate ion mix, naturally brings the pH to within a pH unit or two of a pKa of formate of 3.75 or when using acetate to a pH of 4.75. Combinations of conjugate bases are optionally used in the ion exchange process, as further described *infra*. Notably, commercially available polyethylenimine 250 is in either a fully protonated form with a pH of about 1 with greater than 90, 95, or 99% of the counterions to the protonated amines being chloride anions or is available in a fully deprotonated state with no charges amines at a pH greater than about 12. Commercially available polyethylenimine with a pH of less than six is not available in any form except where greater than 99% of the counterions, to protonated amines of the polyethylenimine, are chloride. Production of polyethylenimine 250 is further described *infra*. Any acid is optionally used herein, where the counterion is the conjugate base of the of acid, as further described *infra*.

Example III

(Process III)

Referring now to Figure 13, the third alkalization polymer tuning process 1300 is further described. Generally, the third alkalization polymer tuning process

1300 combines elements of the first alkalization polymer tuning process 1100 and the second alkalization polymer tuning process 1200 to use both ion exchange 1320 and addition of a base 620 to raise the pH 612 to a tuned pH / protonation level of the polyethylenimine 250. More specifically, the third alkalization polymer tuning process 1300 provides a polymer 110, such as the polyethylenimine 250 in an acidic form 216 and raises the pH 612 of the formulation, such as through one and preferably two or more of: (1) ion exchange 1320, which tunes the counter ions 1360; (2) addition of sodium hydroxide 622, which yields sodium hydroxide ions 624; and/or (3) adding a base 620, which tunes the salt 1370 / counterions. For example, if formic acid/formate is added to raise the pH 612, the formate anion 324 is generated in the aqueous salt solution in the formulation about the polyethylenimine 250 and/or the formate anion 324 is provided as the counterion to the polyethylenimine 250, such as replacing, with an equilibrium coefficient, the chloride ion 322. Similarly, any acid/conjugate base molecule or acid/base pair is optionally used in place of and/or in combination with the formic acid : formate acid/base pair, which allows tuning of at least one of: (1) the salt type and/or salt concentration in the formulation about the polyethylenimine 250 and/or (2) the type and/or concentration of the counterions associated with the protonated sites 310 of the polyethylenimine 250.

Example IV

(Process IV)

Referring now to Figure 14, the acidification polymer tuning process 1400 is further described. The acidification polymer tuning process 1400 is essentially any combination of the first alkalization polymer tuning process 1100, the second alkalization polymer tuning process 1200, and the third alkalization polymer tuning process 1300, where the pH is lowered rather than raised. For clarity of presentation and without loss of generality, only one of the inverse pH adjustment processes is here illustrated in Figure 14. Generally, the acidification polymer tuning process 1400 provides a polymer 110, such as polyethylenimine 250, in the basic pH form 218; the pH is lowered 614; and the

counterions and/or salts in solution are tuned 1350 in serial and/or parallel steps. In the lowering pH step 614, one and preferably two or more pH lowering processes are used, such as: (1) using ion exchange 1320 to tune the counterions 1360; (2) adding hydrochloric acid 632 to yield H^+ and Cl^- in the formulation; and/or (3) adding acid 630 to tune the salt 1370 formed in solution. Again, in a manner related to that described in the third alkalization polymer tuning process 1300, addition of an acid-conjugate base pair, such as a formic acid – formate pair, tunes the counterion 320, such as associated with the protonated sites 310 of the polyethylenimine 250. Further, the pKa of the acid-conjugate base pair buffers the formulation. For instance, addition of the formic acid-formate acid/base pair results in lowering of the pH 614 while providing a buffer capacity, related to the amount of the acid/base pair added, about the pKa of the formic acid-formate pka of 3.75. Similarly, addition of the acetic acid-acetate acid/base pair buffers the pH of the formulation of the polyethylenimine 250 about the pKa of 4.75 for the acetic acid-acetate acid/base pair. Notably, the tuned counterions-salts 1350 are an equilibrium of tuned counterions 1360 with tuned salts 1370, as further described *infra*. Generally, the acidification polymer tuning process 1400: (1) lowers the pH with ion exchange and/or addition of an acid; (2) optionally provides the proton for the protonation 310 via the use of a weak acid-conjugate base acid/base pair, such as the use of formic acid; (3) tunes availability of the type and/or concentration of the counterion 320 via choice of the weak acid/base pairs or choice of strong acid type; (4) is optionally used in a process of creating no or limited salt, such as described in the process of using a conjugate base to acidify 900 the polymer 200, as described *supra*; (5) provides a buffer related to the pKas of the added acids; and/or (6) optionally and preferably removes a filtering step associated with removing excess salt in the formulation, such as resulting from either use of the initial fully acidic form of polyethylenimine 256 and/or the second subprocess of using a conjugate base to alkalize 1000 the polymer 200, which yields a salt, as described *supra*.

Referring again to Figure 14 and Figure 3 and generally referring to the salt solution 330 and the counterions 320 associated with the polymer 200, the salts

in the salt solution 330 are in equilibrium with the counterions 320 associated with the polymer 200 in terms of position. That is, a first counterion and a first salt in solution optionally swap positions, such as relative to a protonation site 310 of the cationic 211 polymer. However, the first counterion type and the second counterion type have preferred locations. For instance, chemically the formate 324 will tend to displace the chloride ion 322 as the counterion. Further, since the polyethylenimine 250 preferably has thousands of protonated sites 310, the probability is great that the counterion type with the greater attraction to the protonated site 310 will be associated with the polymer 200. This probability of knowing which anion type is the counterion 320 and which anion type is in the salt solution 330 only increases with chain length of the polymer 200, such as the polyethylenimine 250. Generally, one can quantify the counterion 320 to salt solution 330 mean likelihood as being greater than 1, 2, 5, 10, 20, 25, 50, 75, 80, or 90 percent a counterion or *vise-versa*, such as by applying the relevant position equilibrium.

Example V

(Process V)

Referring now to Figure 15 and referring again to Figure 9, a conjugate base polymer tuning process 1500 is illustrated. Generally, two or more stock solutions are optionally mixed to form the tuned polymer 210, which is particularly beneficial when starting with the basic form of polyethylenimine 252 or any polymer 200. Generally, a polymer is provided 110, such as polyethylenimine 250 in the basic pH form 218 and the pH is lowered 614, such as with an ion exchange conjugate acid/base pair 1322, which tunes the counterions 1360 and tunes the salt 1370. An example is provided for clarity of presentation and without loss of generality. If the basic form of polyethylenimine 252, such as in a first stock solution, is mixed with an acid/base pair or simply an acid, such as formic acid, in a second stock solution the basic form of polyethylenimine 252 is provided with an proton, from the acid, to yield a tuned protonation level of the polyethylenimine 254 and the counterion 320 is provided as the conjugate base. In a more specific example, if the basic

form of polyethylenimine 252, such as in a first stock solution, is mixed with a second stock solution of formic acid, the formic acid concentration quantitatively yields an amount of the protonation sites 310 and yields the counterion 320 of formate 324 at a quantitative level, such as according to the interaction illustrated in the first subprocess of using a conjugate base to acidify 900 the polymer 200 illustrated in Figure 9. Notably, this allows for direct quantitative measurement and/or comparison of the efficacy of differing counterions 320 associated with the polymer 200. For instance, a first formulation is made with a controlled amount of protonation 310 and a controlled amount of the counterion formate 324, which is tested against a bacteria or virus, such as using a government regulated process, as described *supra*. Similarly, a second formulation is made with a controlled amount of protonation 310 and a controlled amount of the counterion acetate 326, which is tested against a bacteria or virus, such as using a government regulated process, as described *supra*. Hence, each protonation level 310 and each controlled amount of counterion 320 is thus optionally and preferably controlled and quantitatively tested, as further described *infra*. Further, combinations of counterions 320 are optionally tested. For instance, in a first test a controlled amount of formate is added and tested yielding a first efficacy measure and in a second test, 100 ppm iodide anion is added, tested, and a second efficacy is measured, yielding, by difference, the effectiveness of the iodide anion. Further, the conjugate base polymer tuning process 1500 described herein combines with and/or is used as a step in any of the first general process 600, the second general process yielding a tuned counterion solution 700 / formulation, the first alkalization polymer tuning process 1100, the second alkalization polymer tuning process 1200, the third alkalization polymer tuning process 1300, and/or the acidification polymer tuning process 1400, such as where a change in pH step is used, a counterion 320 is introduced, and/or a salt solution 330 is controlled.

Still referring to Figure 15, the tuned counterions 1360, the tuned salts 1370, no formed salt state 1380, and/or the added salt state 1390 are all in equilibrium with each other. However, the chemical properties of the ions, the activity of the solution, relative concentrations, and/or the probabilities associated with a

large number of protonated sites 310 allows for calculation and/or probability determinations of which ions, such as the counterions 320, are associated with the polyethylenimine 250, as described *supra*.

Example VI

(Preprocessing)

Referring now to Figure 16, a polymer slurry acid reduction process 1600 is illustrated. Essential, the polymer slurry acid reduction process 1600 is optionally used in conjunction with any of the above described methods; however, a preferred use of the polymer slurry acid reduction process 1600 is to concentrate the polymer 200 and/or reduce the overall acid molarity, which results in less salt production, where too much salt may interfere with efficacy of the tuned polymer 210 in terms of being antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234. Generally, an acidic form of the polymer is provided 216, such as the acidic form of polyethylenimine 256. The acidic form of polyethylenimine 256 is concentrated or simply the polymer is concentrated 1610, such as by one or more of: reducing the acid concentration 1611, filtering the acidic form of polyethylenimine 256 in hydrochloric acid to yield a slurry of polymer 1612, filtering 1613, decanting 1614, centrifuging 1615, or any process that reduces the overall molarity of the hydrochloric acid in any intermediate or final formulation of the tuned polymer 210. Essentially, removal of a portion of the hydrochloric acid results in a reduction of the amount of the chloride ion 322 and hence the reduction of the salt sodium chloride when the above described processes are implemented, where some of the processes exchange the chloride counterion 322 for another ion and/or alter the pH to form sodium chloride salt, such as by addition of a base, such as sodium hydroxide where the sodium cation combines with the chloride anion to form sodium chloride in any physical state, such as in solution and/or as a precipitate. As illustrated, the resulting polymer/slurry is optionally dried 1620, as described *supra*, and/or is pH adjusted up to a target pH 1630, as described *supra*. Stated again, the filtering step 1613 and/or the step of concentrating the polymer 1610 reduces the total base molarity needed adjust the pH, so less salt is generated with the use of the polymer slurry acid reduction process 1600, especially when raising

the pH of the formulation from a very acidic pH, such as less than 3, 2, 1.7, 1.6, 1.5, or 1. Prior steps to providing the acid form of the polymer are further described *infra*.

Example VI

(Preprocessing)

Referring now to Figure 17, a polymer - salt pair salt reduction process 1700 is illustrated. Generally, the polymer - salt pair salt reduction process 1700 optionally uses any one of more steps of the polymer slurry acid reduction process 1600, described *infra*, such as the step of concentrating the polymer 1610 and/or drying the slurry 1620, which may result in formation of a solid salt layer 1622. With or without formation of the salt layer 1622, the initial acidic form of the polymer 216, such as the acidic form of polyethylenimine 256, is optionally washed 1710, such as with an aqueous solution, to remove/reduce salt 1720. Again, optionally the pH is adjusted upward 1630 at the same or later time to set the pH to a target pH 1640 and/or to yield a tuned (desired mix / type / concentration) of counter ions 1360, as described throughout. Again, the polymer - salt pair salt reduction process 1700 is used with any method described herein to remove formed salt.

Polymer Tuning

Referring now to Figures 18-28, details on counterion tuning are provided for clarity of presentation and without loss of generality by way of the examples provided, *infra*.

Example I

Referring now to Figure 18, a polymer tuning with a weak acid process 1800 is illustrated. In this example, any strong or weak acid is optionally substituted for the illustrated process of adding the counter ion formate 324 to the polymer 200 or as illustrated to the polyethylenimine 250. Referring now to equation 1A, in Figure 18, the reaction of:

linear PEI + formic acid ➔ protonated linear PEI + formate ion (*eq. 1*)

is repeated with chemical illustrations in equations 1B and equation 1C. Generally, the formate counter ion 324 from the formic acid equilibrium with formate is added to a proton on the secondary amine 1912 of the repeating unit 1910 and/or a proton from the formic acid protonates 310 the secondary amine 1912 to form a cationic polymer repeating unit.

Example I

Referring now to Figure 19, a polymer tuning with a weak acid and salt production process 1900 is illustrated. Generally, the weak acid and salt production process 1900 is the polymer tuning with a weak acid process 1800 with the addition of another acid, such as a strong acid, such as hydrochloric acid at any concentration and/or in any state. Again in this example, any strong or weak acid is optionally substituted for either of the illustrated processes of adding hydrochloric acid or the counter ion formate 324 to the polymer 200 or as illustrated to the polyethylenimine 250. Referring now to equation 2, in Figure 19, the reaction of:

PEI + HCl + formic acid ➔ protonated PEI + chloride ion + formate ion (*eq. 2*)

yields, as illustrated: (1) two protonation sites 310 on the protonated PEI having a non-chloride counterion, one proton on average from the hydrochloric acid and one from the formic acid and (2) two counterions 320, a chloride anion 322 and a formate anion 324 from the two acids, respectively. Generally, the chloride ion 322 and the formate counter ion 324 from the formic acid-formate equilibrium is added as being loosely bound to the protons on the secondary amines 1912 of the repeating unit 1910 and/or protons from the polyethylenimine 250 and/or formic acid protonates 310 the secondary amine

1912 to form a cationic polymer repeating unit. Herein, the result of a protonated polymer having non-chloride counterions 258 is distinct from a protonated polymer having only chloride counterions, both in chemical structure and application efficacy. In practice, the *n* monomers of polyethylenimine are associated with any number *a* and *b* of the chloride and formate anion, where *n*, *a*, and *b* are positive integers

Tuning Counterion Availability

Referring now to Figure 20 and Figures 21-28, relative concentration of conjugate base tuning 2000 is illustrated. Generally, a process of relative concentration of conjugate base tuning 2000 includes one or more of the steps of: (1) determining availability of the conjugate bases 2010 / anions / counterions, such as the availability of a first conjugate base 2012, a second conjugate base 2014, or *n* conjugate bases, where *n* is a positive integer greater than 0, 1, 2, 3, 4, or 5; (2) adjusting concentration of the conjugate bases, such as a first conjugate base concentration 2022, a second conjugate base concentration 2024, or *n* conjugate base concentrations, where *n* is a positive integer greater than 0, 1, 2, 3, 4, or 5; and/or (3) optionally adjusting for electronegativity and/or equilibrium coefficients 2030, such as determining/using an equilibrium between a conjugate base associated with the polymer 2032 and the conjugate base being a salt in solution 2034 and/or the affect of electronegativity on an activity coefficient for the protonated site 310 / counterion 320, which combined and/or separately allow for tuning a ratio of counterion types 2040, such as a concentration of first conjugate base on the polymer association 2042, a concentration of a second conjugate base on the polymer association 2044; or concentrations of *n* conjugate bases on the polymer associations, where *n* is a positive integer greater than 0, 1, 2, 3, 4, or 5. For clarity of presentation and without loss of generality, several examples are provided, *infra*, to further illustrate the process of relative concentration of conjugate base tuning 2000.

Example I

Referring now to Figure 21, a process of determining a first conjugate base availability 2100 is illustrated. Generally, the amount of any counterion 320 associated with the polymer 200 in a formulation of the tuned polymer 210 is optionally and preferably controlled. For clarity of presentation and without loss of generality, control of the counterion formate 324 is illustrated, which again is optionally repeated for any counterion and/or two or more counterions 320. Optionally, the counterion concentrations and/or availabilities are optionally independently quantitatively controlled, such as through implementation of any one or more of the processes described herein. In the case of formate 324, formate has a pKa of 3.774. Based upon the pKa, the fraction percentage of any form or any monoprotic, diprotic, and/or triprotic acid is calculable. In Figure 21, the fraction percentage of formic acid and formate are illustrated as a function of pH. As indicated, if a fraction percentage of formate of 85.1 percent is desired, then the pH is adjusted to pH 4.5. Conversely, the pH is selected and the fraction percent of the conjugate base(s) are known. Hence, using any of the processes described herein, the formate concentration is 85.1 percent of the total molarity of the formic acid-formate acid/base pair at pH 4.5. Notably, the overall formate and/or conjugate base concentration is optionally raised or lowered to alter the total amount/concentration of formate 324 / conjugate base available as a counterion 320 in a formulation of the tuned polymer 210, as further described in the following example.

Example II

Referring now to Figure 22, second conjugate base availability determination 2200 is illustrated. In this example, availability of acetate 326 is determined just as described for the determination of formate 324 in the prior example, only using a pKa of acetate of 4.76. While formate 324 had an availability of 85.1 percent at pH 4.5, as described in the preceding paragraph, acetate 326 only has an availability of 35.5 percent at the same pH of 4.5. Thus, if both formate 324 and acetate 326 were added in equal molar values, the ratio of formate-to-acetate is 85.1 : 35.5 at pH 4.5, or a ratio of 2.4 : 1. However, the total mass / concentration of each counterion is optionally varied/controlled, as further described in the following example.

Example III

Referring now to Figure 23, adjusting a conjugate base concentration 2300 is illustrated. Generally, any counterion 320 concentration is raised / lowered by altering the total mass in a formulation of the polymer 200. In this illustrative example, acetate 326 is illustrated at a first acetate concentration 2310 and at a second acetate concentration 2320 that is two times the first concentration. Thus, by increasing the acetate concentration, the ratio of formate-to-acetate is controlled, as illustrated in the next example. Stated another way, while the fraction of acetate to total acetate + acetic acid is still 35.5 percent at pH 4.5, as illustrated in the prior example, the amount of acetate available is controllably altered by changing the total mass of acetate + acetic acid in the formulation and/or in a formulation step. For instance, doubling or halving the total mass of acetate + acetic acid, the amount of acetate double or halves, respectively.

Example IV

Referring now to Figure 24, relative tuning of two conjugate bases 2400 is illustrated. Particularly, by adding 2.4 times as much acetic acid – acetate to a formulation as formic acid – formate, the acetate concentration is made equal to the formate concentration (35.5 * 2.4 = 85.1). Thus, the relative concentration ratio of formate-to-acetate is made to be one-to-one (1:1). Thus, the formate concentration is raised or lowered to alter the total amount of formate 324 available as a counterion 320 in a formulation of the tuned polymer 210. Similarly, the concentration of any conjugate base or first counterion is optionally and preferably tunable to any level, such as greater than 0, 0.1, 0.5, 1, 5, 10, 20, 30, 40, or 50 percent of the counterions 320 and/or a second counterion; less than 100, 90, 80, 70, 60, or 50 percent of the counterions 320 and/or a second counterion; and/or within ± 1, 2, 5, 10, 25, or 50 percent of a fractional percentage of a given counterion to all counterions and/or to a second counterion of 10, 20, 30, 40, 50, 60, 70, 80, or 90 percent, as is further illustrated in the next example.

Example V

Referring now to Figure 25A, a first counterion ratio 2500 on the polymer 200 with a backbone 2510 of atoms and/or monomers 253 is illustrated with a first conjugate base 2520 (CB_1)-to-a second conjugate base 2530 (CB_2) ratio. As illustrated the first conjugate base-to-second conjugate base ratio is three to one, 3:1. Referring now to Figure 25B, a second counterion ratio 2505 on the polymer 200 is illustrated after tuning the conjugate base ratios / counterion ratios, as described *supra*. Particularly, as illustrated the first conjugate base-to-second conjugate base ratio is now tuned to one to one, 1:1, where any tuning ratio is optionally formulated, as described *supra*.

Referring now to Figure 26, relative concentration of conjugate base tuning with three counterion types 2600 is illustrated. Here, formate fractions 2610, benzoate fractions 2620, and acetate fractions 2630 are illustrated as a function of pH, in a manner similar to that illustrated in Figures 21 and 22. The processes described in relation to Figures 20-25 are optionally repeated for three or more base pairs, such as illustrated for formate 324, benzoate, and acetate 326 herein. Thus, ratios of three base pairs are optionally controlled with a ratio of zero-to-one for the first conjugate base to zero-to-one for the second conjugate base to zero-to-one for the third conjugate base (0 to 1 : 0 to 1 : 0 to 1) at any analog step interval, such as within ±0.05 of 0.1, 0.2, 0.25, or 0.5. Referring now to Figure 27, the same process optionally uses a polyprotic acid 2700 with multiple conjugate bases, such as illustrated for the fractions of citrate 2640, such as $citrate^{3-}$ 2642, $citrate^{2-}$ 2644, and $citrate^{1-}$ 2646 with or without mixing with another conjugate base, such as the illustrated formate fraction 2610.

Referring now to Figure 28, relative tuning of multiple conjugate bases/counterions 2800 is illustrated. As illustrated, the polymer 200, which a chemical backbone 2510, is associated with the first conjugate base 2520 (CB_1), the second conjugate base 2530, a third conjugate base 2540, and a

fourth conjugate base 2550 at a ratio of five to four to two to one (5:4:2:1), where any ratios of conjugate bases are optionally and preferably formulated.

The inventors note that de-chlorinating / de-salting / tuning counterion availability decreases toxicity to humans, vertebrates, and invertebrates. Particularly, when polyethylenimine 250 is protonated with traditional means of combining with concentrated hydrochloric acid, essentially all of the counterions to the formed protonated sites are chloride. Notably, the Environmental Protection Agency states that the National Aquatic Life Criteria for chloride is 860 mg/L for an acute exposure and 230 mg/L for a chronic exposure. Even with removal of all excess hydrochloric acid from formed protonated polyethylenimine, the concentration of chloride counterions, for a pH 4.5 / twenty-eight percent protonated polyethylenimine, is 1120 mg/L (1120 ppm). Generally, the chloride counterion concentration, with manufacturing with hydrochloric acid, exceeds the Environmental Protection Agency requirements for any protonated polyethylenimine in a pH range of 1 to 9. Thus, replacing chloride counterions with other, less toxic, counterions is optionally and preferably performed to meet the U.S. Environmental Protection Agency standards. Any of the methods described herein are optionally and preferably implemented to meet the U.S. Environmental Protection Agency standards.

ANTIBACTERIAL, ANTIVIRAL, AND ANTIFUNGAL TESTING

The inventors have determined that formate/formic acid, acetate/acetic acid, citrate/citric acid, and iodide/iodine each have antibacterial properties in combination with linear PEI, where the PEI is in solution and/or a solid film. Linear PEI with both chloride and formate counterions yielded 99.9999 percent activation using the EPA 01-1A test protocol for durability testing for both MRSA and E. coli on a glass substrate and in separate testing on stainless steel substrate with contact times of 10 minutes. Similarly, combinations of formate, acetate, and iodide with chloride yielded percent inactivation ranging from 99.4 to 99.99 using ISO 22196 and JIS 1902 test protocols for MRSA, E. coli, and fungi on substrates of (separately tested) plastic, textile, and glass with contact

times of 1 minute to 10 minutes and were even effective against c. Diff with percent inactivation of 98 and 99.4 percent with contact times of 8 and 24 hours, respectively. Representative antibacterial test results are provided in Tables 1(A-D), *infra*.

5

Table 1A: Linear PEI, Chloride, and Formate Antibacterial Film Testing

Non-durability Testing	Substrate	Contact Time	Chloride and Formate	Test Protocol	Percent Inactivation
Gram-positive MRSA	Plastic	2 min	X	IS0 22196	99.99
Gram-negative E. coli	Plastic	2 min	X	IS0 22196	99.99
Gram-negative E. coli	Textile	10 min	X	JIS 1902	99.4
C. diff	Glass	24 hours	X	IS0 22196	99.4
Gram-negative E. coli.	Stainless	5 min	X	PAS 2424	99.99
Gram-negative P. Aeruginosa	Stainless	5 min	X	PAS 2424	99.93
Gram-positive MRSA	Stainless	5 min	X	PAS 2424	99.92
Gram-negative E. hire	Stainless	5 min	X	PAS 2424	99.94
Gram-negative E. coli.	Stainless	5 min	X	PAS 2424	99.99
Gram-negative E. coli.	Glass	10 min	X	01-1A	99.999
Gram-negative P. aeruginosa	Glass	10 min	X	01-1A	99.999
Gram-positive MRSA	Glass	10 min	X	01-1A	99.999
Gram-negative E. hire	Glass	10 min	X	01-1A	99.999

Table 1B: Linear PEI, Chloride, and Acetate Antibacterial Film Testing

Non-durability Testing	Substrate	Contact Time	Chloride and Acetate	Test Protocol	Percent Inactivation
Gram-positive MRSA	Glass	2 min	X	IS0 22196	99.99
C. diff	Glass	8 hours	X	IS0 22196	98
Gram-negative E. coli.	Glass	10 min	X	01-1A	99.99
Gram-negative P. aeruginosa	Glass	10 min	X	01-1A	99.99

Gram-positive MRSA	Glass	10 min	X	01-1A	99.99

Table 1C: Linear PEI, Chloride, and Iodide Antibacterial Film Testing

Non-durability Testing	Substrate	Contact Time	Chloride and Iodide	Test Protocol	Percent Inactivation
Gram-positive MRSA	Plastic	1 min	X	IS0 22196	99.87
Gram-negative E. coli	Plastic	1 min	X	IS0 22196	99.79
Gram-negative E. coli	Textile	10 min	X	JIS 1902	99.4

Table 1D: Linear PEI, Chloride, and Formate Antibacterial Film Testing

Durability Testing	Substrate	Contact Time	Chloride and Formate	Test Protocol	Percent Inactivation
Gram-positive MRSA	Glass	10 min	X	01-1A	99.9999
Gram-negative E. coli	Glass	10 min	X	01-1A	99.9999
Gram-positive MRSA	Stainless	10 min	X	01-1A	99.9999
Gram-negative E. coli	Stainless	10 min	X	01-1A	99.9999

The inventors have determined that formate/formic acid, acetate/acetic acid, citrate/citric acid, and iodide/iodine each have antiviral properties in combination with linear PEI. Using government approved testing and/or internal tests paralleling government approved tests, linear PEI with chloride and formate counterions yielded 99.6 to 99.9 percent inactivation of enveloped viruses; linear PEI with chloride and acetate counterions yielded 99.95 to 99.99 inactivation of enveloped and non-enveloped viruses; and linear PEI with chloride and iodide counterions yielded 89.7 percent inactivation of the very difficult non-enveloped polio virus, where the tests were performed on a variety of substrates with contact times ranging from 5 to 10 minutes. Representative antiviral test results are provided in Tables 2(A-C), *infra*.

Table 2A: Linear PEI, Chloride, and Formate Antiviral Testing

Durability Testing	Substrate	Contact Time	Chloride and Formate	Test Protocol	Percent Inactivation
Vaccinia enveloped Virus	Plastic	5 min	X	IS0 22702	99.96
Beta Corona enveloped Virus	Stainless	5 min	X	IS0 22702	99.9
Polio Non-enveloped Virus	Plastic	10 min	X	EN 14476	99.6
Vaccinia enveloped virus	Stainless	10 min	X	PAS 2424	99.96
Beta Corona enveloped virus	Artificial Skin	10 min	X	PAS 2424	99.9

Table 2B: Linear PEI, Chloride, and Acetate Antiviral Testing

Durability Testing	Substrate	Contact Time	Chloride and Acetate	Test Protocol	Percent Inactivation
MS2 Surrogate for Non-enveloped Virus	Glass	5 min	X	IS0 22702	99.99
Enveloped H1N1	Spray test	5 min	X	EXI .2.10.26.17	99.95
Non-enveloped Rhinovirus	Spray test	5 min	X	EXI .2.10.26.17	99.95

Table 2C: Linear PEI, Chloride, and Iodide Antiviral Testing

Durability Testing	Substrate	Contact Time	Chloride and Iodide	Test Protocol	Percent Inactivation
Non-enveloped Polio	Textile	10 min	X	IS0 22702	89.7
Polio Non-enveloped virus	Stainless	10 min	X	PAS 2424	89

The inventors have determined that formate/formic acid, acetate/acetic acid, citrate/citric acid, and iodide/iodine each have antifungal properties in combination with linear PEI. Using government approved testing, linear PEI

with chloride and formate counterions yielded 99.6 to 99.9 percent inactivation of fungus. Referring now to Figure 3A, representative antifungal test results are provided.

Table 3A: Linear PEI, Chloride, and Formate Antifungal Film Testing

Non-durability Testing	Substrate	Contact Time	Chloride and Formate	Test Protocol	Percent Inactivation
Fungi	Textile	2 min	X	JIS 1902	99.6
Fungi	Glass	5 min	X	IS0 22196	99.99
Fungus Candida auris	Stainless	5 min	X	PAS 2424	99.94

CHEMICAL BACKBONE 2510

Referring now to Figures 29 to 36, the backbone 2510 is further described. Herein, the polymer 200 is used as an example of a chemical backbone 2510 of a charged chemical structure 2910. Stated again, the polymers 200 described herein are used to carry a plurality of charges, such as cationic charges, along a chemical backbone 2510. However, the chemical backbone 2510, of which the polymer 200 is an example, is optionally any sequence of covalently bonded atoms, such as described *infra*.

Referring now to Figure 29, a process of treating a substance with a charged chemical structure 2900 is illustrated. For clarity of presentation and without loss of generality, a polymer 200 is used herein to represent the charged chemical structure 2910. However, the charged chemical structure is optionally a protein 202 or more generically any chemical backbone 2510. Generally, the chemical backbone 2510 is a series of covalently bonded atoms, amino acids, monomers, and/or functional groups that optionally and preferably carry multiple charges, as further described in Figures 30-36. As with the polymer tuning process 100 illustrated in Figure 1, the protein 202 and/or the chemical backbone 2510 are optionally and preferably tuned, such as by any process described herein for tuning the polymer 200. As with the polymer 200, the now multi-charged / polycationic protein and/or multi-charged / polycationic

chemical backbone is optionally and preferably used to treat a substance 140, such as after an optional change in dominant state 130, where the treatment yields antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234 properties. For clarity of presentation and without loss of generality, whenever a polymer 200, such as a tuned polymer 210 and/or a polyethylenimine 250 is referred to, reference to the more generic chemical backbone 2510 is optionally and preferably inferred.

Referring now to Figures 30(A-D), the charged chemical backbone 2510 optionally and preferably contains charged sites, such as a charged nitrogen. For clarity of presentation, four amine types are illustrated. Referring now to Figure 30A, a primary amine 3002 is illustrated, where, in a positively charged state, a nitrogen is bonded to three hydrogens and one additional molecular fragment, referred to here as a first molecular fragment, R_1. Often, the first molecular fragment terminates in a carbon bound to the nitrogen, where the carbon is of any hybridization. In the positively charged state, the primary amine 3002 is illustrated as protonated 310 and is illustrated with a counterion 320. Referring now to Figure 30B, a secondary amine 3004 is illustrated, where, in a positively charged state, the nitrogen is bonded to two hydrogens and two additional molecular fragments, referred to here as a first molecular fragment, R_1, and a second molecular fragment, R_2. Again, the second molecular fragment and/or is any molecular fragment and optionally terminates in a carbon bound to the nitrogen. Referring now to Figure 30C, a tertiary amine 3006 is illustrated, where, in a positively charged state, the nitrogen is bonded to a single hydrogen and three additional molecular fragments, referred to here as a first molecular fragment, R_1; a second molecular fragment, R_2; and a third molecular fragment, R_3. Referring now to Figure 30D, a quaternary amine 3008 is illustrated, where the nitrogen is bonded to four molecular fragments, designated R_1, R_2, R_3, and R_4.

Still referring to Figures 30(A-D), the polymers illustrated herein, such as the polyethylenimine 250, linear polyethylenimine 3300, and/or the branched

polyethylenimine 3400 contain primary amines 3002, secondary amines 3004, and/or tertiary amines 3006, optionally and preferably in the absence of quaternary amines 3004, which are relatively toxic in comparison. Quaternary ammonium compounds, or quats, are a group of positively charged ions that are often used as disinfectants, surfactants, and fabric softeners. While quaternary ammonium compounds are generally considered safe for use in household and industrial settings, concentrations are environmentally limited as the quaternary ammonium compounds may be toxic at high concentrations, with long exposure times, and/or if ingested. Herein, any one or more quaternary ammonium compounds/molecules are optionally used in combination with any of the polymers 200, the polyethylenimine 250, and/or the multi-charged chemical backbones 2510 described herein.

Referring now to Figure 31A and Figure 31B, an exemplary repeating group, such as found in the polymer 200, the polyethylenimine 250, and in some cases the chemical backbone 2510 is illustrated. Referring still to Figure 31A and referring again to Figure 2, the illustrated repeating monomer 253 is the repeating unit of polyethylenimine 250 in a basic form 3100. Referring now to Figure 31B, the repeating unit of polyethylenimine 250 in an acidic form 3105 is illustrated. In practice, polyethylenimine 250 has any number of unprotonated monomers 3110 and any number of protonated monomers 3105, such as based on pH and/or formulation methodologies, such as pH, temperature, and/or time of reaction, as further described *infra*. In polyethylenimine 250, the repeating unit includes a chain of covalently bonded atoms in a repeating sequence of carbon-carbon-nitrogen. Again, in Figure 31B, the acidic form of the nitrogen is protonated and has a counterion and is referred to as a protonated amine and more specifically a protonated secondary amine. However, the repeating monomer is optionally of any structure. Instead of the repeating carbon-carbon-nitrogen group of the polyethylenimine illustrated in Figure 31B, a repeating carbon-carbon-carbon-nitrogen group of polypropylenimine 3110 is illustrated in Figure 31C. Even more generally, referring now to Figure 31D, the repeating group is optionally of any structure bonded to the nitrogen referred to here as R_1-nitrogen, or simply a repeating

unit of a nitrogen containing backbone 3120. For instance, the polyethylenimine polymer has a repeating carbon-carbon-nitrogen backbone, the polypropylenimine polymer has a repeating carbon-carbon-carbon-nitrogen backbone, and more generally the polymer 200 has a repeating nitrogen containing backbone, such as R_1-nitrogen, or R_1-nitrogen-R_2, where R_1 and R_2 are each independently any organic molecular fragment, such as of any length of carbons, and/or of any length of carbons covalently bonded to and/or interspersed with any other atoms, such as carbon-oxygen-carbon-carbon-carbon-, which is bonded to the nitrogen. Optionally, the nitrogen is not in the backbone structure, but is rather attached to the backbone structure of the repeating group, such as an $-NH_2$ or $-NH_3$ group attached to a carbon in the backbone structure. Optionally, the nitrogen is indirectly covalently bonded to the backbone structure. Herein, where the nitrogen, which is optionally protonated, is attached in any manner to the backbone structure of the repeating unit, the combined nitrogen containing fragment and the backbone fragment of the polymer is referred to as a nitrogen containing repeating backbone monomer 3130 / fragment of the polymer.

Referring now to Figure 32, acidification of a base 3200 is illustrated. More specifically, an example is provided showing the relationship between the basic repeating unit of polyethylenimine 250 and a protonated repeating unit of the polyethylenimine 250. Generally, the base, which is a proton acceptor and/or an electron donor, interacts with an acid, such as hydrochloric acid, which is a proton donor and/or an electron acceptor to form a conjugate acid of the base and a counterion.

Referring now to Figures 33(A-D), linear polyethylenimine 3300, referred to as linear PEI, is sequentially illustrated in a base form, a slightly acidified form, a more acidified form, and an acidified form, respectively. Referring again to Figure 33A, polyethylenimine 250 is illustrated with primary amines 3002 at the polymer termini and a multitude of secondary amines 3004, such in the repeating monomer 253. Again, the linear polyethylenimine 3300 contains a

chemical backbone 2510 of repeating units of carbon-carbon-nitrogen. Referring again to Figure 33B, the partially acidified polyethylenimine 250 is illustrated as partially protonated 310 with a counterion 320, in the illustrated case a chloride ion 322 / chloride counterion. Referring again to Figure 33C, optionally in small sections of the polyethylenimine 250, a dimer is present of one charged nitrogen monomer unit and one uncharged nitrogen monomer unit 413. Notably, the frequency of the charged nitrogen monomer is every monomer unit, every second, third, fourth monomer unit, and/or at random intervals along the length of the polyethylenimine 250. The polymer is optionally referred to a percent protonated, such as greater than 1, 2, 5, 10, 30, or 50 percent protonated and/or as less than 99, 98, 95, 90, 70, or 50 percent protonated. Referring again to Figure 33D, a fully protonated version of the polyethylenimine 250 is illustrated. Generally, referring again to Figures 33(A-D), as the pH is decreased, the percentage of protonated sites on the polyethylenimine 250 generally increases and *vice-versa*.

Referring now to Figure 34A and Figure 34B, branched polyethylenimine 3400 is illustrated, which is an example of another chemical backbone 2510. The branched polyethylenimine 3400 contains the primary amines 3002 and the secondary amines 3004 of linear polyethylenimine 3300 and contains tertiary amines 3006. Optionally and preferably, any number of the primary amines 3002, secondary amines 3004, and/or tertiary amines 3006 are protonated, as illustrated in Figure 34B. As illustrated, at least some of the nitrogens in the tertiary amines are covalently bonded to three carbon-carbon-nitrogen fragments. Again, the linear polyethylenimine 3300 and branched polyethylenimine 3400 contain no quaternary amines 3008, but quaternary amines 3008 are optionally added to a formulation of the polyethylenimines 250 or any polymer 200 to enhance antimicrobial 231, antibacterial 232, antiviral 233, and/or antifungal 234 properties.

Referring now to Figure 35 and Figure 36, for clarity of presentation and without loss of generality, two exemplary chemical backbones 2510 are illustrated.

Example I

Referring now to Figure 35, a first example of a chemical backbone 3500 is illustrated with amino acids / protein sections in place of the polymer 200. As illustrated, the chemical backbone 2510 has a first optional section 3510 of a set of amino acids, which form the chemical backbone 2510 and/or are covalently bonded, directly or indirectly, to the chemical backbone. As illustrated, the amino acids include one or more of: glycine 3511, alanine 3512, serine 3513, and/or asparagine 3514, which are each protonatable, which yields a chemical backbone 2510 with multiple cationic charges, in a manner related to the multiple cationic charged of the polymer 200 / tuned polymer 210. More generally, the first optional section 3510 of the chemical backbone 3500 contains and/or is bonded directly/indirectly to one or more amino acids 3515 and/or protonatable groups 3516. As illustrated, the chemical backbone 2510 has a second optional section 3520 that intermixes one or more nitrogen groups 3517, such as a secondary amine 3004 and/or a tertiary amine 3006, into the chemical backbone 2510 and/or as molecule fragments bonded directly and/or indirectly to the chemical backbone 2510. More generally, the chemical backbone 2510 includes and/or is bonded directly/indirectly with a series of one or more amino acids, protein fragments, RNA fragments, DNA fragments, and/or peptides. Generally, the chemical backbone 2510 contains/carries a plurality of protonatable sites, such as one or more secondary amines 3004; one or more tertiary amines 3006; one or more protonatable amino acids; and/or one or more protonatable sites. Generally, the chemical backbone is of any length of greater than 10, 20, 50, or 100 covalent bonds in series.

Example II

Referring now to Figure 36, a second example of a chemical backbone 3600 is illustrated with a series of covalent bonds in the chemical backbone 2510 from a first terminus 3610 to a second terminus 3620 of an optional number of *n* termini in a branched molecule, where *n* is a positive integer of at least two. Generally, the chemical backbone 2510 contains, directly/indirectly, any

number of protonatable groups 310. Generally, the chemical backbone 2510 contains any combination of carbon, oxygen, sulfur, and nitrogen atoms covalently bonded in a series of greater than 5, 10, 15, 20, 50, 100, 500, or 1000 covalent bonds, where any of the covalent bonds are optionally double bonds. The illustrated series of covalently bonded atoms is illustrative in nature only and is non-limiting. The chemical backbone 2510 and/or any side chain/group bonded directly/indirectly to the chemical backbone 2510 contains greater than 3, 4, 5, 10, 20, 50, 100, 200, 500, 1000, 5000, 10,000, or 50,000 protonatable sites, which are tunable in any manner set forth herein for polymer tuning 210. As illustrated, the chemical backbone 2510 optionally contains a set of one or more side chains 3610, such as a first side chain 3612, a second side chain 3614, and a third side chain 3616, where any of the side chains optionally contain, are bonded to, and/or are indirectly bonded to any number of protonatable groups, which are optionally tuned by any manner set forth herein for tuning the polymer 210. Generally, a set of anions are optionally used in place of and/or in combination with the set of cations / protonatable groups set forth herein.

POLYMER TUNING

Referring now to Figure 37, polymer tuning 100 is further described. As described in Figure 1, a polymer is provided 110, such as any of the polymer types described*, supra*. Herein, control parameters 3710 that yield a tuned polymer state 3740, of the polymer tuning 100 process, are described. Notably, different tuning processes yield differing antibacterial, antiviral, and/or antifungal properties/efficacies, which may be due to folding/unfolding properties of the polymer as a function of temperature, salt concentration, pH, mixing rate, and/or reaction times.

Still referring to Figure 37, control parameters 3710 in polymer tuning includes a process 3712, such as the first general process 600, the second general process yielding a tuned counterion solution 700 / formulation, the first alkalization polymer tuning process 1100, the second alkalization polymer

tuning process 1200, the third alkalization polymer tuning process 1300, the acidification polymer tuning process 1400, the conjugate base polymer tuning process 1500, and/or any process that yields a tuned polymer state. Generally, the process 3712 optionally includes controlling: concentration 3714 of the polymer, the solvent 3716, temperature 3718, pressure 3720, time of any step 3722, mixing 3724, pH 3726, an acid type 3728, a counterion type 3730, solvent evaporation 3732, a delivery process 3734, and/or any chemical, mechanical, electrical, and/or physical parameter to yield the tuned polymer state 3740. The solvent 3716 is optionally water, ethanol, isopropanol, and/or any organic solvent.

Still referring to Figure 37, the tuned polymer state 3740 includes any one or more of: a tuned charge 3800; additive killing 3741 properties; an activity 3742, such as an activity coefficient, γ, of a surrounding solvent and/or solution; longevity of killing 3743 properties; durability 3744, such as the persistence of an antimicrobial and/or antiviral property through time and/or through a repetitive wet/dry rub process; persistence 3745 / persistive killing properties; biocidal properties 3746; a counterion distribution 3738, such as number/concentrations of counterion types/classes; a salt concentration 3750, in solid and/or liquid form; crystallization properties 3752; viscosity 3754, and/or stickiness 3756. Details on the tuned polymer state 3740 are further described, *infra*. Generally, the provided polymer 110 is tuned 3740, such as through use of the control parameters 3710, to yield a biocidal polymer 3760. Additive killing 3741, longevity of killing 3743, and persistence 3745 of killing 3745 are further described, *infra*.

Charge/Charge Effectiveness

Referring still to Figure 37 and referring now to Figure 38, polymer tuning 100 is illustrated with tuning charge 3800 of the polymer 200. For clarity of presentation and without loss of generality, a process of tuning cationic charge 3810 is described, where again, the tuning is optionally tuning an anionic charge and/or a zwitterion charge. In tuning cationic charge 3810, several of

the control parameters 3710, described *supra*, are implemented. For example, pH 3722, temperature 3718, pressure 3720, and/or mixing 3724 are controlled to tune protonation 3815 of the polymer. The tuned protonation 3815 and/or the tuned cationic charge 3810 is simultaneously and/or subsequently tuned for charge effectiveness 3820. Charge effectiveness 3820 is dependent on one or more of salt concentration 3750, ionic strength 3751, de-salting 3753, and activity 3742. Tuning cation charge 3810 is further described, *infra*, in reference to Figures 39-41 and tuning charge effectiveness is further described, *infra*, in reference to Figures 42-46.

Charge

It is observed that at a fixed pH, total charge varies on the polymer 200 dependent upon the control parameters 3810 implemented. For instance, when a polymer 200, such as polyethylenimine 250, is treated with concentrated hydrochloric acid for 96 hours instead of 8 hours, a measured percent chlorination on the resulting polymer increases from 38 to 43 percent by weight, which is a protonation increase from 65 to 100 percent or a 54 percent increase in protonation. Without wishing to be bound to any particular theory, two explanation are provided herein.

A first explanation of increased protonation with reaction time at a temperature of 130 degrees is provided. Referring now to Figure 39, multiple charges of a polymer at a single pH 3900 are illustrated. In Figure 39, a cationic polymer 300 is illustrated at a first time with protonated sites 241 and a first region 3910 and a second region 3920 that are not readily accessed for protonation. It is observed, that for a fixed pH of solution, the degree of protonation 241 increases to differing total protonation levels as a function of time/temperature, as described *supra*. One explanation is that at an elevated temperature, such as at greater than 30, 50, 75, 100, or 125 degrees Celsius, the cationic polymer 300 at least partially unfolds into a more elongated form 3930, which allows the acidic solution to more completely react with the polymer 200 at difficult to access sites, such as a first hindered site 3910 and/or a second hindered site

3920 that are sterically sheltered by the cationic polymer 300 from surrounding solution, to form more cationic sites 241. Notably, once protonated, the additional protonated sites 241 are stable when the temperature of the solution is subsequently reduced. In any event, increasing a reaction time at an elevated temperature in a protonation step of the polymer 200, such as polyethylenimine 250, results in an increase of protonation of the polyethylenimine 250 by 54 percent, such as when protonated with hydrochloric acid at a pH of 1.2, which may be due to increase accessibility of secondary amines on the polyethylenimine 250 with a change in structure / unfolding / unwrapping 3930 of the polyethylenimine 250 at the elevated increased temperature for the longer elapsed time period.

A second explanation of increased protonation with reaction time at a temperature of 130 degrees is provided. Referring now to Figure 40A, three chains of polyethylenimine 250 are illustrated with a first distance, d_1, between chains. The first distance between chains results in a physical obstacle for access to sterically blocked sites 2010 of some of the internal secondary amines, which limits protonation of the sterically blocked sites 2010 by the protonating acid. Referring now to Figure 40B, the three chains of polyethylenimine 250 are illustrated with an increased temperature, which results in a second distance, d_2, between chains, where the second distance is greater than the first distance, such as greater than 1, 2, 5, 10, 25, 50, 100, or 200 percent greater. The increased distance between chains results in increased protonation sites 243 with treatment by the concentrated acid. Again, it is observed that increasing protonation of the polymer 200, such as polyethylenimine 250, results with a longer reaction time at an elevated temperature of 130 degree Celsius, such as when protonated with hydrochloric acid at a pH of 1.2, which may be due to increase accessibility of secondary amines on the polyethylenimine 250 with a change in structure / unfolding / unwrapping of the polyethylenimine 250 at an increased temperature.

Referring now to Figure 41, the increased protonation 4100 of polyethylenimine 250 from 65 to 100 percent with an increased time, in hydrochloric acid at a pH of 1.2, from 8 hours 4110 to 96 hours 4120 is illustrated. Similarly, changes in stirring, temperature, applied pressure, cooling time, and/or mixing, such as via sonication, alter protonation times. Generally, a preferred protonation level of the polyethylenimine is with 1, 2, 4, 6, or 8 percent of any of 25, 30, 35, 40, 45, 50, 55, 60, and 65 percent protonated. The method of forming polyethylenimine 250 also affects protonation.

Still referring to Figure 41, the inventors have determined that the percent protonation of the polyethylenimine is dependent upon the method of protonation / deprotonation of the polyethylenimine, such as being a function of time at given temperatures and pressures and/or the presence of one or more additional components in the formulation. Further, driving off varying percentages of a carboxylic acid formed in the diacylation of a starting product, such as poly(2-ethyl-2-oxazoline), $-(C_5H_9NO)_n-$, and/or poly(2-alkyl-2-oxazoline) alters properties a film formed from the resulting polyethylenimine.

De-Salting / Activity

Referring now to Figures 42-46, the effectiveness of the charged sites on the polymer 200 is further described. When protonating polyethylenimine, a very acidic solution of hydrochloric acid is reacted with the polyethylenimine and/or a polyethylenimine precursor at an elevated temperature for a period of hours. The net result is not only an acidic solution, but also a solution of very high salt concentration with a very low activity coefficient. Reduction of the salt concentration and/or alternatively forming the polyethylenimine without increasing the salt concentration enhances the effectiveness of interaction of the charged sites of the polyethylenimine interacting with bacteria and/or viruses, as further described herein.

Referring now to Figure 42, an effective charge of the polyethylenimine 250 is illustrated with salt removal. As illustrated, an initial effective charge of 100

units is illustrated with an activity coefficient, γ, of 0.5. As the polyethylenimine solution is desalted, such as by any of the methods described *supra*, the effective charge of the protonated sites on the polyethylenimine 250 increases. For example, the effective charge increases by 20, 40, 60, 80, and 100 percent as the activity coefficient of the polyethylenimine solution increases to 0.6, 0.7, 0.8, 0.9, and 1.0, respectively, through removal of ions in the solution. Without wishing to be bound to any particular theory, a theory is that as the effective localized charges, protonated sites 310, of the cationic polymer 300 are drawn to negative charges on a virus, where interaction, physically and/or electrically, between the cationic polymer 300 and the virus hinders viral activity, as further described *infra*. Preferred activity coefficients, such as for the protonated secondary amines of polyethylenimine, are optionally and preferably greater than 0.7, 0.8, 0.9, 0.95, 0.98, 0.99, and/or 0.995 and/or less than 1.000, 0.999 and/or 0.998

Virus / Virus Charge

Referring now to Figure 43A and Figure 43B, viruses 4300 are illustrated. Particularly, a non-enveloped virus 4302 is illustrated in Figure 43A and an enveloped virus 4304 is illustrated in Figure 43B. Examples of non-enveloped viruses 4302 include: hepatitis A, hepatitis E, norovirus, rhinovirus, and astrovirus. Examples of enveloped viruses 4304 include: SARS, MERS, alphaviruses, hepatitis C, and zika virus. The tuned polyethylenimine / tuned polymer 210 is demonstrated as being effective against both non-enveloped viruses 4302 and enveloped viruses 4304, as described in reference to Figures 2(A-C), *supra*.

Still referring to Figure 43A, generally, a virus 4300 includes an inner region of a nucleic acid 4320, such as an RNA strand, that is circumferentially (spherically) encapsulated within a capsid shell 4310 or a set of capsids / nucleocapsid proteins. The capsid shell 4310 contains positive charges facing the negatively charged areas of the nucleic acid 4320. For instance, arginine and/or lysine, in the capsid shell 4310, each have a positively charged NH_2 end

that faces inward. However, at pH 7 ± 3, the arginine and/or the lysine also contains a negatively charged COO^- end, which can face outward. Generally, the virus 4300 has an outer surface that is zwitterionic and/or in many cases has a plurality of local negatively charged locations 4312, which may interact with the positively charged protonated sites 310 of the cationic polymer 300. Possible interactions of the negative charges of the outer surface of the virus 300 interact with the cationic polymer 300 are further described, *infra*. Referring now to Figure 43B, features of the non-enveloped virus 4302 are present in the enveloped virus 4304, while the enveloped virus further includes an encapsulating lipid bilayer 4360, such as derived from a host, where the lipid bilayer typically has additional features, such as glycoproteins 4370. Without wishing to be bound to any particular theory, three examples of interactions of charged polymers with viruses are provided, *infra*.

Example I

Referring now to Figure 44A, a first example of interaction between a tuned cationic polymer 300 and/or any charged polymer with a virus 4300 is illustrated. As illustrated, the positively charged protonated sites 241 of the polymer 200 are electrically attracted 4410 to the negatively charged locations 4312 of the virus 4300. The electrical attraction 4410 optionally occurs many times as both the polymer 200 and the virus 4300 have multiple charged sites, which in this first example causes the tuned cationic polymer 300 to circumferentially encompass the virus 300, thus blocking interaction of the virus with a host, which effectively inactivates the virus 300.

Example II

Referring now to Figure 44B, a second example of interaction between a tuned cationic polymer 300 and/or any charged polymer with a virus 4300 is illustrated. As illustrated, the positively charged protonated sites 241 of the polymer 200 are electrically attracted 4410 to the negatively charged locations 4312 of the virus 4300. The electrical attraction 4410 optionally occurs many times as both the polymer 200 and the virus 4300 have multiple charged sites,

which in this second example causes the tuned cationic polymer 300 to be electrically attracted to multiple sites of the virus, such as around greater than 10, 20, 30, 40, 50, 60, or 70 percent of a circumference of the virus 4300. As illustrated in Figure 44B, the polymer 200 sterically blocks part of the virus, which may result in partial inactivation of the virus.

Example III

Referring now to Figure 45, a third example of interaction between a tuned cationic polymer 300 and/or any charged polymer with multiple viruses 4300 is illustrated. As illustrated, the positively charged protonated sites 241 of the polymer 200 are electrically attracted 4410, as described above, to the negatively charged locations 4312 of more than one virus 4300. The electrical attraction 4410 optionally occurs many times on a single virus 4300. However, as the tuned cationic polymer 300 is long, such as tens, hundreds, or thousands of monomers long, a first cationic polymer 4511 is optionally electrically attracted simultaneously to a set of viruses 4520, such as to a first virus 4521, a second virus 4522, and a third virus 4523. Similarly, a second cationic polymer 4512, of a set of cationic polymers 4510, is optionally attracted to a common virus attracted to the first cationic polymer 4511, such as the second virus 4522, while simultaneously being attracted to a fourth virus 4524, where the fourth virus 4524 is not interacting with the first cationic polymer 4511. Similarly, any number of cationic polymers optionally interact with any number of viruses to form a virus-polymer conglomerate 4500, such as where the number of cationic polymers in the virus-polymer conglomerate 4500 is greater than 1, 2, 5, 10, 50, 100, 500, 1000, 10,000, or 100,000. The virus-polymer conglomerate 4510 is also optionally referred to herein as a virus globule and/or a virus-polymer globule, which may be large enough to settle out of solution and/or form a solid. The conglomerate optionally functions to inactivate a percentage of the virus, such as greater than 50, 60, 70, 80, 90, 99, 99.9, or 99.99% of the viral activity.

Activity

Now that electrically driven virus-protein attractions have been discussed, the importance of the activity, γ, and/or the activity coefficient, γ, of a solution containing the tuned cationic polymer 300 is further addressed. Referring now to Figure 46A, in a first high activity case 4600, a virus 4300 having negatively charged locations 4312 and a tuned cationic polymer 300 having positively charged protonated sites 241 is illustrated with a high activity 4610 and a corresponding large ionic attractive force 4620 between the virus 4300 and the tuned cationic polymer 300. Referring now to Figure 43B, in a second lower activity case 4650, the virus 4300 and the tuned cationic polymer 300 are again illustrated, but with additional ions in solution, which leads to a lower activity coefficient 4660 and a correspondingly weaker ionic attractive force 4670 between the virus 4300 and the tuned cationic polymer 300. Herein, the high activity is optionally an activity coefficient, for a given protonated site of the polymer, of greater than 0.2, 0.3, 0.4 and optionally and preferably greater than 0.5, 0.6, 0.7, 0.8, 0.9, 0.95. or 0.98 and less than 0.9999. The greater the activity coefficient, the stronger the large ionic attractive force 4620. Optionally and preferably, the high activity coefficient 4610 is greater than 0.05, 0.1, or 0.2 larger than the low activity coefficient 4660. An example of the low activity coefficient is the activity of the charged polyethylenimine 250 after reaction with a strong acid, such as hydrochloric acid, in a protonation step of protonating the polymer, such as at a pH less than three. An example of the high activity coefficient, is the activity after any de-salting, chloride reduction step, and or a step of using an anion exchange bead, as described *supra*. Stated again, a higher activity coefficient and/or lower conductivity of a formulation aids in maintaining an actual ionic attractive force between the polymer 200 and the virus 4300, which is optionally measured as a percent inactivation / percent kill of the virus, such as in the above described government tests. Similarly, increased salt, non-reduced salt, and/or a low activity coefficients, such as in formation of a protonated polymer without reduction of salt, hinders complexing activity / attraction of the charged polymer with the virus 4300. Thus reducing salt to increase the activity coefficient, γ, to greater than 0.8, 0.9, 0.95, 0.96, 0.97, 0.98, 0.99, 0.995 and/or less than 0.9999 or 0.9995 increases the effectiveness of the formulation (as described herein) against viruses and/or encapsulated viruses. Similarly, lowering a conductivity of the formulation to

0.1 to 10 mS/cm, to less than 10, 100, 500, 1000, or 10,000 mS/cm, and/or to within a range of greater than 0.5, 1, or 2 mS/cm and less than 50, 25, 10, 8, 6, 4, or 3 mS/cm is used to increase effectiveness of the charged sites of the polymer interacting with charged sites of the virus, which increases inactivation of the virus rates, as described herein.

Liquid/Film Biocide

Referring now to Figures 47-50, delivery of a biocide with a controlled total charge is described.

Referring now to Figure 47, a method of charge control 4700 / control of delivery of tuned cationic polymer 300 and/or the tuned cationic polymer 300 in a formulation is illustrated. In this example, provided for clarity of presentation and without loss of generality, a biocide of known charge density is delivered in a solvent with a known volume, which yields a total cationic charge, such as at a pH with an activity, so that a calculable / controlled charge is delivered to a microbe / bacteria / virus / fungus / surface. Generally, a liquid biocide 4710, such as any of the polymers 200 described herein, is contained in a delivery container 4720 along with a solvent 4730. Optionally and preferably, the solvent contains water and/or any organic solvent, such as a solvent that readily evaporates at room temperature and pressure. In one case, an activity 3742 / activity coefficient of a polymer–solvent combination is controlled, such as described *supra*. Optionally and preferably, the tuned polymer 210 is at least one of the polymers 200 in the delivery container 4720. Optionally and preferably, the tuned polymer 210 has a charge density 4730, such as total cationic charge per milliliter of solution and the pH 3722 is known, buffered, and/or controlled.

Still referring to Figure 47, in a step, the tuned polymer 210 and/or the tuned polymer 210 in a formulation is used in a step of coating a substance 4740, such as with a mist / spray 4742 and/or in any delivery manner, such as with a wipe. Optionally and preferably, the step of coating a substance 4740 delivers

a controlled volume 4744, such as within 1, 2, 5, 10, 25, or 50 percent of 1, 2, 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, 2000, or 5000 µL. Delivery of a controlled volume 4744 is optionally and preferably used to deliver a total charge 4732, such as a total cationic charge per delivery dose.

Still referring to Figure 47, in a step, the tuned polymer 210 is used to form a film 4750, such as through the coating a substance 4740 step. The step of forming the film 4750 optionally delivers a solvent with a known evaporation rate 4752 and/or a known evaporation percentage 4754 as a function of time and/or temperature to form a film with a film state 4760, such as a charged density 4762, such as a total cationic charge per gram or liter, which is often expressed in total cationic charge / mg and/or total cationic charge / µL. The formed film state 4760 optionally and preferably has a film durability 4764, such as meeting any of the above described durability measures.

Delivery

The tuned polymer 210 is optionally delivered in any manner that: (1) delivers a known volume, total charge, and/or total cationic charge and/or (2) covers a surface or object over a given surface area, such in a delivery manner and/or delivery volume that delivers any of the above described parameters per sq. inch and/or per cubic inch. For clarity of presentation and without loss of generality, examples of delivery methods are provided here.

Example I

Referring now to Figure 48(A-C), a first treatment method is illustrated. Referring still to Figure 48A, a spray bottle 4800 is illustrated for delivery of the tuned polymer 210, such as in a formulation solution. The spray bottle 4800 includes a container 4720. Contents 4820 of the container 4720 include at least a solvent 4730 and the tuned cationic polymer 302, which is optionally suspended and/or dissolved in the solvent 4730. Contents 4820 optionally include any number of formulation components, such as a buffer, scent, an

inactive ingredient, an emulsifier, a stabilizer, a thickener, and/or an antioxidant. Optionally and preferably any formulation described herein contains more than one antibacterial and/or antiviral component, such as via inclusion of a quaternar4y amine and/or any form of a polyDADMAC. Generally, any dispenser 4840 is used to dispense the contents 4820 of the container 4720, such as into the air and/or onto a substrate as a spray / mist / liquid / foam. Common dispensers 4840 include trigger spray dispensers, pump spray dispensers, aerosol spray dispensers, airless spray dispensers, and/or foam spray dispensers. As illustrated, the dispenser 4840 includes a nozzle 4844 and a trigger pump 4842. Referring now to Figure 48B, the container 4720 is pressurized, such as with a compressed gas 4740 / expansion fluid, and is operated with a spray nozzle 4840. Referring now to Figure 48C, the dispenser is a wipe 4860, which dispenses the tuned cationic polymer 302 onto a surface / substrate. The wipe 4860 optionally and preferably contains the solvent 4730, which optionally and preferably rapidly evaporates after application to the surface, such as greater than 50, 75, or 90 percent evaporation in less than 60, 30, 20, or 10 seconds.

Still referring to Figure 48A and Figure 48B, optionally and preferably the charge density, referring to the total cationic charge, of the contents 4820 is 0.001 to 0.999 meq/g. An equivalent (eq) is the amount of a substance needed to react with or supply one mole of hydrogen ions (H^+) in an acid–base reaction. An equivalent, represent as eq or Eq, is the amount of a substance that reacts with (or is equivalent to) an arbitrary amount (typically one mole) of another substance in a given chemical reaction, such as 1 eq = 1 mol. Optionally and preferably, the total cationic charge of the contents 4820 is greater than 0.001, 0.005, 0.01, 0.02, or 0.03 meq/g or meq/mL and less than 100, 50, 25, 10, 7, 5, 3, 1, 0.9, 0.8, 0.5, 0.2, 0.1, 0.07, or 0.05 meq/g or meq/mL. For reference, Lysol™ (Parsippany, New Jersey, United States) has a charge of 0.003 meq/mL, but the charges in Lysol™ are from toxic quaternary ammonium molecules as opposed to the relatively non-toxic polymers described herein.

Still referring to Figure 48A and Figure 48B, optionally and preferably, a charge, referring to the total cationic charge, of the contents 4820 is controlled per application, dose, delivery time, and/or delivery volume. A preferable dose of total cationic charge of the contents is optionally and preferably within 10, 20, 50, or 100 percent of 1.45 C/mL or 1.45 C/g. Optionally, the total cationic charge of the contents per dose is greater than 0.05, 0.1, 0.5, or 1 C/mL or C/g and less than 50, 20, 10, 5, 4, 3, or 2 C/mL or C/g.

Example II

Referring now to Figure 49, a second treatment method 4900 is illustrated. Generally, a substrate is treated 4910 with a method, such as coating 4912, spraying 4914, dipping 4916, and/or electrocoating 4918, to form a film 4750, such as a liquid film 4751, which optionally at least partially evaporates to form a semi-solid film 4753 and/or a solid film 4755. Herein, a semi-solid film has properties of both solids and liquids, such as viscosity and the ability to flow under stress while still maintaining some structural integrity. A semi-solid film offers a versatile and adaptable material for a wide range of applications where a balance between the properties of liquids and solids is desired.

Example III

Referring now to Figure 50A and Figure 50B, a third treatment method 5000 is illustrated where a liquid film 4751 and a solid film 4755, respectively, are formed on a substrate 5010. Herein, a substrate is optionally and preferably any material or surface upon which the tuned cationic polymer 302 is applied or deposited. Figures 50C and 50D illustrate an adhesion promoter 5020 interspersed in the film 4750, such as the liquid 4751 and/or solid films 7542, and between the film 4750 and the substrate 5010 respectively. In either case, the adhesion promoter 5020 is not a separate glue layer; rather, the adhesion promoter 5020 is added to the solution/formulation and aids bonding to the substrate 5010 and/or physically and/or chemically at least partially separates from the solution/formulation, such as to form a bonding layer as illustrated in Figure 50D. An example of an adhesion promoter 5020 is polyDADMAC 554.

Optional and preferred concentrations of the polyDADMAC 554 / adhesion promoter 5020 are percentages of a concentration of the charged polymer, such as the polyethylenimine 552. Optionally and preferably the adhesion promoter concentration, such as by mass or parts per million, is greater than 5, 10, 20, 25, or 30 percent that of the charged polymer and/or less than 150, 100, 75, or 50 percent that of the charged polymer. The adhesion promoter 5020 in a solution and/or a formulation is further described *infra*.

Film

As described herein, without wishing to be bound to any particular theory, one theory is cationic charges of the polymer 200 bind to, penetrate into, and/or disrupt function of a bacteria, a virus, and/or a fungus. Again, herein, a total cationic charge is a charge of all of the positively charged groups, which does not include anionic charge from any counterion and/or any anion on the polymer. Indeed, herein, a total cationic charge of the formulation and/or a total cationic charge of a film is a charge of all of the positively charged groups in the formulation, such as in the contents 4820 of the container or the total cationic charge of the film, such as the solid film 4755, which does not include anionic charges, such as from any counterion and/or any anions in the contents in the delivery container 4720.

Through tuning of the polymer, such as in terms of molecular weight and percent protonation and through control of a delivery volume of the polymer, a tuned total cationic charge is delivered to a surface, such as to a unit area of a surface and/or in a volume. For example, once the contents 4820 of the container 4720 including at least a solvent 4730 and the tuned cationic polymer 302 dries to form a film and/or a solid film, the total cationic charge of the film per square inch is in the range of 0.001 C/sq. in. to 20 C/sq. in. and is optionally and preferably greater than 0.001, 0.003, or 0.1 C/sq. in. and less than 10, 5, 4, 3, 2, or 1 C/sq. in, which optionally and preferably includes residual cationic charges in the solvent and/or additional elements in the contents 4820 of the container 4720, such as protons, cationic metals, Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, iron

cations, ammonium ions, and/or cations. Exemplary effective doses of polyethylenimine (LPEI) and/or polyDADMAC are provided in Table 3, where the effective doses pass one or more of the government tests described *supra*.

Table 3: Total Cationic Film Charge

	Concentration (ppm)	Ionization (Percent)	Volume (µL)	Film Charge (C/sq. in.)
Polyethylenimine	4000	100	1000	4.80
Polyethylenimine	4000	100	300	1.44
Polyethylenimine	2000	30	150	0.16
Polyethylenimine	1000	30	50	0.003
PolyDADMAC	4000	100	1000	2.39
PolyDADMAC	4000	100	300	0.72
PolyDADMAC	2000	100	150	0.18
PolyDADMAC	1000	100	50	0.03
LPEI and PolyDADMAC	4000 each	30 / 100	300	3.21
LPEI and PolyDADMAC	4000 each	30 / 100	300	2.16
LPEI and PolyDADMAC	2000 each	30 / 100	300	0.34
LPEI and PolyDADMAC	1000 each	30 / 100	50	0.03

Optionally and preferably, a total cationic charge used in a biocide treatment, such as in a unit of liquid dispensed and/or in a film formed is greater than 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, or 1.8 coulombs and/or is less than 100, 75, 50, 25, 10, 5, 4, 3, 2, or 1 coulombs, such as where a unit of fluid delivered is within 10, 25, or 50% of any of 0.1, 0.2, 0.5, 1, 2, 3, 5, or 10 mL. The cationic charge in the film from the solvent used to form the film and/or from a salt present in the film is optionally greater or less than 0, 0.01, 0.03, 0.05, 0.1, 0.5, 1, 2, or 5 coulombs per square inch.

PEI Production

Referring now to Figures 51-55, generation of polyethylenimine 250 is described and generation of formulations containing polyethylenimine 250 are described.

Referring now to Figure 51, a general process 5100 of forming polyethylenimine 5110; purifying polyethylenimine 5120; tuning polyethylenimine 5130; generating a formulation 5140, such as containing polyethylenimine and/or any quaternary amine; and forming a film 4750, such as containing polyethylenimine is illustrated. Many parameters affect properties of the formulation and/or the film containing polyethylenimine and/or a quaternary amine, including temperature control 3718, pressure control 3720, and/or humidity control 3721. Additional parameters affecting efficacy of formulations described herein are further described *infra*.

Referring now to Figure 52, a production process 5200 of forming a poly(2-alkyl-2-oxazoline) is illustrated. Generally, an oxazoline 5210, is used as a starting material, where oxazoline is a five membered ring with the formula C_3H_5NO. Oxazolines 5220, with an emphasis on the plural, are a family of compounds. Herein, 2-alkyl-2-oxazoline 5230 refers to oxazoline 5210 with an alkyl group, such as attached to a carbon between the nitrogen and the oxygen in oxazoline 5210. For instance, the alkyl group is a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, hexyl, heptyl, octyl, nonyl, phenyl, or benzyl group, to name but a few potential alkyl groups. Generally, alkyl group contains 1, 2, or more than 2, 3, 4, 5, or 6 carbons. Generally, an oxazoline with an attached alkyl group, such as 2-alkyl-2-oxazoline, is subjected to ring opening polymerization 5240 to form a polymer, particularly a poly(2-alkyl-2-oxazoline) 5250. This process is further illustrated in Figure 53A. The acyl group is optionally removed from any 1 or more monomers to form deacylated monomers and/or protonated and deacylated monomers 5280, such as with reaction with an acid, as further described *infra*. Optionally any amount of a 2-alkyl-2-oxazoline is added to and/or maintained in any formulation described herein.

Referring now to Figure 53B, an acylated monomer 5260 of the poly(2-ethyl-2-oxazoline) 5252 and/or the poly(2-alkyl-2-oxazoline) 5250 is optionally

deacylated to form a second monomer type, where the second monomer type is optionally protonated, thus forming a first copolymer 5290. For instance, a first monomer, the acylated monomer 5260 with a -N-CH_2-CH_2- backbone, is optionally deacylated to yield a second monomer with a -N-CH_2-CH_2- backbone that is deacylated and deprotonated 5270. Optionally, the second monomer is protonated to form a deacylated and protonated monomer 5280 with a -NH^+-CH_2-CH_2- backbone, with the nitrogen being protonated. Herein, the first copolymer 5290 has repeating units of: (1) the acylated monomer 5260 with a -N-CH_2-CH_2- backbone and (2) the deacylated and protonated monomer 5280 with the -NH^+-CH_2-CH_2- backbone, albeit where any number of the deacylated and protonated monomers 5280 are optionally unprotonated to form the deacylated and deprotonated -N-CH_2-CH_2- backbone, such as at higher pH values. The two monomer types are optionally arranged in any order and with any repeating lengths in the copolymer. Optionally, any ratio of the first monomer-to-the second monomer is used in an antipathogen formulation. However, a preferred ratio of the deacylated and protonated monomer 5280 with the -NH^+-CH_2-CH_2- backbone-to-the acylated monomer 5260 with a -N-CH_2-CH_2- backbone exceeds 0.1, 0.25, 0.5, 1, or 2-to-1. The inventors recognize that the three monomers in the polymer are optionally referred to as a tripolymer; however, the inventors further recognize that one skilled in the art would recognize that the second monomer of the copolymer is simply in different equilibrium forms dependent upon pH. Thus, for clarity of presentation the copolymer terminology is used herein.

Referring again the Figure 53B and referring now to Figure 54, in a case where the acylated monomers 5260 are fully converted into deacylated monomers that are unprotonated 5270 and protonated 5280 in various ratios, the first copolymer 5290 is fully converted to polyethylenimine, in various states of protonation, as described herein.

Referring again to Figure 53A, five oxazolines 5220, illustrative of many possible oxazolines 5220, are illustrated: a 2-methyl-2-oxazoline 5221 (MeOx),

a 2-ethyl-2-oxazoline 5222 (EtOx), a 2-propyl-2-oxazoline 5223 (PropOx), a 2-isopropyl-2-oxazoline 5224 (i-propOx), and a 2-phenyl-2-oxazoline (PhOx). Generally, a cationic ring-opening polymerization (CROP) is used to generate a poly(2-alkyl-2-oxazoline) 5250. Generally, a CROP reaction proceeds through an S_N1 or S_N2 propagation / polymer chain growth process, such as where a terminus of a polymer chain attacks cyclic monomers to form a longer polymer. In the case of subjecting the cationic ring-opening polymerization to 2-ethyl-2-oxazoline, a poly(2-ethyl-2-oxazoline) 5252 is formed. The poly(2-ethyl-2-oxazoline) 5252 is further described in Figure 54.

Referring again to Figure 53A, an acyl group 5251 is illustrated, such as bound to a monomer of the poly(2-alkyl-2-oxazoline) 5250. An acyl group 5251 contains a double-bonded oxygen atom and an organyl group (R-C=O), which is also written as -C(=O)-R, such as where the free bond on the carbon is covalently linked to a nitrogen in monomer(s) of the poly(2-alkyl-2-oxazoline) 5250. As illustrated, the R group of the acyl group 5251 is optionally any hydrocarbon chain 5253, such as the illustrated ethyl group. However, more generally the hydrocarbon chain is any chain of carbons with at least some hydrogens attached to the chain of carbons, such as where an alkyl group or hydrocarbon chain is a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, hexyl, heptyl, octyl, nonyl, phenyl, or benzyl group. An acyl group with a ethyl hydrocarbon chain 5261 attached to a nitrogen of a monomer of the polymer is illustrated in Figure 53B. In Figure 53B, the ethyl hydrocarbon chain 5261 is an example of an alkyl / alkyl group. In chemistry, an alkyl group is a carbon chain missing one hydrogen, where in chemistry the alkyl term is intentionally unspecific to allow for many possible substitutions.

Referring now to Figure 54A, cleaving an acyl group in a deacylation process 5400 from a poly(2-alkyl-2-oxazoline) 5250 is illustrated. Generally, an acyl group 5251, also referred to herein as a pendant, is bound to the nitrogen in the monomer. An acyl group 5251 is a moiety derived by the removal of at least one hydroxyl group from an oxoacid. An acyl group 5251 contains a double-

bonded oxygen atom and an organyl group (R-C=O). As illustrated, one or more monomers of the poly(2-alkyl-2-oxazoline) are deacylated and/or deacylated and protonated and/or a polyalkylenimine is generated, such as in an at least partially protonated state.

Still referring to Figure 54A, the poly(2-alkyl-2-oxazoline) is optionally used to form a copolymer. For example, the poly(2-alkyl-2-oxazoline) contains a first monomer with an acylated monomers comprising a $-COR_1$ group attached to a nitrogen of a $-N-CH_2-CH_2-$ element, wherein R_1 comprises an alkyl group. When the poly(2-alkyl-2-oxazoline) is reacted with an acid, such as a strong acid or a concentrated hydrochloric acid, the poly(2-alkyl-2-oxazoline) forms at least some second monomers. The second monomers are unprotonated and/or are protonated monomers comprising a $-NH_2^+-CH_2-CH_2-$ group. Generally, the acylated monomers and the protonated monomers comprising any order in the copolymer. If the reaction is run to completion, the poly(2-alkyl-2-oxazoline) is fully deacylated and a polyalkylenimine is formed, such as in a protonated state, as further described *infra*.

Still referring to Figure 54A, an acid, H+, and water are reacted with the poly(2-alkyl-2-oxazoline) 5250 to form a protonated polyalkylenimine 5410 and a carboxylic acid 5420. Herein, for clarity of presentation and without loss of generality, hydrochloric acid 632 is used as the acid. However, any acid, and optionally and preferably any strong acid is used in conjunction with or in place of the hydrochloric acid 632. Notably, use of another acid will substitute another counterion for chloride. For instance, if sulfuric acid, H_2SO_4, is used in place of the hydrochloric acid 632, then the counterion ion is HSO_4^- instead of Cl^-. Thus, the conjugate base of any acid, and preferably any strong acid, is the generated counterion to the protonated sites 310 of the protonated polyalkylenimine 5410 / polyalkylenimine / polyethylenimine 250. For instance, if nitric acid, HNO_3, is used as the acid in the deacylation process 5400, then the counterion is nitrate, NO_3^-; if sulfuric acid, H_2SO_4, is used as the acid, then hydrogen sulfate, HSO_4^- and/or sulfate, SO_4^{2-}, is the resulting counterion; if hydrogen sulfate, HSO_4^-, is

used as the acid, then sulfate, SO_4^{2-}, is the resulting counterion; and if phosphoric acid, H_3PO_4, is used as the acid then dihydrogen phosphate, $H_2PO_4^-$, and/or hydrogen phosphate HPO_4^{2-}, is/are the resulting counterion(s). Similarly, the acid is optionally supplied on an ion exchange material, ion exchange bead, and/or an ion exchange resin. For example, if the ion exchange resin, as described *supra*, contains a sulfonic acid group, R-SO_3H, then the counterion is hydrogen sulfate, HSO_4^- and/or sulfate, SO_4^{2-}. Essentially, ion exchange resins with sulfonic acid groups, -SO_3H, function as a strong acid catalyst to facilitate hydrolysis of the acyl group 5251 in the poly(2-alkyl-2-oxazoline) 5250 by providing protons, H^+. Naturally, differing counterions and/or differing ratio of counterion types alters antibacterial, antiviral, and/or antifungal inactivation efficacy, as described *supra*. The deacylation process results in: an at least partially deacylated poly(2-alkyl-2-oxazoline), an at least partially deacylated and an at least partially protonated/acidified poly(2-alkyl-2-oxazoline), and/or an at least partially protonated polyalkylenimine in the solution and/or a formulation derived therefrom.

Still referring to Figure 54A, the deacylation process 5400 of the poly(2-alkyl-2-oxazoline) 5250 yields a carboxylic acid 5420 in addition to the protonated polyalkylenimine 5410. The inventors have determined that the yield of the protonated polyalkylenimine 5410 depends on not only temperature, acid concentration, reaction time, and pressure, but also on the removal of the carboxylic acid group 5420 from the reactor, which effectively drives the deacylation process 5400 forward, such as according to LeChatelier's Principle. Thus, optional and preferable removal of at least 0.01, 0.02. 0.05, 0.1, 0.25. 1, 2, or 5 percent and preferably greater than 50, 75, 80, 90, 95, 99, or 99.9 percent of the carboxylic acid group 5420 from the reactor, such as through evaporation out of the container, is preferred, preferably while the poly(2-alkyl-2-oxazoline) 5250 is still in contact with the acid / strong acid. Further, the inventors have determined that the amount of the carboxylic acid 5420 in a final formulation affects the inactivation efficacy of the antibacterial, antiviral, and/or antifungal formulation.

Referring now to Figure 54B, an exemplary case of reacting poly(2-ethyl-2-oxazoline) 5252 with acid and water to form a protonated polyethylenimine 251 and propanoic acid 5422, a carboxylic acid 5420, is illustrated. Removal of at least 0.01, 0.02. 0.05, 0.1, 0.25. 1, 2, or 5 percent and preferably greater than 50, 75, 80, 90, 95, 99, or 99.9 percent of the carboxylic acid group 5420 from the reactor and/or from a final formulation is preferred as the propanoic acid and propanoate formed therefrom at preferred formulation pH's in the range of 2.5 to 8 and/or within 0.25 or 0.5 pH units from a pH of 4, 5, or 6 is preferred. Optionally, propanoate is used as a counterion to the protonated sites 310 of the polyethylenimine 250 at any concentration, such as greater than 0, 1, 2, 5, or 10 percent and/or less than 100, 99.9, 99, 98, 95, or 90 percent of all counterions in a final formulation. The propanoic acid / propanoate exchange further functions as a buffer to pH change in this case.

Referring now to Figure 55, formula preparation 5500, such as with polyethylenimine 250, is described. Polyethylenimine is formed 5110 chemically through the reaction of poly(2-ethyl-2-oxazoline) with an acid, such as hydrochloric acid 632, and water 5512, as described *supra*. The inventors have determined that the yield of the resulting protonated polyethylenimine 251 is dependent upon reaction conditions, such as heat 3719. Optionally and preferably, a reaction chamber / reactor, containing the reactants, is maintained at greater then 80, 90, 100, 110, 120, or 130 °C for greater than 1, 2, 4, 7, 8, 15, 16, 23, or 24 hours. Maintaining the reactor at an increased pressure 3721, such as at greater than 1, 1.1, 2, 2.1, 2.2, 2.5, 3, 3.5, 3.9, or 4 bar is preferred, which results in effectively higher temperatures, such as greater than 131, 135, 140, or 145 °C. The reaction removes the pendant 5120. After an optional reaction time at elevated temperature, the reactor is optionally and preferably opened up to atmosphere, which reduces the pressure 3723. Maintaining the reactor at an elevated temperature, such as at greater than 50, 70, 80, 90, 100, 110, 120, or 130 °C for greater than 1, 2, 4, 7, 8, 15, 16, 23, or 24 hours drives off the propionic acid / propanoic acid 5422 / carboxylic acid 5420, which pulls the reaction forward and allows more of the poly(2-ethyl-2-oxazoline) to react

with the acid to form more of the protonated polyethylenimine. By maintaining, the now vented reactor, at an elevated temperature, the poly(2-ethyl-2-oxazoline) is deacylated and the pendant is removed 5120 from the poly(2-ethyl-2-oxazoline), at greater then 90, 95, 98, 99, 99.5, 99.9, or 99.99 percent and/or the reaction is driven forward to greater than 95, 99, 99.9, or 99.99 percent yield. Opening the reactor to atmospheric pressure and maintaining the reactor at elevated temperatures also functions to vent the hydrochloric acid 633, such as into a filter or acid trap, which reduces the amount of acid in the reactor by greater than 0, 0.1, 0.5, 1, 2, 3, 5, or 10 percent. The removal of hydrochloric acid and/or the propanoic acid 5422 functions to purify the generated polyethylenimine 250. Each of the elevated temperature, elevated pressure, and carboxylic acid removal processes are observed to alter inactivation properties of formulations using the polyethylenimine 250, which might be due to unfolding of the polyethylenimine 250 and/or effective activity of protonated sites on the polyethylenimine 250. Further purification of the polyethylenimine 5130 is described *infra*.

Still referring to Figure 55, steps of purifying the polyethylenimine 5130 are further described. As described, *supra*, reducing the pressure 3723 and maintaining the reactor at an elevated temperature drives off the pendant 5120 from the reactor. Optionally and preferably, the supernatant 5134 is separated from the precipitate 5132, such as the polyethylenimine precipitates. This reduces the hydrochloric acid concentration by an addition amount, such as a further greater than 1, 5, 10, 25, 50, or 75 percent. Further, temperature control 5136 is used to evaporate water and/or the water concentration is reduced 5513 through a drying step. The drying step also further reduces the hydrochloric acid 635 by greater than 1, 2, 5, 10, 25, 50, 75, 90, 95, or 99 percent. Controlling humidity 5138, such as to less than 90, 75, 50, 25, or 10 percent further aids the removal of water 5513 and also is optionally used to still further remove the hydrochloric acid 635, which is attracted to any remaining water. Optionally venting, a reduction in pressure, stirring, and/or rinsing the precipitate is used to still further reduce the hydrochloric acid content to less than 25, 10, 5, 2, 1, 0.1, or 0.01 percent by mass. The reduction of the chloride

counterion concentration and/or the chloride counterion concentration is observed to alter the antibacterial / antiviral efficacy of formulations generated using the purified polyethylenimine. Similarly, introduction of antibacterial/antiviral counterions is observed to alter the antibacterial / antiviral efficacy of formulations generated using the purified polyethylenimine.

Still referring to Figure 55, once the polyethylenimine is purified, such as to a solid with any percentage of propanoic acid 5422 by weight remaining, the polyethylenimine is optionally and preferably tuned 5140, as described *supra*. A formulation is prepared 5150, as further described *supra*, and optionally a film is formed 4750, such as by spraying and drying.

Referring now to Figure 56, processing the polyethylenimine 5600 is described. Generally, the polyethylenimine is purified 5120 and tuned 5130, as described *supra*. A formulation 5140 is subsequently prepared. Generally, optionally and preferably the formulation 5140 is prepared by mixing any amount of the polyethylenimine 250 with any solvent 5142, such as ethanol, any amount of polyDADMAC 554, any amount of any quaternary amine 5150 / quaternary ammoniums, any acid(s) 5160, any buffer, and/or any amount of formate 324, acetate 326, $citrate^-$ and $citrate^{2-}$ 327, chloride 322, iodide 325, bromide, nitrate, sulfate, nitrite, hydrogen carbonate, hydroxide ion, sulfide, and/or S^{2-}, any of which are optionally used as counterions and/or in a control of counterions 5162, such as type, to any positively charged species in the final formulation. Generally, any anion is present at a mass concentration of greater than 0, 0.01, 0.03, 0.05, 0.1, 1, or 5 percent. Generally the ratio of a first ion to a second ion is greater than 1:1, 1:0.5, 1:0.1, or 1:0.01; the ratio of the first ion and/or the second ion to a third ion is greater than 1:1, 1:0.5, 1:0.1, or 1:0.01; the ratio of the third ion to a fourth ion is greater than 1:1, 1:0.5, 1:0.1, or 1:0.01; the ratio of the third ion and/or a fourth ion to a fifth ion is greater than 1:1, 1:0.5, 1:0.1, or 1:0.01. The pH 3722 of the formulation is optionally and preferably set to a pH greater than 2, 3, 4, or 5; to a pH less than 14, 12, 10, 8, 7, or 6; in a pH range of 2-10, 3-9, 3-8, 3-7, and/or 4-6; and/or to a pH within any of ± 0.25, 0.5,

0.75, 1.0, or 2 of any of 3, 4, 5, 6, or 7. Further, the salt content 5170 affects the ability of the charges on the polyethylenimine 250 to interact with surfaces of the bacteria and/or virus. Generally, the salt content 5170 / salt concentration is optionally and preferably set to greater than 0, 1, 2, 5, 10, 50, or 100 ppm and/or to less than 3000, 2500, 2000, 1000, 750, 500, 250, 100, 50, 40, 30, or 20 ppm, which further tunes efficacy of the formulation antibacterial / antiviral effectiveness. Optionally and preferably, denaturant components of the ethanol, such as methyl ethyl ketone, acetone, and/or methanol, are left in the formulation at concentrations of greater than 1, 2, 5, 10, 50, or 100 ppm. However, the denaturants are optionally removed from the formulation. A surfactant is optionally provided in the formulation. Examples of a surfactant include Tween 20, a polyethylene glycol sorbitan monolaurate, and/or a non-ionic detergent. The formulation 5140 is optionally used directly, such as in a spray, and/or is used to form a film 4750. The film 4750 is further described *infra*.

Film

A film 4750 is optionally formed from the formulation, such as by drying. The film 4750 of preferred formulations have properties, described *infra*. Generally, the formulation / formulation of the solution prior to drying comprises: a polyethylenimine concentration of 0 ppm; greater than 10, 50, 100, 1000, 2000, 3000, 4000, 5000, 7500, or 10,000 ppm; and/or less than 15,000, 10,000, 7,500, 5500, 4500, or 3500 ppm; a polyDADMAC concentration and or a concentration of any one or more quaternary ammonium compounds of 0 ppm; greater than 10, 50, 100, 1000, 2000, 3000, 4000, 5000, 7500, or 10,000 ppm; and/or less than 15,000, 10,000, 7,500, 5500, 4500, or 3500 ppm; an acid / conjugate base pair with a concentration of 0 ppm; greater than 1, 5, 10, 50, 100, 200, 300, 400, 500, 750, or 1000 ppm; and/or less than 5,000, 2500, 1000, 500, 300, or 200 ppm, where the acid / conjugate base pair is optionally any one or more of the acids named herein; and/or a counterion or set of counterions, a pH, a buffer, a salt type or salt types, a salt concentration, a denaturant, carboxylates, and/or a buffer as described herein, such as for the polyethylenimine 250 and/or for the formulation 5140. Film properties are tuned

for safety, efficacy, durability, additive killing, and/or longevity of killing using particular formulations in the ranges set forth herein.

Film Properties

Referring now to Figure 57, an optional and preferred film property is additive killing 5700. Herein, additive killing refers to treating a surface and killing / inactivating a percent of an original dose of bacteria, virus, and/or fungus present on the surface or applied to the film 4750 at a single point in time. In Figure 57, a representative formulation of 4000 ppm polyethylenimine 250, 2000 ppm polyDADMAC 554, 3600 ppm formic acid in balance with formate at a pH of 4.0, and 500 ppm hydrochloric acid is used to kill / inactivate greater than 1, 5, 9, 10, 50, or 99 percent of the bacteria Staphylococcus aureus and/or the germ Pseudomonas aeruginosa in 10 minutes or less and greater than 99.9999 percent of the Staphylococcus aureus and/or the Pseudomonas aeruginosa in 100 minutes or less. Generally, various formulations, as described herein, using at least the polyethylenimine 250 as prepared using a methodology as described herein are used to reduce activity of the a bacteria contacting the film by: greater than 1, 5, 9, 10 or 20% and less than 99.99995% in less than one minute; greater than 99.999% in less than four days; and greater than 99.99995% in less than seven days.

The additive killing, longevity of killing, and/or the durability of killing of bacteria, viruses, and/or fungi is optionally and preferably performed in any order and/or simultaneously. For instance, a film is applied to a surface, the bacteria and virus are both treated simultaneously. Similarly, if a film is applied to a surface and bacteria subsequently are applied to the surface and inactivated, then viruses co-placed with the bacteria on the film are treated at the same time as the bacteria. Similarly, if a virus/bacteria is placed on a film, subsequent placement of second or later batched of virus/bacteria are later treated by the film, such as when newly introduced virus/bacteria make contact with the film.

Formulations

Referring now to Figure 58, preparation / use of a formulation 5800 is described. Generally, a charged polymer is provided 5810, such as in a solvent and/or a solvent is added 5820 to the charged polymer and/or to the formulation 5140 in step(s) of generating a formulation 5830. Optionally and preferably, one or more buffers are added 5840 to the formulation 5840 at any concentration. Optionally and preferably, one or more salts are added 5850 to the formulation 5140 and/or are removed from the formulation 5140, such as to tune the conductivity and/or activity coefficient, which aids inactivation/killing properties of the formulation 5140 against pathogen(s). Optionally and preferably, one or more acids and/or bases are added 5860 to the formulation 5140. Optionally and preferably, one or more adhesion promoters 5870 are added to the formulation, which, in combination with the above steps, alters the critical load of adhesion of the formulation 5140 in a dried film state to the substrate 5010. Generally, the formulation is formulated to contain a desired total cationic charge and/or a total anionic charge per mL, per dose, or per volume, where the total cationic charge and/or the total anionic charge is offset by counterions in the formulation. The types of charge sources affects inactivation of pathogens performance as described throughout. Generally, the formulation 5140 is sprayed 5880 and/or wiped on a surface to form a film 5890, such as a liquid film and/or a semi-solid / solid film after drying as described herein. Silanes and polyDADMAC are examples of adhesion promoters, where polyDADMAC has an additional advantage of being an antipathogen in the formulation.

Adhesion

Referring now to Figure 59, adhesion 5900 is illustrated, such as between a dried film containing the polyethylenimine / charged polymer and the substrate 5010. In the illustrated example, a 4000 ppm polyethylenimine in a solvent of 25% water and 75% ethanol with a 2000 ppm polyDADMAC adhesion promoter is illustrated, where the formulation also included 100 ppm citric acid, which provides an antipathogen counterion as well as a pH buffer. Again, the charged polyDADMAC additionally functions as an antipathogen. The adhesion or critical load of adhesion to a glass substrate was 180, 120, and 80 μN at a pH

of 4, 5, and 6, respectively. Generally, the achieved, optional, and/or preferred adhesions of the dried formulation on a substrate are greater than 10, 20, 30, 50, 75, 100, or 125 and/or less than 1000, 600, 500, 400, 300, 250, 200, or 150 μN.

Generally, critical load of adhesion or simply adhesion refers to the minimum applied load at which a coating or thin film begins to detach or delaminate from its substrate, signifying the point where the adhesive forces between the two materials are overcome and failure occurs. A critical load of adhesion is typically measured using a scratch test method, as was done to obtain the illustrative values in Figure 59 for the illustrative specified formulation, where the load is progressively increased until a visible sign of detachment is observed. The critical load is considered a measure of the adhesion strength between the coating and substrate. The critical load of adhesion is typically measured in units of force (Newtons, N) as it represents the maximum load that can be applied to an adhesive joint before it fails, and is usually expressed as force per unit area (Pascals, Pa) when considering the adhesion strength of a material. Force, stress, scratch test results, coating thickness, substrate, scratch stylus form, and surface roughness are all optionally and preferably considered/included in determination of the adhesion strength.

For clarity of presentation and without loss of generality, multiple examples of film properties of formulations, as described herein, are provided here.

Example I

In a first example, additive killing / inactivation of less than 99.99% of bacteria/virus in 10 minutes and greater than 99.999% within 100 minutes uses a film incorporating at least one quaternary ammonium, such as polyDADMAC at a concentration of 500 ppm, and a total positive charge of at least 10, 50, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 10000, and/or 15,000 C/in^3, where the total positive charge is the charge of all of the positive sites in the formulation, which are offset by anionic counterions in the formulation. In

one embodiment, the film is formed on one square inch of substrate with greater than 100, 200, 299, 300, 400, or 500 µL of polyethylenimine at a concentration of with 500 ppm or 3000, 4000, or 5000 ppm or of at least 500, 1000, or 2000 ppm. It is found that the polyDADMAC aids bacterial / viral inactivation as the polyDADMAC with or without a surfactant helps the formulation adhere to a substrate, such as metal or glass.

Example II

In a second example, the number/density of charged sites / protonated sites is set at a high enough level to inactivate the bacteria/virus. Without wishing to be held to any one theory, the charged sites of the film electrostatically bind to and/or block corresponding oppositely charged sites of the bacteria / virus. It is found that a film with greater than 1×10^5 and preferably greater than 5×10^{10} or 5×10^{15} protonated sites/in^2 functions to inactivate / block at least 99.999% of bacteria within 1 hour. The protonated sites, such as a protonated secondary amine of polyethylenimine and/or a protonated site of a quaternary amine, such as a quaternary ammonium and/or polyDADMAC each have corresponding negatively charged counterions. The inactivation of the bacteria is observed to be effective against a second, third, fourth or greater number of sequential introductions of the bacteria at intervals in excess of 1, 12, 24, or 72 hours.

Example III

In a third example, during the deacylation step of a starting material for polyethylenimine, such as poly(2-ethyl-2-oxazoline), efficacy of a final formulation is altered / improved by driving out of the formulation the released carboxylic acid, such as removal of greater than 1, 2, 5, 10, 25, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.9 percent and/or less than 100, 99, 98, 97, 95, or 90 percent of the carboxylic acid. Removal of the carboxylic acid is optionally performed through use of elevation temperature and pressure in an acidic environment, which has the additional benefit of driving the deacylation process toward/to completion.

Example IV

In a fourth example, longevity of inactivation of bacteria/virus is demonstrated with formulations as described herein, especially those with adherence properties of the film to a substrate with greater than 1, 5, 10, 25, or 50 μN. Adherence is aided through use of low concentrations of polyDADMAC, such as greater than 10, 20, or 50 ppm and less than 2000, 1000, 500, 250, or 100 ppm in combination with polyethylenimine at concentrations greater than 500 or 1000 ppm. Presence of some of the non-chloride counterions also aids the longevity of inactivation, such as to bacteria / virus introduced to a film resulting from the formulation at periods greater than 1, 2, 3, 4, 7, 14, 21, or 28 days after film formation.

Example V

In a fifth example, it is determined that coating a garment with greater than 1 and preferably greater than 2, 5, 10, 20, or 50 milligrams of the formulation, such as including at least polyethylenimine, such as with the adherence properties described *supra*, function to inactivate current and/or newly introduced bacteria, as described herein.

Example VI

In a sixth example, formulations resulting in dried film thickness in a range of 0.2 to 20 μm range, for greater than 95% of the film coverage are preferable, especially when coupled with a charge density, from a calculation of only the protonated sites of the film/formulation, of greater than 200, 400, or 600 C/in^3 and/or less than 7000, 14000, or 25000 C/in^3.

Example VII

In a seventh example, formulations, as described herein, that show persistence of inactivation, such as greater than 99.9% inactivation, after rubbing or cleaning the surface are preferred, such as with the EPA 01-1A protocol. Formulations particularly effective at persistence of inactivation include those

with polyDADMAC at a pH of 4 or 4.5 ± 0.25 or 0.5 pH units and/or those with a surfactant.

Example VIII

In an eighth example, a method for treating a pathogen, comprising the steps of: (1) reacting, in a solution, poly(2-alkyl-2-oxazoline) with a strong acid to form a polyethylenimine and a carboxylic acid, the polyethylenimine comprising protonated sites; (2) increasing relative concentration of the polyethylenimine through removal of at least 95% of the carboxylic acid from the solution; (3) adjusting pH of the solution to greater than 2.5; (4) producing a film on a substrate from the solution; (5) contacting first bacteria with the film; and (6) reducing activity of the first bacteria contacting the film by: greater than 9% and less than 99.99995% in less than one minute; greater than 99.999% in less than four days; and greater than 99.99995% in less than seven days.

Example IX

In a ninth example, a method for treating a pathogen, comprising the steps of: (1) removing, with an acid, less than 99% of -COR_1 acyl groups from nitrogen sites of a poly(2-alkyl-2-oxazoline) in a solution to form a partially protonated and partially acylated poly(2-alkyl-2-oxazoline), where R_1 comprises an alkyl group; (2) adjusting a pH of the solution to greater than 2.5; and (3) optionally forming a film from the solution with any of the properties described herein, such as charge density, additive killing properties, and longevity of killing properties, with or without any additives as described herein, such as a quaternary ammonium salt, a surfactant, and/or an organic acid.

Example X

In a tenth example, a partially protonated and partially acylated poly(2-alkyl-2-oxazoline) polymer, such as a poly(2-etyhl-2-oxazoline) is used with or without a polyethylenimine or related polyalkylenimine. Further, any of the reactions /

procedures described herein, such as counterion control, is optionally performed for individual stock solutions (stocks) for one or the other of the partially protonated and partially acylated poly(2-alkyl-2-oxazoline) polymer or the polyethylenimine / polyalkylenimine.

Example XI

In an eleventh example, addition of a polyDADMAC salt to a partially protonated polyethylenimine is observed to increase a thickness of a resulting film. For instance, adding 500, 1500, or 2000 ppm of the polyDADMAC salt to a solution used to form a film also using 5000 ppm polyethylenimine with a polyethylene glycol sorbitan monolaurate surfactant increased the film thickness, for a 200 µL sample on one square inch, to greater than 200 nm, such as with an average thickness of between 200 and 800 nm. The polyDADMAC salt in combination with a surfactant also increased durability of the film, such as a resistance to losing film thickness and/or film charge when rubbed.

Example XII

In a twelfth example, addition of a polyDADMAC salt and/or a surfactant to a partially protonated polyethylenimine is observed to a static coefficient of friction, μ_s, to greater than 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2.0, or 2.2 depending on the concentrations of elements of the film.

Example XIII

In a thirteenth example, increasing a tackiness of a film of the formulations described herein is observed to increase inactivation of the film properties against bacteria and viruses. For instance, a solution of a polyDADMAC dried to yield a coefficient of friction, μ_s, of greater than 0.8, 1, 1.2, 1.4, or, 1.6 and/or to less than 4, 3.5, 3, 2.5, or 2.0 is observed to enhance antibacterial/antiviral properties in terms of longevity of action, additive killing of action, and/or effectiveness after extended periods, such as killing properties retained after greater than 1, 2, 4, 6, 12, 18, 24, 48, or 96 hours.

Example XIV

In a fourteenth example, a combination a polyethylenimine, a quaternary ammonium, and/or a surfactant yields a film, when at least partially dried that resists scratching. For instance, an application of a force of greater than 10, 20, or 30 μN and less than 200, 100, or 75 μN and preferably 50 μN yields a mean scratch depth of more than 1, 5, 10, or 100 nm and less than 3000, 2000, 1000, or 750 nm. Optionally and preferably the film is dried, such as through removal of more than 10, 25, 50, 75, or 90 percent of a solvent and optionally less than 99.9, 99.5, 99, 98, 97, 96, or 95 percent of the solvent. Optionally and preferably, the film comprises a ratio of a polyethylenimine to an adhesion promoter, such as polyDADMAC, exceeding 1:1, 2:1, or 3:1 by mass and/or by parts per million. The surfactant aids coverage of a substrate and the polyDADMAC aids adhesion to the substrate.

Example XV

In a fifteenth example, a chloride concentration of a biocide formulation is maintained at a concentration greater than 0, 100, or 200 ppm and/or less than 2000, 1500, 1000, 800, 600, 500, 400, or 300 ppm, to aid in toxicity management of the formulation and/or to meet EPA guidelines/requirements.

Example XVI

In a sixteenth example, a formulation comprising at least one of polyethylenimine, a quaternary ammonium, and/or a polydiallyldimethylammonium salt, such as with any one or more of the formulation additives described herein, is used to inactivate/kill greater than 5, 10, 25, 50, 75, 90, 95, 99, or 99.9 percent of a fungus, such as a Candida Auris within less than 1, 2, 5, 10, 30, 60, 360, or 2400 minutes of contact with the formulation.

Example XVII

In a seventeenth example, a method for treating a pathogen, comprises the steps of: polymerizing a molecule to form a polymer, the molecule comprising an alkyl in a molecule comprising a 2-alkyl-2-oxalozine other than a 2-ethyl-2-oxazoline; preparing a formulation comprising a derivative of the polymer and an organic solvent, the derivative comprising protonization of greater than ten percent of monomers of the polymer; and inactivating at least ninety percent of the pathogen within ten minutes of contact of the pathogen with the formulation.

Example XVIII

In an eighteenth example, a method for treating a pathogen, comprising the steps of: acidifying, in a solution, a molecule, the molecule comprising an alkyl, the molecule comprising a poly(2-alkyl-2-oxazoline) other than poly(2-ethyl-2-oxazaline, the step of acidifying forming an at least partially deacylated poly(2-alkyl-2-oxazoline), the at least partially deacylated poly(2-alkyl-2-oxazoline) comprising an at least partially acidified state; adding at least one organic solvent to the solution to yield a formulation; tuning, for a protonated secondary amine of the at least partially deacylated poly(2-alkyl-2-oxazoline), the formulation to comprise an activity coefficient (γ) in a range of 0.6 to 0.9991; and inactivating at least ninety percent of the pathogen within ten minutes of contact of the pathogen with the formulation.

Example XIX

In a nineteenth example, an apparatus for treating a pathogen, comprises a liquid formulation comprising: (1) a copolymer, the copolymer comprising: acylated monomers comprising a $-COR_1$ group attached to a nitrogen of a $-N-CH_2-CH_2-$ element, wherein R_1 comprises an alkyl group; and protonated monomers comprising a $-NH_2^+-CH_2-CH_2-$ group, the acylated monomers and the protonated monomers comprising any order in the copolymer; (2) a total cationic charge of the copolymer in a range of 0.4 to 15 C/cm^3, the total cationic charge at least 98% offset by anionic counterions; and (3) an antipathogen property resultant in killing at least 99% of the pathogen within ten minutes of contact of the pathogen with the liquid formulation.

Example XX

In a twentieth example, a method for treating a pathogen, comprises the steps of: forming a polyethylenimine with hydrochloric acid in a solution, the polyethylenimine comprising secondary amines, the secondary amines being at least partially protonated; adding sodium hydroxide to the solution of the polyethylenimine to increase a pH of the solution by at least one pH unit, such as to above 9; removing at least five percent of non-amine cations, such as sodium ions, in the solution from the solution; lowering the pH, subsequent to the step of removing, of the solution, such as to below 6, to yield a protonation level of the secondary amines in excess of fifty percent; and inactivating at least 50% of a bacteria within 10 minutes of contact of the bacteria with the solution.

Example XXI

In a twenty-first example, a method for treating a fungus, comprises the steps of: providing, in a formulation, a polyethylenimine at a pH of less than two, a majority of protonated sites on the polyethylenimine associated with chloride counterions; exchanging at least ten percent of the chloride counterions for hydroxide anions bound to an anion exchange material resultant in bound chloride ions on the anion exchange material; separating the anion exchange material from the solution, the step of separating resultant in at least a ten percent reduction of the chloride counterions associated with the polyethylenimine; and inactivating at least fifty percent of the fungus within ten hours through treatment of the fungus with the formulation.

Example XXII

In a twenty-second example, a method for treating a pathogen, comprises the steps of: (1) providing a polyethylenimine in a solution, the polyethylenimine comprising a total cationic charge of protonated secondary amines of the polyethylenimine, the solution comprising anionic counterions associated with the protonated secondary amines; (2) tuning a protonated site of the

polyethylenimine, in a formulation of the solution containing ions, to an activity coefficient (γ) in a range of 0.93 to 0.999; (3) forming from the formulation an antibacterial film, the antibacterial film comprising a mean thickness of less than 5 μm and the total cationic charge of the protonated secondary amines in a range of 0.1 to 1.8 C/in^2; and (4) inactivating greater than 99% of first bacteria within less than five minutes of contact with the antibacterial film.

Example XXIII

In a twenty-third example, a method for treating pathogens on a substrate, comprises the steps of: providing a solid polyethylenimine in a basic pH form comprising protonated and unprotonated nitrogens, a ratio of the protonated nitrogens-to-the unprotonated nitrogens less than 5:95; solubilizing the solid polyethylenimine in a strong acid to form a solution of a polyethylenimine, the polyethylenimine comprising protonated nitrogen sites and counterions to the protonated nitrogen sites; removing at least ten percent of the strong acid from the solution; forming a formulation from the solution, the formulation further comprising at least one of ethanol and isopropyl alcohol; spraying the formulation onto the substrate to form a film, the film reducing activity of the first bacteria contacting the film by: greater than 9% and less than 99.99995% in less than three minutes; and greater than 99.99995% in less than seven days.

Example XXIV

In a twenty-fourth example, a method for treating a substrate with a biocide comprises the steps of: combining in a formulation: a salt of a polydiallyldimethylammonium, an organic solvent, and water; controlling a concentration of the polydiallyldimethyl-ammonium to greater than 50 mg per liter in the formulation to yield a self-spreading surface tension, of the formulation, of less than 50 mN/m; limiting, in the formulation, the concentration of the polydiallyldimethylammonium to less than 1500 mg per liter to yield a sprayable liquid comprising a viscosity of less than 250 centipoise; spraying the substrate with the formulation to yield an antibacterial film, the antibacterial film comprising: (1) 15 to 500 μg per square inch of the

polydiallyldimethylammonium; and inactivating, according to a test protocol of JIS X 2801 (2006), at least 90 percent of first bacteria with the antibacterial film.

Example XXV

In a twenty-fifth example, an apparatus for coating a substrate, comprises: an antimicrobial film, comprising: a partially protonated polyethylenimine in a range of 0.1 to 5 mg per square inch; a polydiallyldimethylammonium salt; chloride counterions; first conjugate base counterions of a first organic acid, the first conjugate base counterions comprising a first mass of 0.001 to 0.1 mg per square inch; second conjugate base counterions of a second organic acid, the second conjugate base counterions comprising a second mass of 0.05 to 1 mg per square inch; and a total cationic charge of the antimicrobial film in a range of 200 to 3000 C/m^2, the total cationic charge countered by anions in the antimicrobial film, the antimicrobial film comprising an antimicrobial efficacy of greater than ninety percent according to PAS 2424:1024.

Example XXVI

In a twenty-sixth example, a method for forming a biocidal film, comprises the steps of: forming a solution comprising a protonated polyalkylenimine, free chloride ions, and chloride counterions; removing at least fifty percent of the free chloride ions from the solution via density separation to yield a residual chloride concentration; reducing the residual chloride concentration by at least fifty percent with an anion exchange material; decreasing a percentage of protonated sites of the polyalkylenimine associated with the chloride counterions through addition of an organic acid to the solution; and forming, from the solution, the film with properties of: inactivating at least 99 percent of a first bacteria making contact with the film within one hour; and deactivating at least 99 percent of second bacteria within one hour, the second bacteria contacting the film at least one day later.

Example XXVII

In a twenty-seventh example, a method for treating a pathogen, comprises the steps of: removing, with an acid, less than 99% of -COR_1 acyl groups from nitrogen sites of a poly(2-alkyl-2-oxazoline) in a solution to form a partially protonated and partially acylated poly(2-alkyl-2-oxazoline), where R_1 comprises an alkyl group; adjusting a pH of the solution to greater than 2.5; forming a film from the solution, the film comprising a total cationic charge in a range of 0.001 to 10.0 coulombs per square inch, the total cationic charge countered with counteranions; and reducing activity of the pathogen contacting the film by: greater than 9% and less than 99.99995% in less than one minute, greater than 99.999% in less than four days, and greater than 99.99995% in less than seven days.

Example XXVIII

In a twenty-eighth example, a method for treating a pathogen, comprises the steps of: (1) polymerizing a 2-ethyl-2-oxazoline to form a poly(2-ethyl-2-oxazoline); (2) acidifying at least five percent of the poly(2-ethyl-2-oxazoline) to form a first copolymer, the first copolymer comprising: a first monomer, the first monomer comprising an acyl group attached to a -N-CH_2-CH_2- group; and a second monomer, the second monomer comprising a -NH^+-CH_2-CH_2- group; (3) forming a formulation comprising the first copolymer in a solvent, the formulation comprising a charge density of 0.2 to 10 C/mL; and (4) inactivating at least fifty percent of the pathogen within a ten minute window of a contact of the pathogen with the formulation.

Example XXIX

In a twenty-ninth example, any of the formulations described herein are optionally added to a washing machine rinse additive.

Example XXX

In a thirtieth example, a method for inactivating pathogens is described, comprising the steps of: (1) partially deacylating a poly(2-ethyl-2-oxazoline) to

yield a copolymer, the copolymer comprising: first monomers comprising a -$NH^+CH_2CH_2$- group and second monomers comprising a -$COCH_2CH_3$ group attached to a nitrogen in a polymer backbone group comprising -NCH_2CH_2-, the first monomers and the second monomers arranged in any order in the copolymer; (2) preparing a formulation comprising the copolymer and a total cationic charge in a range of 0.2 to 10 C/cm^3, the total cationic charge at least 95% offset by counteranions in the formulation; (3) contacting the pathogens with the formulation; and (4) inactivating at least ninety percent of the pathogens within ten minutes of the step of contacting.

Example XXXI

In a thirty-first example, a method for inactivating pathogens is described, comprising the steps of:

(A) partially deacylating a poly(2-alkyl-2-oxazoline) with an acid to yield a first copolymer, an alkyl in the poly(2-alkyl-2-oxazoline) comprising a hydrocarbon chain, the copolymer comprising: (a) first monomers, the first monomers comprising a -$NH_2^+CH_2CH_2$- group; and (b) second monomers, the second monomers comprising an acyl group, -C(=O)-R', attached to a nitrogen in a polymer backbone group comprising -NCH_2CH_2-, wherein R' comprises the hydrocarbon chain, and/or providing the first copolymer; and/or

(B) partially protonating polyethylenimine to yield a second copolymer and/or providing the second copolymer, the second copolymer comprising: first monomers, the first monomers comprising a -$NH_2^+CH_2CH_2$- group; and second monomers, the second monomers comprising a -$NHCH_2CH_2$- group,

wherein in one or both cases, the first monomers and the second monomers arranged in any order in their respective copolymers; and (2) preparing a formulation, the formulation comprising the first and/or second copolymer and a total cationic charge in a range of 0.2 to 10 C/cm^3, the total cationic charge at least 95% offset by counteranions in the formulation; (3) contacting the pathogens with the formulation; and (4) inactivating at least ninety percent of the pathogens within ninety minutes of the step of contacting.

Generally, the method optionally and preferably controls salts/anions of the solution, to enhance the inactivating step, such as measured by conductivity, activity, sodium concentration, organic acid/base types, and/or counterion concentrations/types. The formulation, optionally sprayed to form a film, has antivirion, antiviral, antibacterial, and/or antifungal properties that persist through time. The copolymer has monomers in equilibrium with other chemical forms, where the equilibrium is a function of pH of the formulation/process of making the formulation. Any additive to the formulation is optionally used, such as an addition of a quaternary ammonium, a polydiallyldimethylammonium salt, and/or a partially protonated polyethylenimine. The formulation is generally an antipathogen, such as finding applications in a washing machine additive, a hand sanitizer, and/or in at least partial coating of a fabric/garment.

Example XXXII

In a thirty-second example, a method for inactivating pathogens is described, comprising the steps of: (1) preparing a copolymer comprising repeating units, the repeating units comprising at least two, and optionally one or three of: first monomers comprising a $-NH_2^+CH_2CH_2-$ group; second monomers comprising a $-NHCH_2CH_2-$ group; and third monomers comprising an acyl group, -C(=O)-R', attached to a nitrogen in a polymer backbone group of the copolymer comprising $-NCH_2CH_2-$, wherein R' comprises a hydrocarbon chain, the repeating units arranged in any order in the copolymer; (2) preparing a formulation, the formulation comprising the copolymer and a total cationic charge in a range of 0.2 to 10 C/cm^3, the total cationic charge at least 95% offset by counteranions in the formulation; and (3) inactivating at least ninety percent of the pathogen within a time period of less than ninety minutes from contact of the pathogen with the copolymer.

Generally, a formulation property and/or a film property results from specific properties of the formulation and/or of a film derived from the formulation. For instance, parameters affecting the formulation/film properties include: concentrations of formulation elements, a pH of the formulation, an amount of

the formulation applied to an area, a temperature of preparation of a formulation element, a reaction time of preparation of a formulation element, separation of reaction products, and/or interaction of formulation elements, such as counterions with charged polymer sites. For instance, a polyethylenimine at 1000 ppm in a first solvent will have different formulation properties than a polyethylenimine at a second concentration, such as 6000 ppm at a different pH and/or with another antipathogen, such as a counterion, a ratio of counterions, a quaternary ammonium, and/or a copolymer. Generally, any antipathogen formulation component is optionally in a concentration range exceeding 1, 2, 10, 100, 500, or 1000 ppm and/or is optionally in a concentration range of less than 15,000, 10,000, 7000, 5000, 3000, 1000, 500, or 200 ppm. The formulation elements are described, *supra*.

Example XXXIII

In a thirty-third example, *Aloe vera* is incorporated into any of the formulations described herein, such as in a hand sanitizer functioning as an antipathogen. An optional and preferred concentration of *Aloe vera* in a formulation is greater than 10, 20, 50, 100, 500, or 1000 ppm.

Example XXXIV

In a thirty-fourth example, a method for treating a pathogen is described, comprising the steps of: (1) spraying a formulation onto a substrate, the formulation comprising: a charged polymer comprising charged monomers, counterions to the charged monomers; and, an adhesion promoter; (2) drying the formulation to yield a film comprising a critical load of adhesion in a range of 10 to 600 μN; (3) contacting the pathogen with the film; and (4) inactivating, with the film, at least fifty percent of the pathogen within thirty minutes of the step of contacting, where the adhesion promoter is optionally an antipathogen, such as a polydiallyldimethylammonium salt.

Still yet another embodiment includes any combination and/or permutation of any polymer and/or copolymer described herein and/or any part of a polymer and/or any counterion and/or any formulation element described herein.

Still yet another embodiment includes any combination and/or permutation of any of the elements described herein.

Herein, any number, such as 1, 2, 3, 4, 5, is optionally more than the number, less than the number, or within 1, 2, 5, 10, 20, or 50 percent of the number.

The particular implementations shown and described are illustrative of the invention and its best mode and are not intended to otherwise limit the scope of the present invention in any way. Indeed, for the sake of brevity, conventional manufacturing, connection, preparation, and other functional aspects of the system may not be described in detail. Furthermore, the connecting lines shown in the various figures are intended to represent exemplary functional relationships and/or physical couplings between the various elements. Many alternative or additional functional relationships or physical connections may be present in a practical system.

In the foregoing description, the invention has been described with reference to specific exemplary embodiments; however, it will be appreciated that various modifications and changes may be made without departing from the scope of the present invention as set forth herein. The description and figures are to be regarded in an illustrative manner, rather than a restrictive one and all such modifications are intended to be included within the scope of the present invention. Accordingly, the scope of the invention should be determined by the generic embodiments described herein and their legal equivalents rather than by merely the specific examples described above. For example, the steps recited in any method or process embodiment may be executed in any order and are not limited to the explicit order presented in the specific examples.

Additionally, the components and/or elements recited in any apparatus embodiment may be assembled or otherwise operationally configured in a variety of permutations to produce substantially the same result as the present invention and are accordingly not limited to the specific configuration recited in the specific examples.

Benefits, other advantages and solutions to problems have been described above with regard to particular embodiments; however, any benefit, advantage, solution to problems or any element that may cause any particular benefit, advantage or solution to occur or to become more pronounced are not to be construed as critical, required or essential features or components.

As used herein, the terms “comprises”, “comprising”, or any variation thereof, are intended to reference a non-exclusive inclusion, such that a process, method, article, composition or apparatus that comprises a list of elements does not include only those elements recited, but may also include other elements not expressly listed or inherent to such process, method, article, composition or apparatus. Other combinations and/or modifications of the above-described structures, arrangements, applications, proportions, elements, materials or components used in the practice of the present invention, in addition to those not specifically recited, may be varied or otherwise particularly adapted to specific environments, manufacturing specifications, design parameters or other operating requirements without departing from the general principles of the same.

Although the invention has been described herein with reference to certain preferred embodiments, one skilled in the art will readily appreciate that other applications may be substituted for those set forth herein without departing from the spirit and scope of the present invention. Accordingly, the invention should only be limited by the Claims included below.

CLAIMS

1. A method for dispensing a biocide, comprising the steps of:

packaging contents in a container, said contents comprising said biocide and a solvent, said biocide comprising a protonated polymer comprising repeating charged units, said contents comprising a total cationic charge density in a range of 0.001 to 0.95 meq/g, at least ten percent of said total cationic charge density from charges of non-quaternary amines of said protonated polymer; and

dispensing said biocide from said container.

2. The method of Claim 1, further comprising the step of:

formulating, prior to said step of packaging, said protonated polymer with protonated polyethylenimine, said protonated polyethylenimine comprising at least ten percent of said protonated polymer by mass.

3. The method of Claim 2, said step of dispensing further comprising the step of:

delivering a dose of said contents from said container, said dose comprising a total cationic charge, said protonated polyethylenimine comprising at least twenty percent of said total cationic charge.

4. The method of Claim 3, said step of dispensing further comprising the step of:

treating a substrate with said biocide, said step of treating inactivating greater than fifty percent of non-enveloped viruses on said substrate in less than ten minutes.

5. The method of Claim 1, said step of packaging further comprising the step of:

incorporating a polyethylenimine into said container as a component of said protonated polymer.

6. The method of Claim 5, further comprising the steps of:

protonating said polyethylenimine with hydrochloric acid in a solution;

removing at least ten percent of chlorides added to said solution in said step of protonating; and

performing said steps of protonating and removing prior to said step of packaging.

7. The method of Claim 5, further comprising the steps of:

protonating said polyethylenimine with hydrochloric acid in a solution, said step of protonating further comprising the step of:

yielding chloride counterions to protonated secondary amine sites of said polyethylenimine; and

reducing a concentration of said chloride counterions by at least ten percent to a concentration of to less than 900 ppm; and

performing said step of protonating prior to said step of packaging.

8. The method of Claim 1, further comprising the step of:

protonating at least five percent of nitrogens of said protonated polymer prior to said step of packaging.

9. The method of Claim 8, further comprising the step of:

formulating said protonated polymer with counterions, less than fifty percent of said counterions comprising chloride.

10. The method of Claim 1, further comprising the step of:

formulating said protonated polymer with a polymer backbone chain comprising at least two carbons and at least one nitrogen per monomer of said protonated polymer.

11. The method of Claim 10, further comprising the step of:

controlling an activity coefficient, γ, of a protonated site of said protonated polymer, to a value greater than 0.7.

12. The method of Claim 11, said step of dispensing further comprising the step of:

delivering protonated polyethylenimine from said container, wherein a total cationic charge of a total concentration of said protonated polyethylenimine in said container represents at least fifty percent of said total cationic charge density in said container.

13. The method of Claim 1, further comprising the steps of:

prior to step of packaging:

protonating at least ten percent of all nitrogens in a first mass of said protonated polymer with hydrochloric acid, said step of protonating yielding chloride counterions to repeating protonated sites of said protonated polymer; and

exchanging at least ten percent of said chloride ions for non-chloride counterions with an anion exchange material.

14. The method of Claim 13, further comprising the step of:

formulating said protonated polymer with at least ten percent protonated polyethylenimine by mass.

15. The method of Claim 1, further comprising the steps of:

prior to step of packaging:

protonating at least ten percent of all nitrogens in a first mass of said protonated polymer with hydrochloric acid, said step of protonating yielding a total mass of said protonated polymer and chloride; and

reducing a chloride mass of said total mass by at least ten percent.

16. The method of Claim 15, further comprising the step of:

formulating said protonated polymer with a protonated polyethylenimine, said protonated polyethylenimine comprising at least ten percent of said protonated polymer by mass.

17. The method of Claim 16, said step of dispensing further comprising the step of:

delivering a total cationic charge in a range of 0.05 to 0.95 C/mL per delivery dose from said container.

18. The method of Claim 1, further comprising the steps of:

formulating, prior to said step of packaging, said protonated polymer with polyethylenimine, said polyethylenimine comprising at least ten percent of said protonated polymer by mass;

protonating said polyethylenimine with hydrochloric acid in a solution, said step of protonating further comprising the step of:

yielding chloride counterions to protonated secondary amine sites of said polyethylenimine; and

reducing a concentration of said chloride counterions by at least ten percent to a concentration of to less than 900 ppm;

performing said step of protonating prior to said step of packaging;

controlling an activity coefficient, γ, of a protonated site of said protonated polymer, to a value greater than 0.7;

delivering a dose of said contents from said container, said dose comprising a total cationic charge, said polyethylenimine comprising at least twenty percent of said total cationic charge; and

treating a substrate with said biocide, said step of treating inactivating greater than fifty percent of non-enveloped viruses on said substrate in less than ten minutes.

19. The method of Claim 1, further comprising the steps of:

raising a pH of a solution of said protonated polymer to greater than five;

performing said step of raising prior to said step of packaging;

removing, after said step of raising said pH of said solution to greater than five, greater than ten percent of chloride anions in said solution; and

reducing, after said step of removing, said pH of said solution by at least one-half of a pH unit.

20. An apparatus for dispensing a biocide, comprising:

a container;

a biocide formulation in said container, said biocide formulation comprising:

said biocide and a solvent, said biocide comprising a protonated polymer comprising repeating charged units;

a total cationic charge density in a range of 0.005 to 0.95 meq/g, at least ten percent of said total cationic charge density from charges of non-quaternary amines of said protonated polymer;

an activity coefficient, γ, of a protonated site of said protonated polymer, of greater than 0.7; and

a counterion chloride concentration of less than 900 ppm.

21. A method for forming an antibacterial/antiviral material, comprising the steps of:

dispensing a dose of dispensed contents from a container, said dose of dispensed contents comprising a cationic polymer, a solvent, cationic sites, and anionic sites, said cationic sites in said dose of dispensed contents comprising a total cationic charge;

reducing a concentration of said solvent in said dose of dispensed contents by mass through evaporation;

forming a film comprising said total cationic charge in a range of 0.001 to 10.0 coulombs per square inch, wherein polymer cationic sites of said cationic polymer of said dose comprises greater than sixty percent of said total cationic charge; and

inactivating, with said film, in accordance with at least one of Japanese Industrial Standard (JIS) Z 2801 (2006) test for antimicrobial activity and ISO 22916, at least one of:

at least 95% of a gram positive bacteria population within 30 minutes;

at least 95% of a gram negative bacteria population within 30 minutes;

at least 95% of an enveloped virus population within 30 minutes of contact;

at least 95% of a non-enveloped virus population within 30 minutes of contact; and

at least 94% of a *Clostridium difficile* bacteria population within 24 hours of contact.

22. The method of Claim 21, said step of dispensing further comprising the step of:

delivering said dose of said dispensed contents with a total cationic charge density in a range of 0.005 to 0.3 meq/g.

23. The method of Claim 22, further comprising the step of:

yielding a charge from sodium cations, in said dose, of greater than 0.3 coulombs per square inch in said film.

24. The method of Claim 22, further comprising the step of:

yielding a total salt cationic site charge, of a salt in said film, of greater than five percent of said total cationic charge.

25. The method of Claim 22, further comprising the step of:

formulating said dispensed contents with a chloride concentration in excess of 240 parts per million.

26. The method of Claim 25, further comprising the step of:

delivering both a polyalkylenimine and a polydiallyldimethylammonium chloride from said container in said step of dispensing.

27. The method of Claim 22, further comprising the step of:

delivering at least one of a polyalkylenimine and a polydiallyldialkylammonium chloride from said container in said step of dispensing.

28. The method of Claim 27, further comprising the step of:

controlling a concentration of a salt in said film to a concentration of greater than eighty parts per million.

29. The method of Claim 27, further comprising the step of:

yielding in said film at least five parts per million of at least one of:

Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, and Fe^{3+}.

30. The method of Claim 27, further comprising the step of:

delivering at least one quaternary ammonium compound from said container in said step of dispensing, wherein a total cationic polymer charge of secondary amines exceeds a total quaternary ammonium compound charge in said film.

31. The method of Claim 27, further comprising the step of:

formulating a formulation comprising said dispensed contents with an organic acid.

32. The method of Claim 27, said step of formulating further comprising the step of:

producing said formulation with a partially deprotonated form of citric acid at a concentration greater than ten parts per million.

33. The method of Claim 32, further comprising the step of:

forming said film with said polyalkylenimine, said polyalkylenimine comprising:

repeating secondary amine monomers;

protonation sites at greater than twenty percent of said secondary amine monomers;

a first counterion comprising chloride at a concentration greater than ten parts per million; and

a second counterion comprising a conjugate base of a carboxylic acid, said second counterion comprising a concentration greater than ten parts per million,

said film in accordance with United States Environmental Protection Agency protocol *#01-1A* for residual self-sanitizing activity of dried chemical residues on hard, non-porous surfaces.

34. The method of Claim 32, further comprising the step of:

reducing a concentration of chloride ions from a solution containing polyethylenimine by at least ten percent prior to adding said polyethylenimine into said container.

35. The method of Claim 34, further comprising the step of:

formulating said polyethylenimine with:

a first counterion type of first cations of said polyethylenimine, said first counterion type comprising a first mass of at least 0.05 percent of said film; and

a second counterion type of second cations of said polyethylenimine, said second counterion type comprising a second mass of at least 0.05 percent of said film, wherein a ratio of said first mass-to-said second mass exceeds a ratio of 1.2 to 1,

said first counterion type differing from said second counterion type,

wherein said first counterion type and said second counterion type do not comprise chloride anions.

36. The method of Claim 21, further comprising the step of:

delivering at least one of polyalkylenimine and a polydiallyldimethylammonium chloride at a concentration of at least five hundred parts per million from said container in said step of dispensing.

37. The method of Claim 36, further comprising the step of:

controlling a concentration of a salt in said film to a concentration of less than one hundred parts per million.

38. The method of Claim 37, further comprising the step of:

reducing a concentration of chloride ions from a solution containing said polyalkylenimine by at least ten percent prior to adding said polyalkylenimine into said container.

39. The method of Claim 37, further comprising the steps of:

protonating said polyalkylenimine with hydrochloric acid in a solution, said step of protonating further comprising the step of:

yielding chloride counterions to protonated secondary amine sites of said polyalkylenimine; and

reducing a concentration of said chloride counterions by at least ten percent to a concentration of to less than 1500 ppm; and

performing said step of protonating prior to a step of packaging said polyalkylenimine in said container.

40. The method of Claim 21, further comprising the step of:

formulating said cationic polymer with a polyalkylenimine, wherein said polyalkylenimine comprises an alkyl group other than ethyl.

41. An apparatus, comprising:

an antimicrobial film comprising:

a polyethylenimine polymer comprising:

repeating secondary amine monomers; and

protonation sites at greater than twenty percent of said secondary amine monomers;

a first counterion comprising chloride at a concentration greater than ten parts per million; and

a second counterion comprising a conjugate base of a carboxylic acid, said second counterion comprising a concentration greater than one part per million,

said antimicrobial film in accordance with United States Environmental Protection Agency protocol *#01-1A* for residual self-sanitizing activity of dried chemical residues on hard, non-porous surfaces.

42. The apparatus of Claim 41, said conjugate base of said carboxylic acid comprising:

formate, wherein a ratio of said formate-to-said chloride comprises a ratio of greater than 0.1-to-1.

43. The apparatus of Claim 42, said antimicrobial film further comprising:

an acetate counterion, said acetate counterion present at a concentration greater than five parts per million.

44. The apparatus of Claim 43, said antimicrobial film further comprising:

an acetate-to-formate ratio of less than 1:1.

45. The apparatus of Claim 44, said polyethylenimine polymer further comprising:

a charge exceeding 1 Coulombs.

46. The apparatus of Claim 44, said antimicrobial film further comprising

iodide at a concentration in a range of greater than 1 part per million and less than 150 parts per million.

47. The apparatus of Claim 42, said antimicrobial film further comprising:

iodide at a concentration in a range of greater than 1 part per million and less than 150 parts per million.

48. The apparatus of Claim 47, said antimicrobial film further comprising:

an acetate-to-formate ratio of less than 1:1.

49. The apparatus of Claim 41, said antimicrobial film further comprising:

at least one quaternary amine at a concentration greater than ten parts per million,

said polyethylenimine polymer comprising a linear polyethylenimine.

50. The apparatus of Claim 41, said antimicrobial film further comprising:

formic acid, wherein a ratio of formate-to-formic acid in said antimicrobial film is greater than 1-to-1.

51. The apparatus of Claim 50, said antimicrobial film further comprising:

a semi-solid film.

52. The apparatus of Claim 41, said antimicrobial film further comprising:

a chloride counterion-to-formate counterion ratio of less than five-to-one; and

a pH in a range of 2.8 to 7.

53. The apparatus of Claim 41, said polyethylenimine polymer further comprising:

an average molecular weight exceeding 1000 g/mol.

54. The apparatus of Claim 41, said polyethylenimine polymer further comprising:

a charge exceeding 2 Coulombs.

55. The apparatus of Claim 41, said antimicrobial film further comprising:

a chloride counterion-to-formate counterion ratio of less than five-to-one;

a pH in a range of 2.8 to 7; and

formic acid, wherein a ratio of formate-to-formic acid in said antimicrobial film is greater than 1-to-1,

wherein said polyethylenimine polymer comprises: an average molecular weight exceeding 1000 g/mol and a charge exceeding 2 Coulombs.

56. The apparatus of Claim 41, said antimicrobial film further comprising:

a viscosity exceeding three thousand centipoise.

57. A method for generating a polycationic polymer, comprising the steps of:

providing polyethylenimine polymers comprising at least five non-protonated amines for every protonated amine;

protonating at least two percent of said non-protonated amines with at least one organic acid, said step of protonating further comprising the step of:

introducing organic conjugate base counterions to said protonated amines; and

decreasing pH of a solution containing said polyethylenimine polymers by at least 0.5 pH units, said solution comprising at least five percent water.

58. The method of Claim 57, said step of protonating further comprising the step of:

yielding formate counterions to said polyethylenimine polymer.

59. The method of Claim 58, further comprising the step of:

tuning a concentration of formate to greater than fifteen percent of a total concentration of formate and formic acid in said solution containing said polyethylenimine polymers.

60. The method of Claim 58, said step of protonating further comprising the step of:

yielding acetate counterions to said polyethylenimine polymers.

61. The method of Claim 60, further comprising the step of:

tuning a formate-to-acetate ratio, in said solution containing said polyethylenimine polymers, to less than ten-to-one.

62. The method of Claim 57, said step of protonating further comprising the step of:

acidifying greater than twenty percent of said non-protonated amines with hydrogens from said at least one organic acid.

63. The method of Claim 57, further comprising the step of:

adding hydrochloric acid to said solution containing said polyethylenimine polymers.

64. The method of Claim 57, further comprising the step of:

increasing iodide concentration of said solution containing said polyethylenimine polymers to greater than ten parts per million.

65. The method of Claim 64, further comprising the step of:

forming a film from said solution containing said polyethylenimine polymers, said film comprising less than five percent water.

66. The method of Claim 57, further comprising the step of:

inactivating greater than fifty percent of a virus upon contact of the virus with said solution containing said polyethylenimine polymers for a period exceeding five minutes.

67. The method of Claim 66, wherein said step of protonating further comprises the step of:

introducing a polyprotic acid to said solution containing said polyethylenimine polymers.

68. The method of Claim 57, further comprising the step of:

inactivating greater than fifty percent of a bacteria upon contact of the bacteria with said solution containing said polyethylenimine polymers for a period exceeding five minutes.

69. The method of Claim 57, further comprising the step of:

forming a film from said solution containing said polyethylenimine polymers, said film comprising less than five percent water.

70. The method of Claim 69, further comprising the step of:

introducing to said solution containing said polyethylenimine polymers both:

a first counterion type to first cations of said polyethylenimine polymers, said first counterion type comprising a first mass of at least 0.03 percent of a polymer mass of said polyethylenimine polymers; and

a second counterion type to second cations of said polyethylenimine polymers, said second counterion type comprising a second mass of at least 0.03 percent of said polymer mass,

said first counterion type differing from said second counterion type.

71. The method of Claim 70, further comprising the step of:

incorporating into said solution containing said polyethylenimine polymers a third counterion type to third cations of said polyethylenimine polymers, said third counterion type comprising a third mass of at least 0.03 percent of said polymer mass,

each of said first counterion type, said second counterion type, and said third counterion type comprising a counterion type separately selected from a set of counterion types, comprising: chloride counterions, formate counterions, acetate counterions, iodide counterions, and citrate counterions,

said first counterion type differing from said third counterion type,

said second counterion type differing from said third counterion type.

72. The method of Claim 69, further comprising the steps of:

increasing a pH containing said polyethylenimine polymers, prior to said step of providing polyethylenimine polymers, from a first pH under 4 to a second pH over 6; and

acidifying, subsequent to said step of increasing said pH, said solution containing said polyethylenimine polymers from said second pH to a third pH under 5.5.

73. The method of Claim 72, further comprising the step of:

reducing, after said step of increasing and prior to said step of acidifying, salt of said solution containing said polyethylenimine polymers by at least twenty percent.

74. The method of Claim 57, further comprising the step of:

forming a solid film from less than five milliliters of said polyethylenimine polymers, said film comprising a cationic charge of greater than three coulombs.

75. The method of Claim 57, further comprising the steps of:

tuning a concentration of formate to greater than fifteen percent of a total concentration of formate and formic acid in said solution containing said polyethylenimine polymers, said step of protonating further comprising the step of yielding acetate counterions to said polyethylenimine polymer;

acidifying greater than twenty percent of said non-protonated amines with hydrogens from said at least one organic acid;

adding hydrochloric acid to said solution containing said polyethylenimine polymers;

increasing iodide concentration of said solution containing said polyethylenimine polymers to greater than ten parts per million;

inactivating greater than fifty percent of a virus upon contact of the virus with said solution containing said polyethylenimine polymers for a period exceeding five minutes;

introducing to said solution containing said polyethylenimine polymers:

a first counterion type to first cations containing said polyethylenimine polymers, said first counterion type comprising a first mass of at least 0.03 percent of a mass containing said polyethylenimine polymers;

a second counterion type to second cations containing said polyethylenimine polymers, said second counterion type comprising a second mass of at least 0.03 percent of said mass containing said polyethylenimine polymers; and

a third counterion type to third cations containing said polyethylenimine polymers, said third counterion type comprising a third mass of at least 0.03 percent containing said polyethylenimine polymers,

said first counterion type, said second counterion type, and said third counterion type each comprising a counterion type selected from a set of cation types, comprising: chloride counterions, formate counterions, acetate counterions, iodide counterions, and citrate counterions, said first counterion type differing from said second counterion type, said first counterion type differing from said third counterion type, and said second counterion type differing from said third counterion type; and

forming a film from said solution containing said polyethylenimine polymers, said film comprising less than five percent water and a total cationic charge of greater than three coulombs.

76. The method of Claim 75, further comprising the steps of:

increasing a pH containing said polyethylenimine polymers, prior to said step of providing polyethylenimine polymers, from a first pH under 4 to a second pH over 6;

reducing, after said step of increasing, salt of said solution containing said polyethylenimine polymers by at least twenty percent; and

acidifying, after said step of reducing, said solution containing said polyethylenimine polymers from said second pH to a third pH under 5.5.

77. A method for generating a biocide, comprising the steps of:

generating a solution of polymers comprising charged amines on nitrogen containing repeating backbone monomers, said solution comprising chloride anions as counterions to greater than fifty percent of said charged amines and a pH less than four; and subsequently:

increasing a pH of said solution by at least one pH unit; and

decreasing said chloride anion concentration of said solution by at least five percent to yield a reduced chloride anion solution,

said reduced chloride anion solution yielding said biocide, said biocide inactivating, with physical contact for greater than two minutes, both: greater than fifty percent of a bacteria and fifty percent of a virus.

78. The method of Claim 77, said step of generating further comprising:

generating said solution of polymers with a repeating monomer, said repeating monomer comprising a carbon-carbon-nitrogen backbone section.

79. The method of Claim 77, said step of generating further comprising:

generating said solution of polymers with polyethylenimine polymers, said charged amines comprising charged secondary amines.

80. The method of Claim 79, said step of generating further comprising the step of:

yielding chloride counterions to greater than eighty percent of said charged secondary amines.

81. The method of Claim 79, further comprising the step of:

raising said pH of said solution to greater than 6 to form a solid salt.

82. The method of Claim 81, said step of raising further comprising the step of:

combining an organic acid with said solution containing said polyethylenimine polymers to raise said pH of said solution to greater than 6.

83. The method of Claim 81, said step decreasing further comprising the steps of:

removing, after said step of raising said pH of said solution to greater than 6, greater than ten percent of said solid salt on said biocide; and

reducing pH, after said step of removing, of said biocide by at least one-half of a pH unit to form a reduced salt solution comprising said biocide.

84. The method of Claim 77, further comprising the steps of:

raising said pH of said solution to greater than 6;

said step decreasing further comprising the step of removing, after said step of raising said pH of said solution to greater than 6, greater than ten percent of chloride anions in said solution; and

reducing pH of said solution by at least one-half of a pH unit.

85. The method of Claim 77, further comprising the step of:

adding anion exchange material to said solution, said anion exchange material simultaneously performing said steps of: (1) increasing said pH of said solution by at least one pH unit and (2) decreasing said chloride anion concentration of said solution by at least five percent.

86. The method of Claim 85, further comprising the step of:

forming a solid film from said solution through evaporation of at least eighty percent of an organic solvent in said solution, said film comprising a total cationic charge in a range of 0.6 to 5 coulombs.

87. The method of Claim 80, further comprising the step of:

reducing said chloride anion concentration by at least thirty percent.

88. The method of Claim 80, further comprising the step of:

reducing a concentration of said chloride counterions to, on average, association with less than seven of every eight of said charged secondary amines.

89. The method of Claim 77, further comprising the steps of:

forming a solid film with said solution; and

maintaining said inactivation after greater than three cycles of rubbing, for greater than ten seconds, the solid film with a pressure exceeding four pounds of pressure.

90. The method of Claim 77, further comprising the step of:

performing both said step of increasing said pH and said step of decreasing said chloride anion concentration simultaneously.

91. The method of Claim 90, said step of performing further comprising the step of:

exchanging ions from an anion exchange material for ions from said solution.

92. The method of Claim 79, further comprising the step of:

removing counterions to said charged secondary amines of said polyethylenimine from said solution with said anion exchange material.

93. The method of Claim 92, said step of removing counterions further comprising the step of:

removing chloride anions from said solution with said anion exchange material.

94. The method of Claim 77, said step of generating further comprising a step of:

combining with said polymers a source of protons other than hydrochloric acid.

95. The method of Claim 80, said step of generating further comprising the step of:

adding a strong acid to said polyethylenimine polymers, said strong acid comprising at least one of:

hydrobromic acid;

hydroiodic acid;

nitric acid;

perchloric acid;

sulfuric acid; and

chloric acid.

96. The method of Claim 95, further comprising the step of:

simultaneously raising pH of said solution and decreasing anions in said solution through addition of an anion exchange material to said solution.