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CA 2842462 A1 2013/01/24

(21) 2 842 462

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2012/07/20
(87) Date publication PCT/PCT Publication Date: 2013/01/24
(85) Entrée phase nationale/National Entry: 2014/01/20
(86) N° demande PCT/PCT Application No.: US 2012/047750
(87) N° publication PCT/PCT Publication No.: 2013/013223
(30) Priorités/Priorities: 2011/07/20 (US61/572,714);
2012/05/03 (US61/642,431)

(51) Cl.Int./Int.Cl. *A61L 27/52* (2006.01),
*A61L 27/54* (2006.01), *A61L 31/14* (2006.01),
*A61L 31/16* (2006.01)

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(54) Titre : MATERIAUX HYDROGEL CONTENANT UN COMPOSE CERAGENINE D'ELUTION
(54) Title: HYDROGEL MATERIALS INCORPORATING ELUTING CERAGENIN COMPOUND

(57) Abrégé/Abstract:
A hydrogel polymer includes a ceragenin compound. The ceragenin compound has a hydrophobicity/hydrophilicity that produces a release rate in a range of 0.1-100 µg/ml for at least 3 days.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 January 2013 (24.01.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/013223 A1

(51) International Patent Classification:
*A61L 27/52* (2006.01) *A61L 31/14* (2006.01)
*A61L 27/54* (2006.01) *A61L 31/16* (2006.01)

(21) International Application Number:
PCT/US2012/047750

(22) International Filing Date:
20 July 2012 (20.07.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/572,714 20 July 2011 (20.07.2011) US
61/642,431 3 May 2012 (03.05.2012) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

WO 2013/013223 A1

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# HYDROGEL MATERIALS INCORPORATING ELUTING CERAGENIN COMPOUND

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of United States Provisional application numbers 61/572,714 filed July 20, 2011, titled "Ceragenin-Containing Hydrogels For Prevention of Bacterial Biofilm Formation" and 61/642,431, filed May 3, 2012, titled Hydrogel Materials Incorporating Eluting Ceragenin Compound," both of which are hereby incorporated herein by reference.

## BACKGROUND OF THE INVENTION

1. The Field of the Invention

**[0002]** The present invention relates to hydrogel materials. The materials include a hydrogel polymer that incorporates ceragenin compound that controllably elute from the hydrogel polymer. The hydrogel materials may be formed into a contact lens.

2. The Relevant Technology

**[0003]** A hydrogel polymer is a polymer that can absorb and retain extremely large amounts of a liquid relative to its own mass. In deionized and distilled water a hydrogel polymer may absorb 500 times its weight (from 30-60 times its own volume). The ability of a hydrogel polymer to absorb water depends on the ionic concentration of the aqueous solution being absorbed. Saline solutions are less absorbed than distilled water. For example, a saline solution of 0.9% is absorbed by a hydrogel polymer on the order of 50 times the polymer's weight.

**[0004]** The total absorbency and swelling capacity are controlled by the type and degree of cross-linkers used to make the polymer. Hydrogel polymers are commonly made by polymerizing acrylic acid blended with sodium hydroxide in the presence of an initiator to form a poly-acrylic acid sodium salt (sometimes referred to as sodium polyacrylate).

## BRIEF SUMMARY

**[0005]** The present invention relates to hydrogel materials that include a hydrogel polymer that controllably elutes a ceragenin compound from the hydrogel polymer over time. The controlled elution of the ceragenin can occur over days, weeks, or months at a release rate that is within a desired range for making the hydrogel material anti-microbial while still maintaining the desired properties of the hydrogel material. In one embodiment, the hydrogel may be incorporated into a medical device or form a coating thereon. For example, the hydrogel may be coated on an implantable medical device or formulated into a contact lens.

**[0006]** Ceragenin compounds are anti-microbial compounds that have cationic groups attached to a sterol backbone. The compounds mimic the three-dimensional structure of naturally occurring anti-microbial peptides and provide a natural mechanism of killing microbes.

**[0007]** According to one embodiment of the invention, a ceragenin compound is incorporated into a hydrogel material and the hydrophobicity/hydrophilicity of the hydrogel polymer and the ceragenin compound are selected to cause the ceragenin compound to non-covalently bond to the hydrogel polymer. The non-covalent bonding prevents the ceragenin compound from being released all at once when placed in water or another fluid medium. The non-covalent bonding allows the ceragenin compound to be released over time. In one embodiment, the non-covalent bonding yields a release rate of 0.1 – 100 μg/ml, 0.5 – 50 μg/ml, or 1 – 10 μg/ml at three days, one week, or one month and/or over a period of at least 3 days, one week, or one month.

**[0008]** Surprisingly and unexpectedly, it has been found that by non-covalently bonding the ceragenin to a hydrogel using hydrophobic/hydrophilic interactions, the ceragenin compound can preferentially elute in the presence of microbes. In other words, the ceragenin can be "consumed" or removed from the hydrogel at a rate that is faster than the elution rate without the presence of the microbes. The data surprisingly show that microbes entering the hydrogel are attracted to the ceragenin compound. Because the ceragenins are non-covalently bonded, the microbes do not become immobilized on the surface of the hydrogel polymer, which is believed to avoid the buildup of biofilm and allow the continued elution of ceragenin and/or kill of microbes. The increased rate of elution or consumption of the ceragenin in the presence of microbes is a highly desirable and surprising and unexpected result.

**[0009]** The ceragenin compound can be incorporated into the hydrogel polymer by (i) polymerizing the hydrogel polymer in the presence of the ceragenin compound, (ii) intimately mixing the ceragenin with the hydrogel polymer (e.g., in dry form), and/or (iii) imbuing the hydrogel polymer with the ceragenin compound dissolved in a solvent.

**[0010]** In addition, it has been found that the ceragenins as used in the present invention surprisingly kill harmful microbes preferentially over normal flora, which means that the ceragenins can be used at lower concentrations compared to other antimicrobials while achieving the same or better effectiveness. This feature avoids many of the deleterious effects of prior art antimicrobials, many of which tend to kill the "good microbes."

**[0011]** The ceragenin eluting hydrogels can be incorporated into or formed into medical devices such as medical devices to be implanted into a human or other animal. For example, the hydrogels can be coated on a medical device. In another embodiment, the hydrogels incorporating ceragenin eluting compounds can be formed into ophthalmic products such as contact lenses. The hydrogel materials are particularly advantageous for medical devices due to the controlled release over periods of time that are needed for medical treatment or for maintaining shelf life of products such as ophthalmic products. For example, the ophthalmic

products can controllably release ceragenin compound in a concentration sufficient to meet regulatory requirements for maximum bacterial loads over weeks or even months in a saline solution having the contact lens submersed therein.

**[0012]** These and other features of the present invention will become more fully apparent from the following description and appended claims, or may be learned by the practice of the invention as set forth hereinafter.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0013]** To further clarify the above and other advantages and features of the present invention, a more particular description of the invention will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. It is appreciated that these drawings depict only illustrated embodiments of the invention and are therefore not to be considered limiting of its scope. The invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

**[0014]** Figure 1 illustrates example ceragenin compounds.

**[0015]** Figure 2 is a schematic representation of a substrate with a hydrogel coating;

**[0016]** Figure 3 is a graph showing elution of a ceragenin from a hydrogel in phosphate buffered saline;

**[0017]** Figure 4 is a graph showing elution of a ceragenin from a hydrogel following autoclaving;

**[0018]** Figure 5 is a graph showing elution of a ceragenin from a hydrogel in phosphate buffered saline and tryptic soy broth;

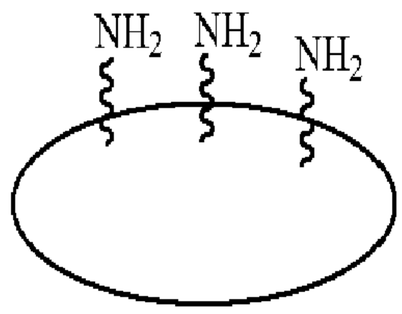
**[0019]** Figure 6 is a graph showing elution of a ceragenin from a hydrogel in buffer and $10^6$ CFU of S. *aureus*; and

**[0020]** Figure 7 is a graph showing elution of a ceragenin from a hydrogel in buffer and $10^6$ CFU of P. *aeruginosa*.

## DETAILED DESCRIPTION

### I. Ceragenins

**[0021]** Ceragenin compounds, also referred to herein as cationic steroidal anti microbial compounds (CSA), are synthetically produced small molecule chemical compounds that include a sterol backbone having various charged groups (e.g., amine and cationic groups) attached to the backbone. The backbone can be used to orient the amine or guanidine groups on one face, or plane, of the sterol backbone. For example, a scheme showing a compound having primary amino groups on one face, or plane, of a backbone is shown below in Scheme I:




Scheme I

**[0022]** Ceragenins are cationic and amphiphilic, based upon the functional groups attached to the backbone. They are facially amphiphilic with a hydrophobic face and a polycationic face. Without wishing to be bound to any particular theory, the anti-microbial ceragenin compounds described herein act as anti-microbial agents (e.g., anti-bacterials, anti-fungals, and anti-virals). It is believed, for example, that the ant-microbial ceragenin compounds described herein act as anti-bacterials by binding to the outer cellular membrane of bacteria and other microbes and inserting into the cell membrane forming a pore that allows the leakage of ions that are critical to the microbe's survival and leading to the death of the affected microbe. In addition, the anti-microbial ceragenin compound described herein may also act to sensitize bacteria to other antibiotics. For example, at concentrations of the anti-microbial ceragenin compounds below the corresponding minimum bacteriostatic concentration, the ceragenins cause bacteria to become more susceptible to other antibiotics by increasing the permeability of the outer membrane of the bacteria.

**[0023]** The charged groups are responsible for disrupting the bacterial cellular membrane, and without the charged groups, the ceragenin compound cannot disrupt the membrane to cause cell death or sensitization. A number of examples of ceragenin compounds of Formula I that can be incorporated into the composite materials described herein are illustrated in Figure 1.

II. Hydrogel Polymers

**[0024]** The hydrogel polymers useful in the present invention include any hydrogel suitable for use in products where there is a need to minimize microbial load. For example, the hydrogels of the present invention may be hydrogels useful in making medical devices, medical device coatings, tissue scaffolds, wound dressings, contact lenses.

**[0025]** Examples of suitable hydrogel polymers include, but are not limited to, polyvinyl alcohol, sodium polyacrylate, acrylate polymers, polyethylene oxide, poly(2- acrylamido-2-methyl- 1 -prop anesulfonic acid) (polyAMP S), polyvinylpyrrolidone, polyacrylamide, silicone, agarose, methylcellulose, hyaluronan, hydrolyzed polyacrylicnitrile, combinations of these. The hydrogels may be copolymers. The copolymers may include hydrophobic and hydrophilic units.

**[0026]** In one embodiment, the hydrogel is suitable for manufacturing a contact lens. Hydrophilic contact lenses can be formed from cross-linked polymers based on hydrophilic derivatives of acrylic or methacrylic acid, hydrophilic vinylic monomers such as vinylpyrrolidone, and the like. The hydrogels preferably include hydrophobic regions made from blocks or monomers that are hydrophobic.

**[0027]** An example of a suitable contact lens hydrogel is disclosed in US Patent 8,011,784, which is incorporated herein by reference.

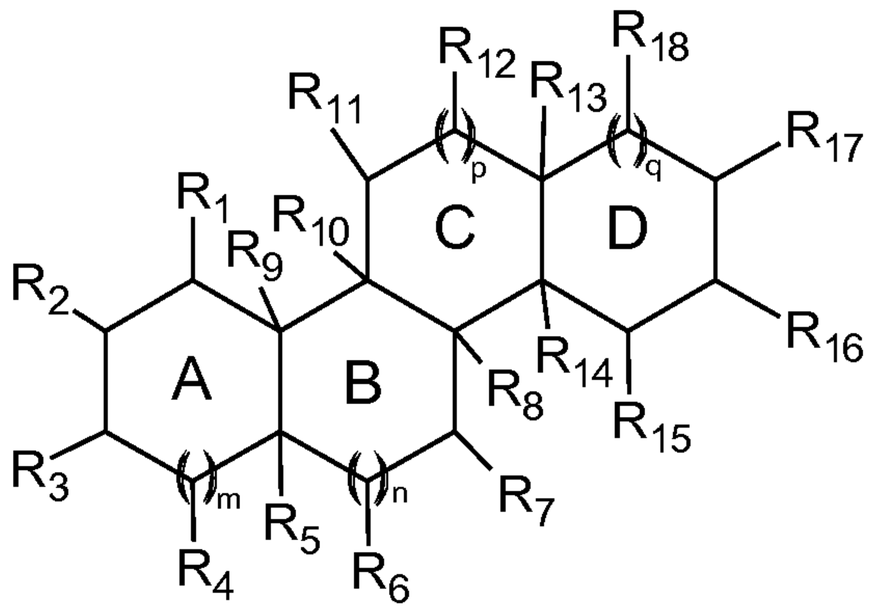
**[0028]** The hydrogel polymers may be formed into a contact lens having a shape and structure suitable for correcting vision. Those skilled in the art are familiar with the shapes and structures of hydrogel polymers that can provide correction for vision. Other devices that can be formed from the hydrogels include wound healing devices such as tissue scaffolds and wound dressing.

III. Eluting Ceragenin Compounds

**[0029]** The ceragenin compound is selected in combination with the hydrogel polymer to non-covalently bond to the hydrogel polymer such that over time the ceragenin is released from the hydrogel in the presence of a solvent such as water or saline and preferably preferentially released or consumed in the presence of microbes.

**[0030]** Those skilled in the art will recognize that the hydrophobicity/hydrophilicity needed to achieve the desired bonding will depend on the type of hydrogel polymer being paired with the particular ceragenin compound. Because ceragenins have a sterol backbone, which can provide a high degree of hydrophobicity, in some embodiments it may be preferred to use a hydrogel that includes hydrophobic regions (e.g., hydrophobic blocks or monomers). The ceragenin compounds may have moieties that impart the desired amount of hydrophobicity to non-covalently bond with the hydrophobic regions of the hydrogel polymers. In one embodiment the ceragenin compound may have a pendant chain to the sterol backbone with a hydrocarbon chain of at least 9 carbons.

**[0031]** The ceragenin compound may have a structure as shown in Formula I:




Formula I

where rings A, B, C, and D form a fused ring system and at least one of the R groups on 2 or 3 of the 4 four fused rings has a cationic group. The other R groups on Figure I can have a variety of different functionalities, thus providing the ceragenin compound with the desired properties for eluting from a hydrogel.

**[0032]** In a preferred embodiment, p = 1 and q = 0 and at least $R_3$, $R_7$, and $R_{12}$ independently include a cationic group attached to the fused ring system and $R_{17}$ is a hydrophobic substituent that includes a hydrophobic group selected to give the ceragenin compound its desired hydrophobic/hydrophilic characteristics, which allows the ceragenin compound to non-covalently bond to the hydrogel polymer and elute out over time and/or be selectively exposed to microbes. The $R_{17}$ substituent may be hydrophobic but still include one or more heteroatoms (O or N) by having sufficient number of carbon atoms attached thereto to form a hydrophobic group. The hydrophobic group may be branched, substituted, or unsubstituted and the branching may occur at the heteroatom (e.g., dialkyl amines). The hydrophobic substituent is preferably attached at $R_{17}$ when q = 0 and $R_{18}$ when q = 1, but may be attached at other locations on the D ring or on R groups at locations on rings A, B, or C of Formula I. Where a hydrophobic substituent has a hydrophobic group attached to a heteroatom of an alkyl group, the hydrophobic group may have from 1 – 20 carbons, preferably 8, 9, 10, 11, 12, or more carbons and 20, 18, 16 or fewer carbons or within a range thereof. The hydrophobic group may also include a hydrophobic moiety such as trimethylsilane. The hydrophobic group may include one or more alkyl groups each having 4 or more, 6 or more, 8 or more, 10 or more or 12 or more carbons. The hydrophobic group can be attached to the sterol structure by an alkyl group linking to the heteroatom. The linkage may be an ester, an ether, an amine, or an amide. Ester linkages are preferred where hydrolysis is desired and/or no charge is desired to impart greater hydrophobicity. There the heteroatom includes an amine, the hydrophobic group is preferably a dialkyl. Examples of a suitable hydrophobic substituents having a hydrophobic group as described herein are C13-alkylamino-C5-alkyl and di-(C1-C20) alkylamino-(C1-C10)-alkyl, which can be covalently bonded to the D ring at R17 or R18 (Formula I).

**[0033]** A number of examples of compounds of Formula I that may be used in the embodiments described herein are illustrated in Figure 1. Suitable examples of ceragenins useful in producing a composition that will elute from a hydrogel include, but are not limited to, CSA-13, CSA-131, CSA-132, CSA-133, CSA-134, CSA-135, , CSA-137, and CSA-138. These compounds can be advantageous for their relatively high hydrophobicity. Examples of CSA's with shorter chain hydrocarbons on $R_{17}$ include, but are not limited to CSA-136, CSA-142, CSA-146, CSA-90, and CSA 44, which are also shown in Figure 1.

**[0034]** With reference again to Formula I, more specifically, each of fused rings A, B, C, and D is independently saturated, or is fully or partially unsaturated, provided that at least two of A, B, C, and D are saturated, wherein rings A, B, C, and D form a ring system; each of m, n, p, and q is independently 0 or 1; each of $R_1$ through $R_4$, $R_6$ , $R_7$ , $R_{11}$ , $R_{12}$, $R_{15}$, $R_{16}$, and $R_{18}$ is independently selected from the group consisting of hydrogen, hydroxyl, a substituted or unsubstituted ($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) hydroxyalkyl, ($C_1$-$C_{10}$) alkyloxy-($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) alkylcarboxy-($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) alkylamino-($C_1$-$C_{10}$)alkyl, ($C_1$-$C_{10}$) alkylamino-($C_1$-$C_{10}$) alkylamino, ($C_1$-$C_{10}$) alkylamino-($C_1$-$C_{10}$) alkylamino- ($C_1$-$C_{10}$) alkylamino, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyl, a substituted or unsubstituted aryl, a substituted or unsubstituted arylamino-($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) haloalkyl, $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl, oxo, a linking group attached to a second steroid, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyloxy, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyloxy-($C_1$-$C_{10}$) alkyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylcarboxy, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylaminocarbonyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylcarboxamido, $H_2N$-$HC(Q_5)$-C(0)-0—, $H_2N$—$HC(Q_5)$-C(0) —N(H) —, ($C_1$-$C_{10}$) azidoalkyloxy, ($C_1$-$C_{10}$) cyanoalkyloxy, P.G.-HN—$HC(Q_5)$-C(0) —0—, ($C_1$-$C_{10}$) guanidinoalkyloxy, ($C_1$-$C_{10}$) quaternaryammoniumalkylcarboxy, and ($C_1$-$C_{10}$) guanidinoalkyl carboxy, where $Q_5$ is a side chain of any amino acid (including a side chain of glycine, i.e., H), P.G. is an amino protecting group, and each of $R_5$, $R_8$, $R_9$, $R_{10}$, $R_{13}$, and $R_{14}$ is independently deleted when one of fused rings A, B, C, or D is unsaturated so as to complete the valency of the carbon atom at that site, or selected from the group consisting of hydrogen, hydroxyl, a substituted or unsubstituted ($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) hydroxyalkyl, ($C_1$-$C_{10}$) alkyloxy-($C_1$-$C_{10}$) alkyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyl, a substituted or unsubstituted aryl, ($C_1$-$C_{10}$) haloalkyl, $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl, oxo, a linking group attached to a second steroid, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyloxy, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylcarboxy, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylaminocarbonyl, $H_2N$—$HC(Q_5)$-C(0) —0—, $H_2N$—$HC(Q_5)$-C(0)—N(H)—, ($C_1$-$C_{10}$) azidoalkyloxy, ($C_1$-$C_{10}$) cyanoalkyloxy, P.G.-HN—$HC(Q_5)$-C(0) —0—, ($C_1$-$C_{10}$) guanidinoalkyloxy, and ($C_1$-$C_{10}$) guanidinoalkylcarboxy, where Q5 is a side chain of any amino acid, PG. is an amino protecting group, provided that at least two or three of $R_{1\text{-}4}$, $R_6$ , $R_7$ , $R_{11}$, $R_{12}$, $R_{15}$, $R_{16}$, $R_{17}$, and $R_{18}$ are independently selected from the group consisting of a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyloxy, ($C_1$-$C_{10}$) alkylcarboxy-($C_1$-$C_{10}$) alkyl, ($C_1$-$C_{10}$) alkylamino-($C_1$-$C_{10}$) alkylamino, ($C_1$-$C_{10}$) alkylamino-($C_1$-$C_{10}$) alkylamino ($C_1$-$C_{10}$) alkylamino, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylcarboxy, a substituted or unsubstituted arylamino ($C_1$-$C_{10}$) alkyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkyloxy ($C_1$-$C_{10}$) aminoalkylaminocarbonyl, a

substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylaminocarbonyl, a substituted or unsubstituted ($C_1$-$C_{10}$) aminoalkylcarboxyamido, a ($C_1$-$C_{10}$) quaternaryammonium alkylcarboxy, $H_2N$-HC($Q_5$)- C(0)-0—, $H_2N$-HC($Q_5$)-C(0)—N(H)—, ($C_1$-$C_{10}$) azidoalkyloxy, ($C_1$-$C_{10}$) cyanoalkyloxy, P.G.-HN-HC(Q5)-C(0)—O—, ($C_1$-$C_{10}$) guanidinoalkyloxy, and a ($C_1$-$C_{10}$) guanidinoalkylcarboxy; or a pharmaceutically acceptable salt thereof. Additional examples of specific CSA compounds are disclosed in Applicant's copending U.S. Application No. 13/288,902 Filed Nov. 3, 2012, which is incorporated herein by reference.

**[0035]** A "ring" as used herein can be heterocyclic or carbocyclic. The term "saturated" used herein refers to the fused ring of Formula I having each atom in the fused ring either hydrogenated or substituted such that the valency of each atom is filled. The term "unsaturated" used herein refers to the fused ring of Formula I where the valency of each atom of the fused ring may not be filled with hydrogen or other substituents. For example, adjacent carbon atoms in the fused ring can be doubly bound to each other. Unsaturation can also include deleting at least one of the following pairs and completing the valency of the ring carbon atoms at these deleted positions with a double bond; such as $R_5$ and $R_9$; $R_8$ and $R_{10}$; and $R_{13}$ and $R_{14}$.

**[0036]** The term "unsubstituted" used herein refers to a moiety having each atom hydrogenated such that the valency of each atom is filled.

**[0037]** The term "halo" used herein refers to a halogen atom such as fluorine, chlorine, bromine, or iodine.

**[0038]** Examples of amino acid side chains include but are not limited to H (glycine), methyl (alanine), —$CH_2$—(C=0)—$NH_2$ (asparagine), —$CH_2$—SH (cysteine), and -CH(OH)-$CH_3$ (threonine).

**[0039]** An alkyl group is a branched or unbranched hydrocarbon that may be substituted or unsubstituted. Examples of branched alkyl groups include isopropyl, sec-butyl, isobutyl, tert-butyl, sec-pentyl, isopentyl, tert-pentyl, isohexyl. Substituted alkyl groups may have one, two, three or more substituents, which may be the same or different, each replacing a hydrogen atom. Substituents are halogen (e.g., F, CI, Br, and I), hydroxyl, protected hydroxyl, amino, protected amino, carboxy, protected carboxy, cyan, methylsulfonylamino, alkoxy, acyloxy, nitro, and lower haloalkyl.

**[0040]** The term "substituted" used herein refers to moieties having one, two, three or more substituents, which may be the same or different, each replacing a hydrogen atom. Examples of substituents include but are not limited to halogen (e.g., F, CI, Br, and I), hydroxyl, protected hydroxyl, amino, protected amino, carboxy, protected carboxy, cyan, methylsulfonylamino, alkoxy, alkyl, aryl, aralkyl, acyloxy, nitro, and lower haloalkyl.

**[0041]** An aryl group is a $C_{6_20}$ aromatic ring, wherein the ring is made of carbon atoms (e.g., $C_6$-$C_{14}$, $C_{6_10}$ aryl groups). Examples of haloalkyl include fluoromethyl, dichloromethyl, trifluoromethyl, 1,1-difluoroethyl, and 2,2-dibromoethyl.

[0034] An aralkyl group is a group containing 6-20 carbon atoms that has at least one aryl ring and at least one alkyl or alkylene chain connected to that ring. An example of an aralkyl group is a benzyl group.

**[0042]** A linking group is any divalent moiety used to link one compound to another. For example, a linking group may link a second compound to a compound of Formula I. An example of a linking group is ($C_1$-$C_{10}$) alkyloxy-($C_1$-$C_{10}$) alkyl.

**[0043]** Amino-protecting groups are known to those skilled in the art. In general, the species of protecting group is not critical, provided that it is stable to the conditions of any subsequent reaction(s) on other positions of the compound and can be removed at the appropriate point without adversely affecting the remainder of the molecule. In addition, a protecting group may be substituted for another after substantive synthetic transformations are complete. Clearly, where a compound differs from a compound disclosed herein only in that one or more protecting groups of the disclosed compound has been substituted with a different protecting group, that compound is within the disclosure. Further examples and conditions are found in T. W. Greene, Protective Groups in Organic Chemistry, (1st ed., 1981, 2nd ed., 1991).

**[0044]** A person of skill will recognize that various ceragenin compounds described herein preserve certain stereochemical and electronic characteristics found in steroids. The term "single face," as used herein, refers to substituents on the fused sterol backbone having the same stereochemical orientation such that they project from one side of the molecule. For example, substituents bound at $R_3$, $R_7$ and $R_{12}$ of Formula I may be all 13-substituted or a-substituted. The configuration of the moieties $R_3$, $R_7$ and $R_{12}$ may be important for interaction with the cellular membrane.

**[0045]** Compounds include but are not limited to compounds having cationic groups (e.g., amine or guanidine groups) covalently attached to a sterol backbone or scaffold at any carbon position, e.g., cholic acid. In various embodiments, a group is covalently attached at anyone, or more, of positions $R_3$, $R_7$, and $R_{12}$ of the sterol backbone. In additional embodiments, a group is absent from anyone, or more, of positions $R_3$, $R_7$, and $R_{12}$ of the sterol backbone.

**[0046]** Other ring systems can also be used, e.g., 5-member fused rings. Compounds with backbones having a combination of 5- and 6-membered rings are also contemplated. Cationic functional groups (e.g., amine or guanidine groups) can be separated from the backbone by at least one, two, three, four or more atoms.

**[0047]** Ceragenins with hydrophobic substituents can be prepared using the techniques described in Applicant's US Patent 6,767,904, with the modification being using longer chain alkyls to form a substituent that is more hydrophobic. For example, instead of using an octyl amine to form the functional group at $R_{17}$ (Formula I where q = 0, a corresponding longer chain amine can be used.

## IV. Non-Covalent Incorporation of Ceragenins Into a Hydrogel Polymer

**[0048]** Ceragenin compounds incorporated into the hydrogel polymer can be non covalently associated with the polymer. Upon contact with moisture, the ceragenin can leach or elute from the polymer. Ceragenins are generally soluble in water, and ceragenins can be associated with hydrogel polymers to control release rates. Selection of appropriate hydrogel polymer and ceragenin structures allows for an extended period of release of the ceragenin.

**[0049]** For example, the group extending from a heteroatom (e.g., N) on $R_{17}$ (Formula I) can be tailored to allow varied rates of elution from the hydrogel polymer. Exemplary groups include lipids, hydrophobic chains (e.g., aliphatic), hydrophilic (e.g., polyethyleneoxide), or any chain that interacts with the polymer is a way that allows modification of the rate of elution. Longer chain lengths will retain the ceragenin within the polymer matrix (in particular the hydrophobic domains). In one embodiment, the ceragenin compound may have a carbon chain of at least 9 carbons attached to the D ring of the sterol group (Formula I). For example, the carbon chain of at least 9 carbons may be attached to $R_{17}$ group of Formula I, or the $C_{24}$ carbon or other similar carbon of a sterol backbone.

**[0050]** The particular ceragenins incorporated into the hydrogel polymer may be soluble or partially soluble in aqueous solutions. Additionally, ceragenins when blended with the water and the appropriate surfactant can be handled in the form of gels, or emulsions. Block copolymers based on ethylene oxide and/or propylene oxide, in particular, Pluronic-type surfactants, are especially useful for this purpose. Pluronic is a product of BASF, a business with offices in Port Arthur, Texas, USA.

**[0051]** Ceragenin compounds can be incorporated into the hydrogel polymer at any suitable step during manufacture of a hydrogel material or product. For example, in an embodiment, a hydrogel can be brought into contact with a solution of ceragenins by immersion, spraying, printing, or coating, etc. In one embodiment, the solvent is a solvent that causes swelling of the hydrogel and the solvent and ceragenin are applied to the hydrogel in try form, thereby causing the hydrogel polymer to swell. Suitable swelling solvents include short chain alcohols such as ethanol, methanol, isopropyl alcohol, and the like. If desired, the solvent used to incorporate the ceragenin can be removed, for example, by evaporation. If necessary the hydrogel can be dried by utilizing forced hot air, oven drying, air at room temperature, microwave drying, or the use of

heated drying drums, vacuum chambers, etc. In some manufacturing systems the normal air flow and temperature sufficiently dry the substrate without a discrete drying process.

**[0052]** Ceragenin compounds are known to be soluble in water. Alternatively, ceragenin compounds are also soluble in such materials as ethanol (and other alcohols), propylene glycol, glycerine, and polyols, or mixtures thereof with or without water can be used in incorporate ceragenin compounds into the hydrogel material. Furthermore ceragenins can be incorporated as gels, emulsions, suspensions, and in dry form.

**[0053]** In another embodiment ceragenin is incorporated into a hydrogel polymer during formation of the hydrogel. Hydrogel polymers can commonly be made from the polymerization of monomers. In these processes, the ceragenin can be included in the monomer blend during polymerization. The ceragenin in final polymer can be noncovalently incorporated into the polymer and will accordingly elute when contacted with a solvent such as water.

## V. Elution

**[0054]** When the ceragenin compound is incorporated into the hydrogel material, the hydrophobicity/hydrophilicity of the hydrogel polymer and the ceragenin compound are selected to cause the ceragenin compound to non-covalently bond to the hydrogel polymer. The non-covalent bonding prevents the ceragenin compound from being released all at once in the presence of a solvent. Rather, the bonding allows the ceragenin compound to be released over time in the presence of a solvent.

**[0055]** The non-covalent bonding depends on the composition of both the hydrogel material and the ceragenin and therefore need to be selected together to produce the desired elution. The selection is typically carried out by selecting a particular hydrogel material having desired chemical and mechanical properties for a particular application. For example, if the hydrogel is coated on a medical device to be implanted in vascular tissue, the hydrogel is selected for compatibility with vascular tissue and blood. If the hydrogel is used to form a contact lens, the hydrogel is selected for its compatibility with the eye and the need to form the hydrogel in a shape that will correct vision. The hydrophobicity/hydrophilicity of the hydrogel material is therefore somewhat constrained by the particular application.

**[0056]** The ceragenin compound is selected to provide non-covalent bonding to the particular hydrogel. The ceragenin may be selected to have R groups that bond non-covalently the functional groups of the hydrogel. For example, a polyacrylate based hydrogel may have a certain percentage of hydrophobic groups and hydrophilic groups in the polymer matrix and the ceragenin compound may be selected to have a hydrophobic $R_{17}$ group that non-covalently bonds to the hydrophobic groups of the hydrogel to cause a relatively consistent elution over a period of days or weeks.

**[0057]** In some cases, the solvent may also influence elution. In one embodiment, the solvent is water. The amount of solvent in the hydrogel material may be greater than that of the hydrogel polymer. In one embodiment, the hydrogel material is at least 50%, 90%, or 99% by weight solvent. In some embodiments, the solvent may be saline.

**[0058]** In one embodiment, the hydrogel polymer and the ceragenin compound are selected to yield non-covalent bonding that provides a release rate of 0.1 – 100 μg/ml, 0.5 – 50 μg/ml, or 1 – 10 μg/m1 at three days, one week, or one month in water or saline. In one embodiment, the foregoing elution rate remains within the foregoing ranges for at least 3 days, one week, or one month. These elution rates are achieved in part by the non-covalent bonding that prevents rapid release of the compound, which results in more compound being available at a later date.

**[0059]** As mentioned above, it has been surprisingly found that non-covalently bound ceragenins in hydrogels selectively elute in the presence of microbes. This is a surprising and unexpected result that makes the use of hydrogel-ceragenin compounds particularly advantageous as compared to other materials, such as ceragenins covalently bonded to the surface of a polymer.

**[0060]** In one embodiment, the ceragenin compound is selected according to its hydrophobicity. Desired hydrophobicities for eluting from polymers with hydrophobic groups are compounds with a CLogP value greater than 6.5, 7.5, 8.5 or in some cases even greater than 10. The CLogP value of various compounds is provided with the compounds in Figure 1.

**[0061]** Those skilled in the art will recognize that the selection of the particular hydrogel and ceragenin compound will depend on the particular application and the appropriate selection can be made by one of skill in the art using the teachings and examples provided herein.

## VI. Medical Devices and Coatings

**[0062]** The hydrogels described herein may be used in various applications, including, but not limited to, medical devices, coatings, bandages, implants, tissue scaffolding, and the like. Figure 2 is a schematic representation of a medical device 100 that includes a substrate 110 and a hydrogel coating 120.

**[0063]** The substrate 110 may be made of any material suitable for supporting and/or adhering to a hydrogel material. The substrate can be polymeric, metallic, an alloy, inorganic, and/or organic. In one embodiment, the substrate is a biocompatible or bioabsorbable material. Suitable biocompatible metallic materials include, but are not limited to, stainless steel, tantalum, titanium alloys (including nitinol), and cobalt alloys (including cobalt-chromium-nickel alloys). Suitable nonmetallic biocompatible materials include, but are not limited to, polyamides, polyolefins (i.e. polypropylene, polyethylene etc.), nonabsorbable polyesters (i.e. polyethylene terephthalate), and bioabsorbable aliphatic polyesters (i.e. homopolymers and

copolymers of lactic acid, glycolic acid, lactide, glycolide, para-dioxanone, trimethylene carbonate, ε-caprolactone, and the like, and combinations of these)

**[0064]** The thickness of the substrate will depend on the device and the material but may be 0.1, 1.0, 10 mm or greater and/or 100, 10, or 1 mm or less and/or within a range thereof.

**[0065]** The thickness of hydrogel coating 120 is generally less than the thickness of substrate 110. Hydrogel coating 120 may have a thickness of 0.01, 0.1, 1.0, or 10 mm or greater and 100, 10, 1.0, or 0.1 mm or less or within a range thereof.

**[0066]** The coating 120 can be continuous or non-continuous. The coating may be applied to the substrate using techniques such as dip coating, spin coating, or the like.

**[0067]** Examples of medical devices that can be formed from a hydrogel containing ceragenin eluting compounds or can have such a hydrogel coated thereon include but are not limited to bone implants, bone pins, bone screws, tissue grafts, airway devices such as endotracheal tubes, implantable devices such as coronary stents, peripheral stents, catheters, arterio-venous grafts, by-pass grafts, pacemaker and defibrillator leads, anastomotic clips, arterial closure devices, patent foramen ovale closure devices, and drug delivery balloons. The hydrogel may be coated on or form any portion of the structures of such devices and is preferably on an outer surface and more preferably on an out service that contacts tissue or a tissue air interface (when the device is implanted).

## VII. Stabilization of Ceragenins by pH

**[0068]** In one embodiment a ceragenin compound can have hydrolysable linkages that attach the cationic groups to the sterol group (e.g., ester bonds). Hydrolysis of these linkages inactivates the ceragenin. To make the ceragenin stable, an acid can be added to achieve a pH less than 6, 5.5, 5, or 4.5 and optionally greater than 2, 2,5, or 3 or a range thereof. Stability before use is important to give a desired shelf life and instability during and after use can be desirable to prevent long term accumulation of ceragenins in biological systems.

**[0069]** As discussed above, it may be advantageous to adjust the degree of neutralization of the hydrogel polymer to improve the stability of the ceragenin. The degree of neutralization of the hydrogel polymer can be adjusted during its manufacturing process, or subsequently. Alternatively, the ceragenin can be suspended or dissolved in an acidic solution; and when the ceragenin suspension or solution is added to the hydrogel polymer the degree of neutralization of the hydrogel would thereby be adjusted.

## VIII. Examples

**[0070]** To better understand the mechanism by which ceragenin compounds can be eluted from a hydrogel to prevent bacterial colonization, we determined the rate at which CSA-138 elutes from a hydrogel suitable for use in contact lenses. To quantify the amount of ceragenin

eluting from the hydrogel, we used LC/MS using a mass-labeled internal standard. However, this method only gave detection limits of about 2 μg/ml, and we were able to effectively kill bacteria at constant elution rates below the detection limit. For example, the elution fell below detection limits within five days of elution from lenses in which CSA-138 had been incorporated at 1%, yet the ceragenins appeared to still be providing suitable kill rates.

**[0071]** To decrease the detection limit for CSA-138, we prepared a radiolabeled version of CSA-138 (CSA-138T2), incorporated it into contact lenses, and quantified its elution from lenses using scintillation counting.

Example 1

**[0072]** Lenses containing 1% CSA-138 were stored in 0.5 mL phosphate buffered saline (PBS) prior to testing. One set of lenses was autoclaved for 45 min before elution studies were performed. For elution studies, lenses were suspended in 2 ml aliquots of PBS, 10% TSB growth medium, 10% TSB growth medium containing $10^6$ CFU of Staphylococcus aureus, or 10% TSB growth medium containing $10^6$ CFU of Pseudomonas aeruginosa. Corresponding aliquots were exchanged every 24 h, including bacterial inocula. Samples were removed every 24 h and analyzed for the presence of CSA-138 using scintillation counting. A standard curve was generated to correlate counts per minute to concentration of CSA-138. All experiments were performed in triplicate.

**[0073]** Though some variations from day to day were observed, a recognizable trend was observed in the elution profile of lenses suspended in PBS (Figure 3). As expected, the elution on the first day was relatively high (about 2.2 μg/ml). Over the course of following 19 days, daily elution changed from approximately 1.6 to 1.4 μg/ml per day.

**[0074]** A comparable elution profile was observed with lenses that were autoclaved prior to the start of the study, except that the initial amount of material that eluted decreased somewhat (Figure 4). This decrease in elution is likely due to enhanced elution into the storage solution during the autoclaving process. Over the course of the study (from day 2 to 20), the amount of CSA-138 that eluted changed from approximately 1.4 to 1.2 μg/ml per day.

**[0075]** It was anticipated that an increase in the osmolality of an aqueous solution would decrease the solubility of CSA-13 and slow elution. We determined the elution profile in 10% TSB in PBS, and as expected elution decreased (Figure 5) to match that seen with lenses that had been autoclaved.

**[0076]** Because kill rates appeared to be happening at such low concentrations, we hypothesized that the presence of bacteria was influencing the elution of CSA-138 from lenses. To test this hypothesis, lenses were incubated with S. aureus or P. aeruginosa and elution was monitored. These experiments were performed for nine and eight days, respectively. Elution of

CSA-138 fluctuated substantially and to a much greater extent than outside of the presence of bacteria (Figures 6 and 7). Because of these variations, the experiments were shortened relative to elution experiments without bacteria. Though there was substantial variation in the elution in the presence of bacteria, it was possible to determine the significance of the differences in elution comparing samples with and without bacteria. After the first day, differences gave a p value of 0.05 and for many of the days, the p value was below 0.01. These results argue that bacteria impact elution of CSA-138 from lenses.

**[0077]** The MIC values of CSA-138 for S. aureus and for P. aeruginosa are 0.5 and 1.0 μg/ml, respectively. The elution of CSA-138 from lenses gives concentrations that are just able to eliminate the inocula introduced. Autoclaving the lenses, increasing the osmolality in the surrounding solution, and the presence of bacteria impact the elution profile modestly.

**[0078]** If one takes the elution profile given in Figure 5 and extends the trend until elution of CSA-138 drops below 1 μg/ml, this would require about 40 days (elution decreases from 1.4 to 1.2 μg/m1 per day between days two and 20; a decrease from 1.2 to 1.0 μg/ml per day would be expected to require another 19 days). Thus, it would be expected that elution of CSA-138 would be sufficient to eliminate reasonable inocula of bacteria for as many as 40 days. As noted in a previous report, elution of CSA-138 from lenses prevents colonization by S. aureus for 30 consecutive days and by P. aeruginosa for 19 days. These studies are performed with relatively high inocula ($10^6$ CFU), and it is anticipated that CSA-138 eluting after 30 days would be sufficient to eliminate smaller inocula.

**[0079]** Optimization of the structure of CSA-138 has yielded a potent antimicrobial agent that associates with contact lens material and elutes at the concentration necessary to eliminate substantial inocula of Gram-positive and -negative bacteria. Considering the number of bacteria to which lenses are typically exposed, it is likely that lower concentrations of CSA-138 could be used while continuing to prevent bacterial growth on lenses.

**[0080]** For purposes of this invention, "physiological conditions" are aqueous conditions where the pH, temperature, and salt concentrations are generally suitable for sustaining life (e.g., for many, but not all devices, physiological conditions is often a pH near 7, temperatures near 37 °C, and salt concentration near 150 mM).

**[0081]** The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

# CLAIMS

What is claimed is:

1. A ceragenin eluting hydrogel material, comprising

a hydrogel polymer;

a ceragenin mimetic compound incorporated into the hydrogel polymer and non-covalently bonded thereto, the ceragenin mimetic compound comprising a sterol backbone and a plurality of cationic groups.

2. A ceragenin eluting hydrogel material as in claim 1, wherein the ceragenin mimetic compound elutes from the hydrogel in excess water at a rate of 0.1 – 100 μg/ml, 0.5 – 50 μg/ml, or 1 – 10 μg/m1 at 3 days, one week, or one month and/or over a period of 3 days, one week and/or one month.

3. A ceragenin eluting hydrogel material as in claim 1, wherein the hydrogel material further comprises at least 50%, 90%, or 99% by weight water.

4. A ceragenin eluting hydrogel material as in claim 3, wherein the water is a saline water.

5. A ceragenin eluting hydrogel material as in claim 1, wherein the hydrogel polymer includes polyvinyl alcohol, sodium polyacrylate, an acrylate polymer, polyethylene oxide, polyAMPS, polyvinylpyrrolidone, polyacrylamide, silicone, agarose, methylcellulose, hyaluronan, or a combination thereof.

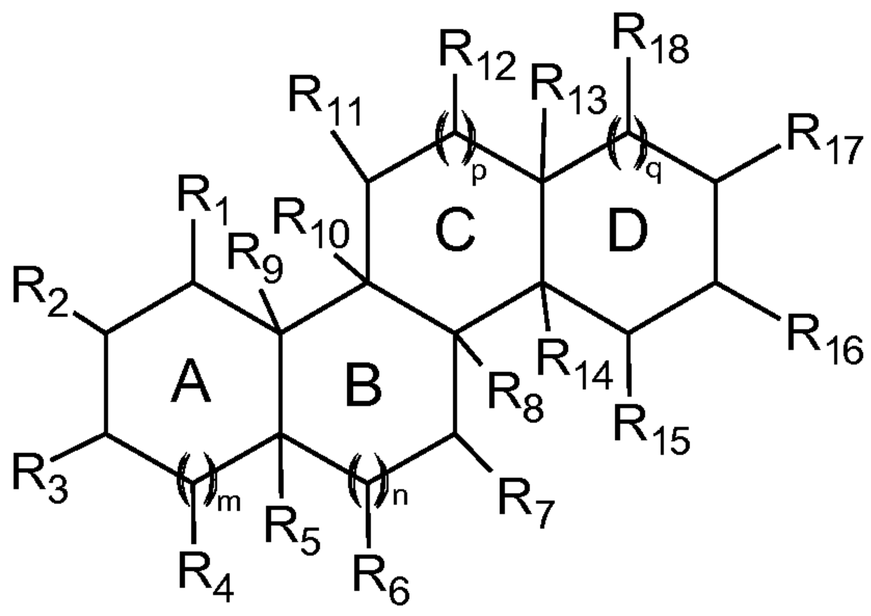
6. A ceragenin eluting hydrogel material as in claim 1, wherein the hydrogel includes a hydrophobic region.

7. A ceragenin eluting hydrogel material as in claim 1, wherein the ceragenin mimetic compound includes a hydrophobic substituent attached to the sterol backbone.

8. A ceragenin eluting hydrogel material as in claim 7, wherein the hydrophobic substituent is linked to the $R_{17}$ position of the ceragenin of Formula I.

9. A ceragenin eluting hydrogel material as in claim 7, wherein hydrophobic substituent includes a carbon chain of at least 9 carbons.

10. A ceragenin eluting hydrogel material as in claim 7, having a structure as in Formula I:




Formula I

wherein,

$q = 0$ and $p = 1$;

$R_3$, $R_7$, and $R_{12}$ independently include a cationic group; and

$R_{17}$ is a hydrophobic substituent having a hydrophobic group with a carbon chain of at least 11 atoms.

11. A medical device comprising a hydrogel material according to claim 1.

12. A medical device as in claim 11, wherein the medical device is selected from the group consisting of bone implant, bone pin, bone screw, tissue graft, endotracheal tube, coronary stent, peripheral stent, catheter, arterio-venous graft, by-pass graft, pacemaker or defibrillator lead, anastomotic clip, arterial closure device, patent foramen ovale closure device, and drug delivery balloon.

13. A medical device as in claim 11, wherein the hydrogel is coated on a substrate.

14. A medical device as in claim 11, wherein the type and concentration of the ceragenin compound is selected to provide a MIC sufficient to inhibit the growth of Staphylococcus aureus type MRSA or VRSA under physiological conditions.

14. The medical device as in claim 11, wherein the hydrogel is formed into a contact lens.

15. The medical device of claim 14, wherein the hydrogel includes silicone.

16. A method of making a ceragenin mimetic compound comprising:

providing a hydrogel material comprising a hydrogel polymer; and

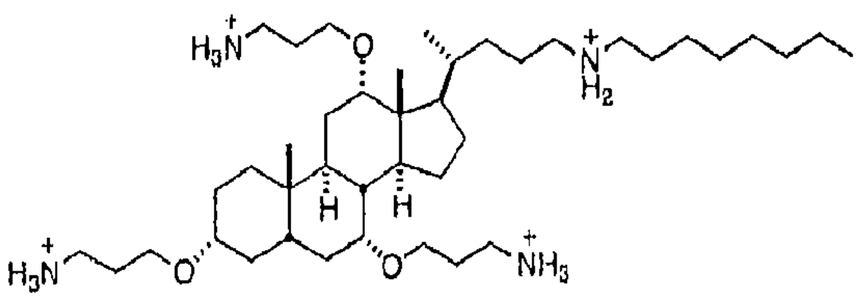
incorporating a ceragenin compound into the hydrogel material so as to non-covalently bond the ceragenin compound to the hydrogel polymer, the ceragenin compound comprising a sterol backbone and a plurality of cationic groups.

17. The method of claim 16, wherein the ceragenin mimetic compound is included in the hydrogel at a concentration sufficient to elute from the hydrogel in excess water at a rate of 0.1 – 100 μg/ml, 0.5 – 50 μg/ml, or 1 – 10 μg/m1 at 3 days, one week, or one month and/or over a period of 3 days, one week and/or one month.

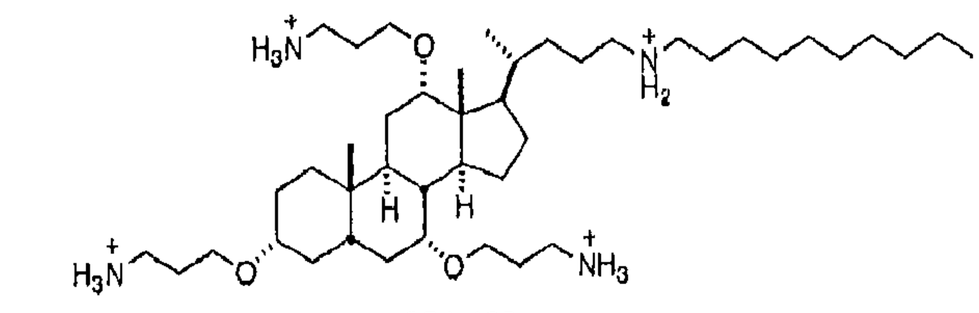
18. A method as in claim 16, wherein the ceragenin mimetic compound is incorporated into the hydrogel by imbuing the ceragenin mimetic compound dissolved in a solvent.

19. A method as in claim 16, wherein the ceragenin mimetic compound is incorporated into a dry hydrogel polymer.

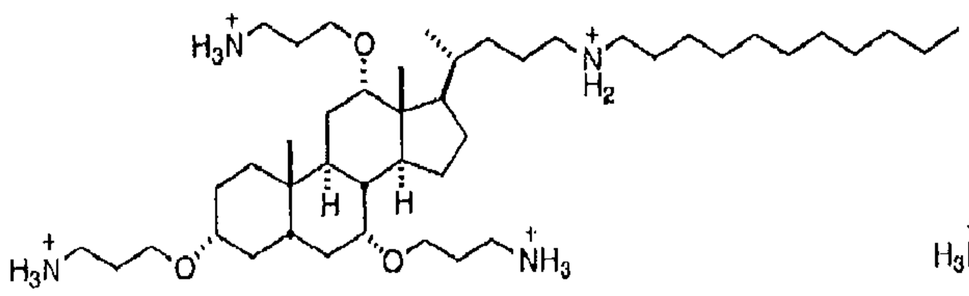
20. A method as in claim 16, wherein the hydrogel is polymerized from one or more monomers in the presence of a ceragenin, thereby incorporating the ceragenin into the hydrogel polymer, wherein the ceragenin is non-covalently bonded to the one at least one of the one or more monomers.



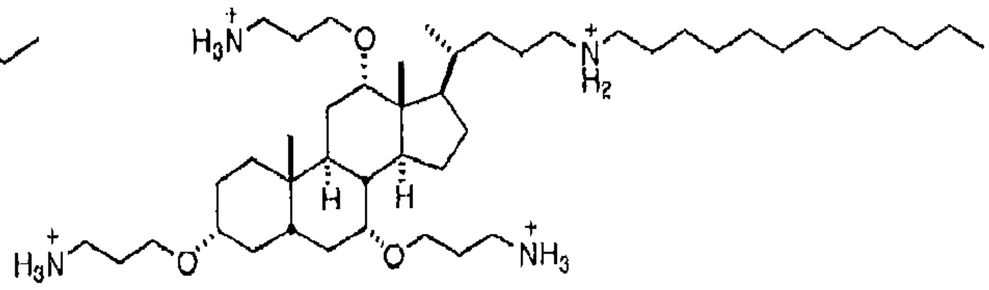
CSA-13
CLogP: 5.228



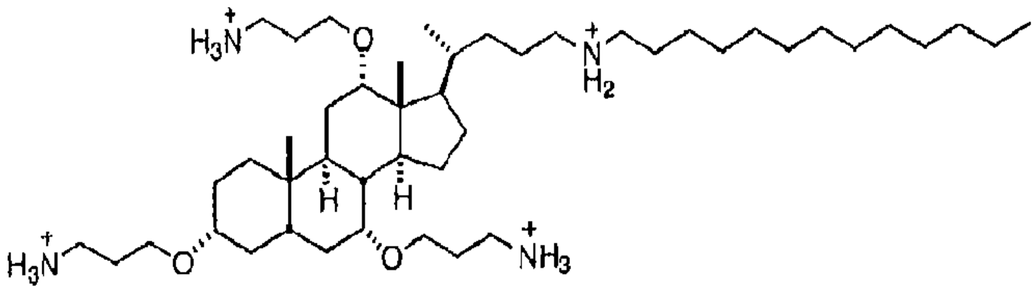
CSA-136
CLogP: 6.286



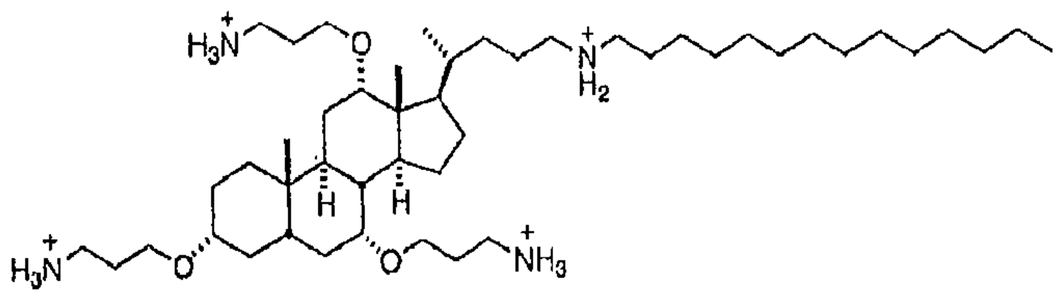
CSA-137
CLogP: 6.815



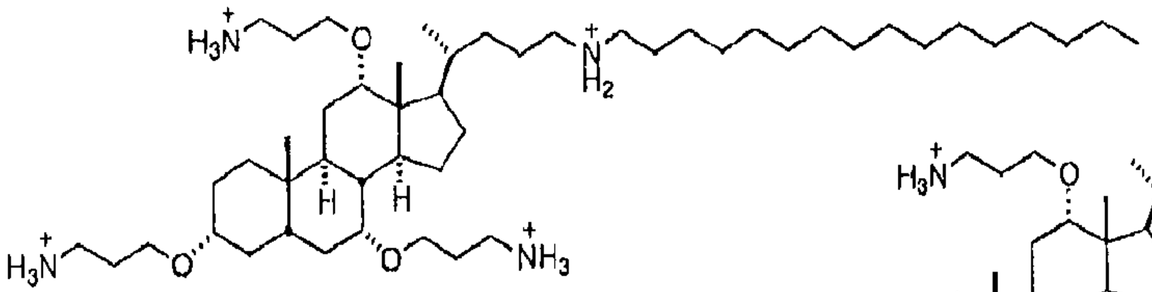
CSA-131
CLogP: 7.344



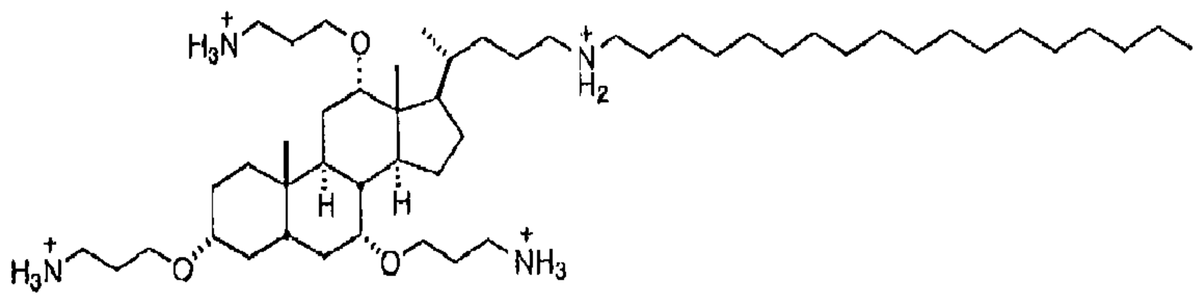
CSA-138
CLogP: 7.873



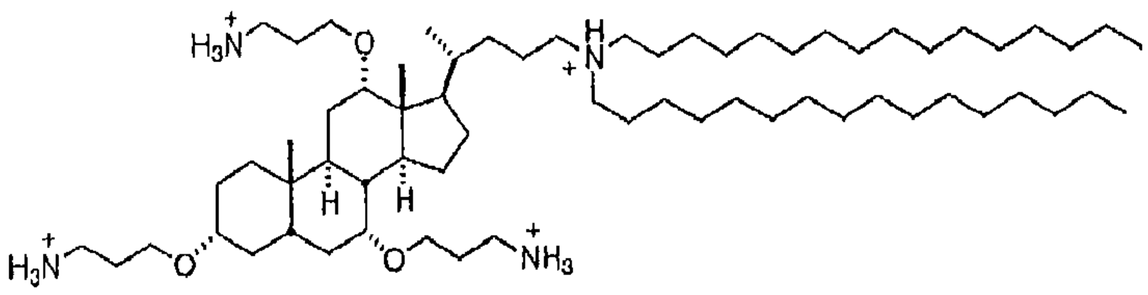
CSA-134
CLogP: 8.402



CSA-135
CLogP: 9.46

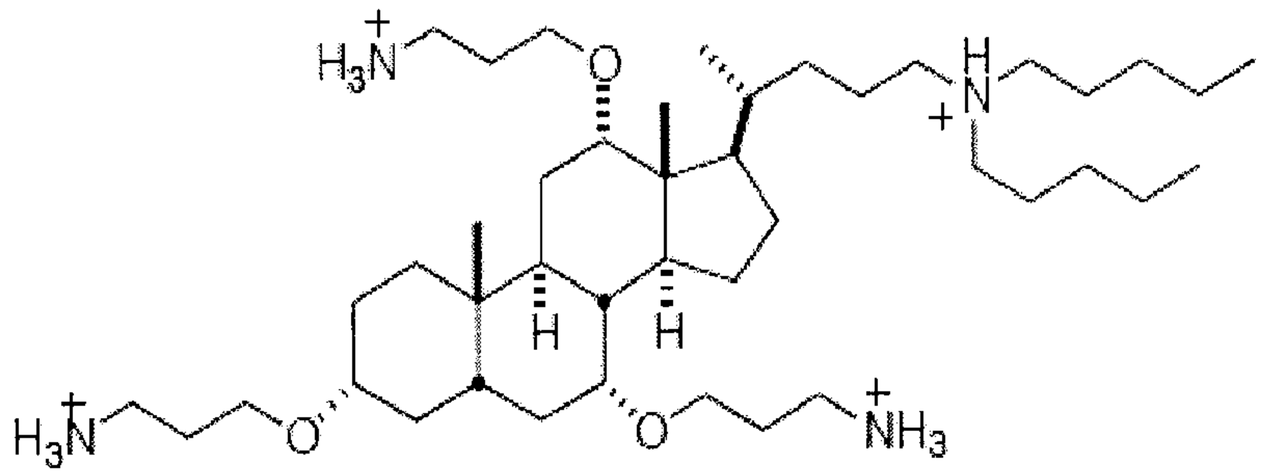


CSA-132
CLogP: 10.518

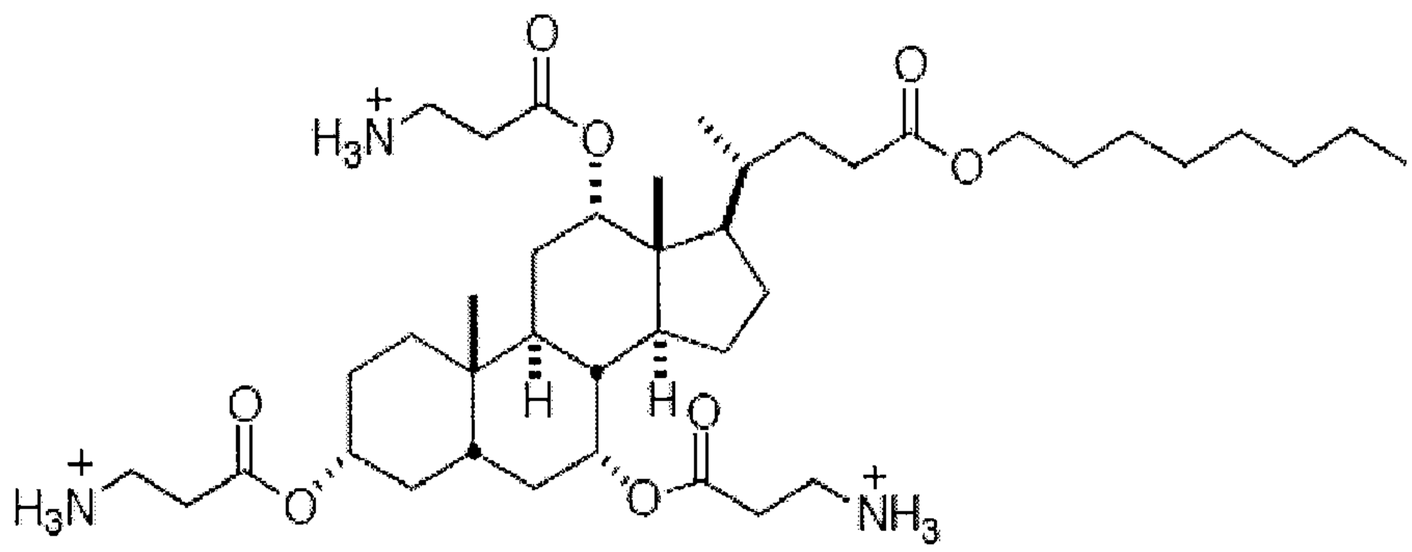


CSA-133
CLogP: 17.7238

Figure 1

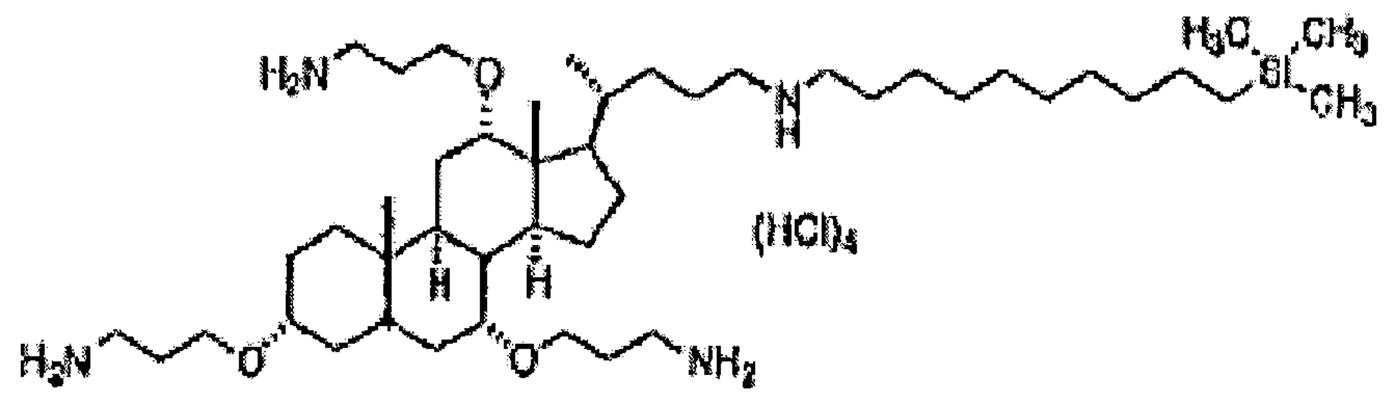


CSA-90
CLogP: 6.0858

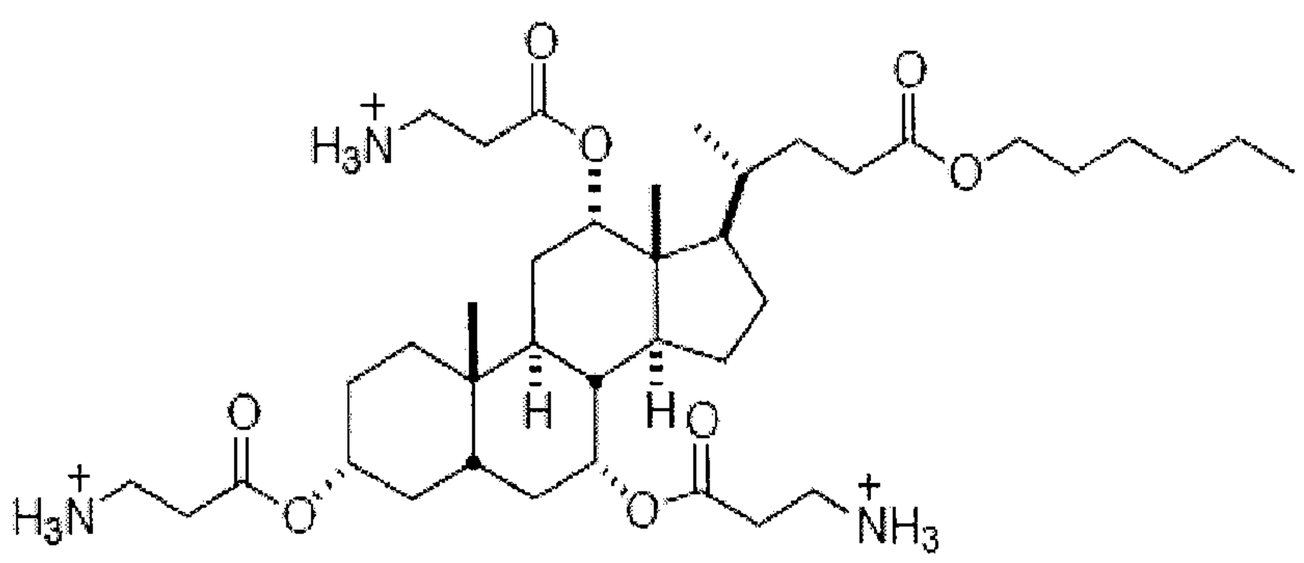


CSA-44
CLogP: 6.351

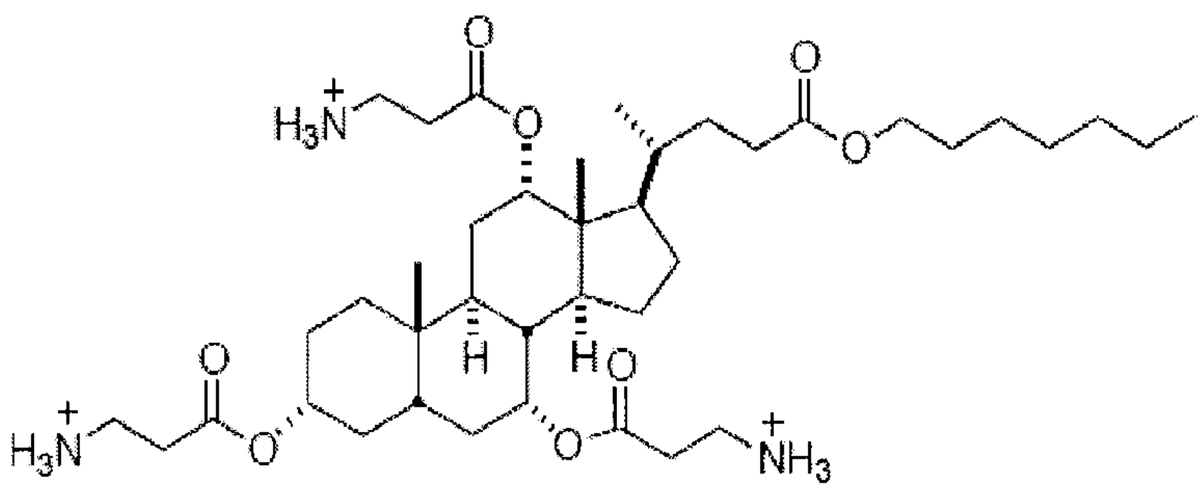
**FIGURE 1** (con't)




CSA-139




CSA-142
CLogP: 5.293




CSA-146
CLogP: 5.822

**FIGURE 1 (con't)**

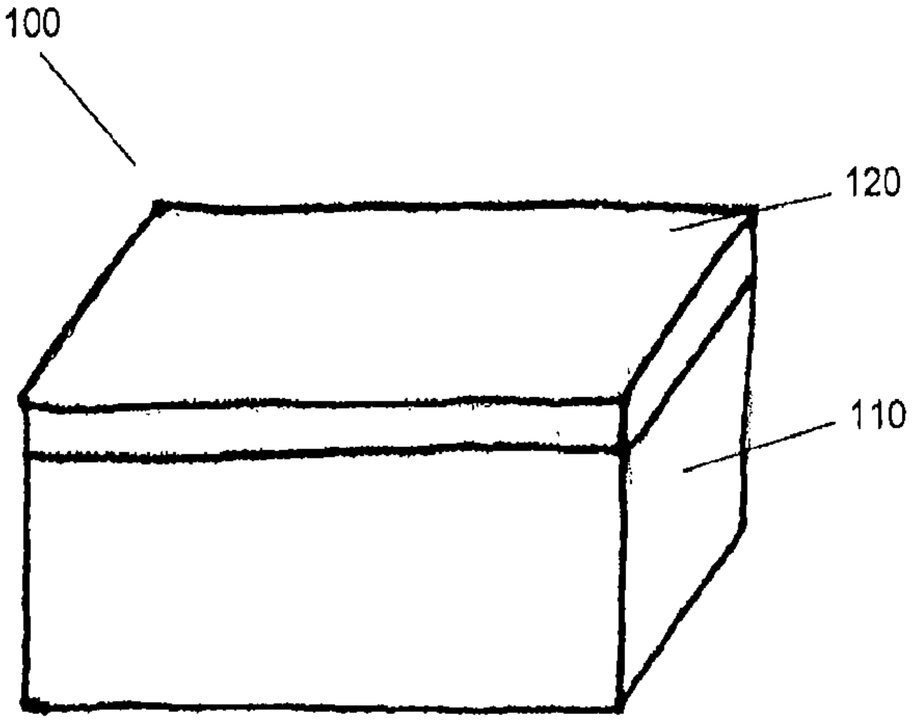



Figure 2




Figure 3

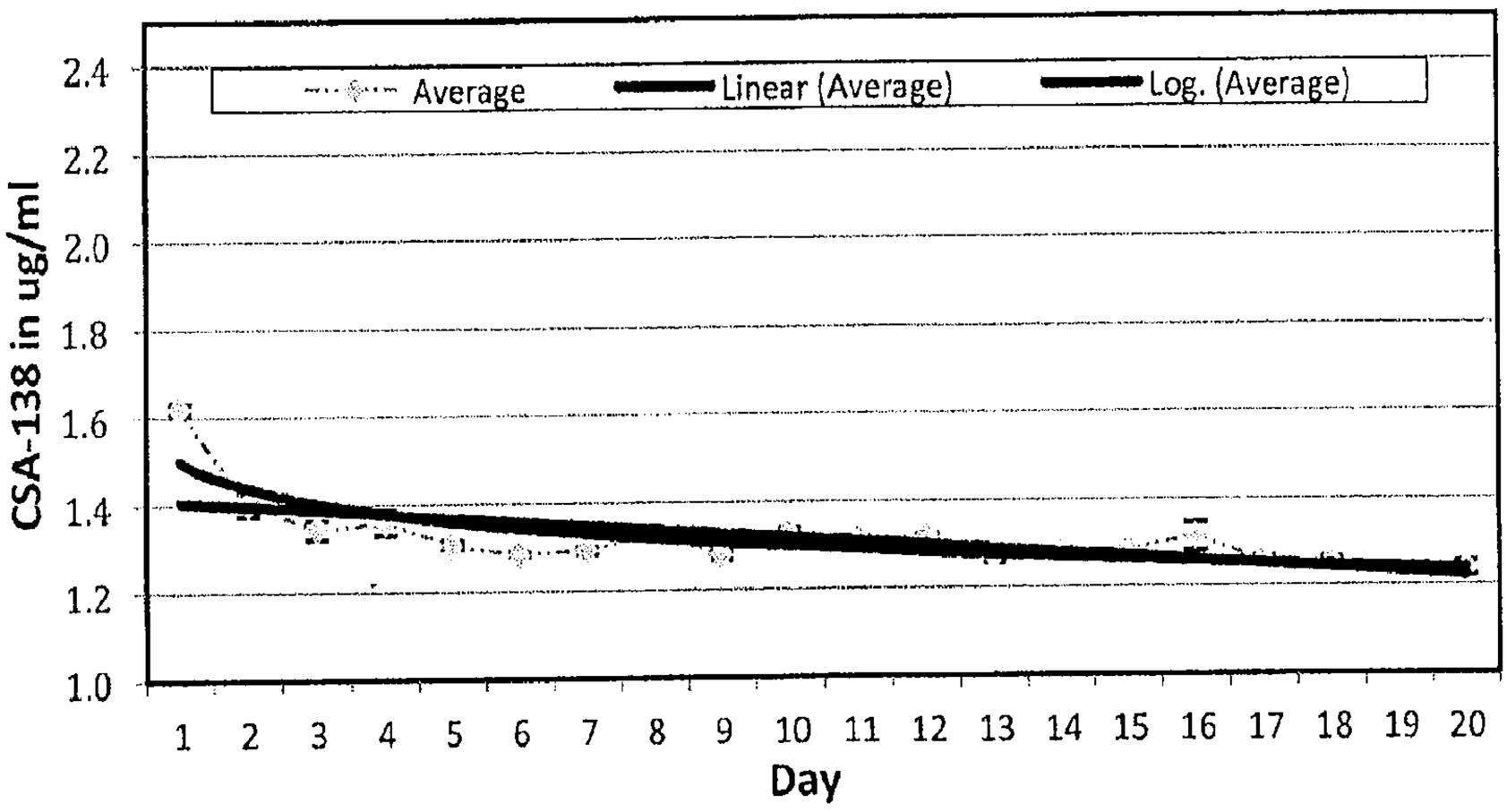



Figure 4

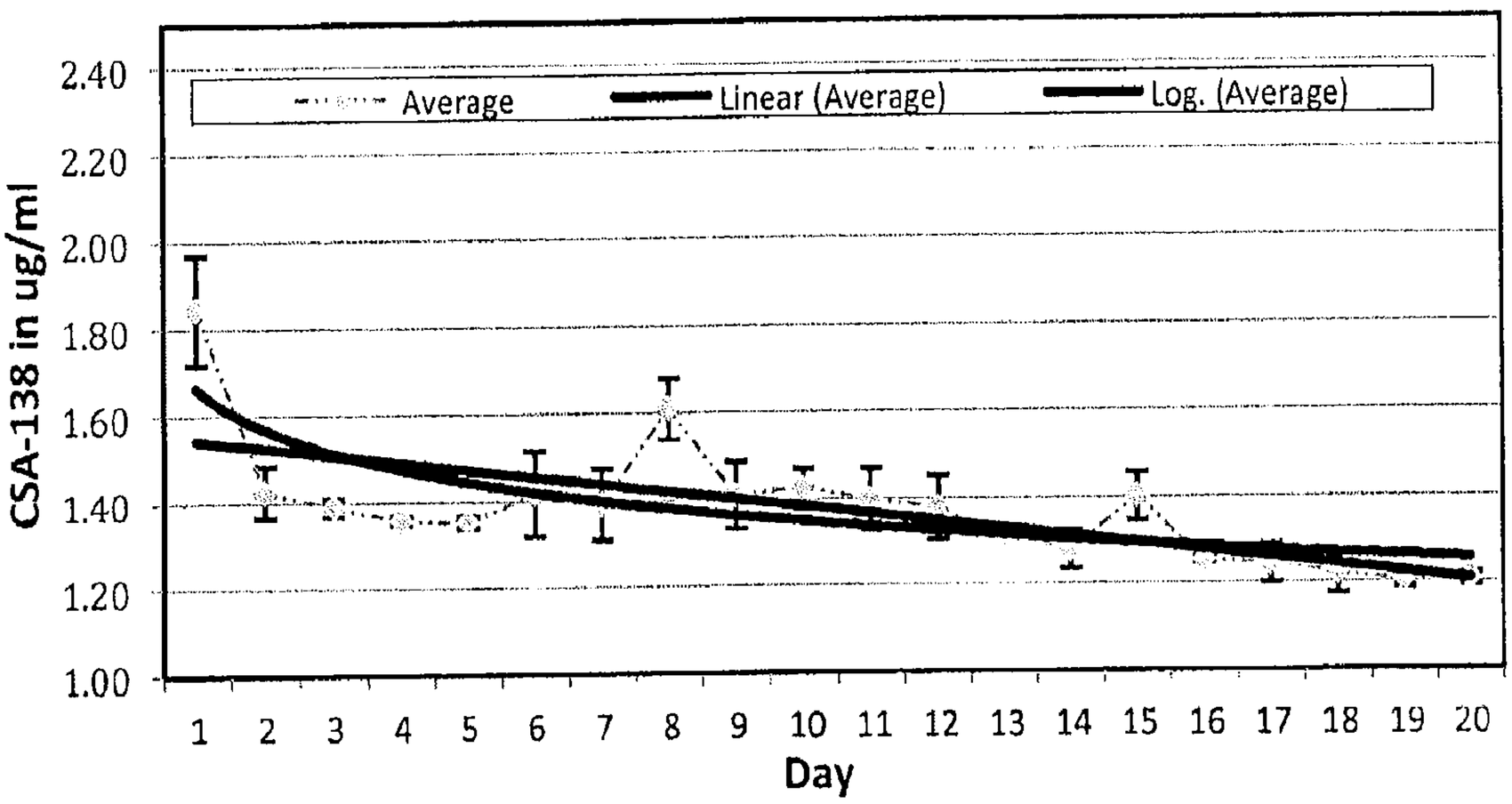
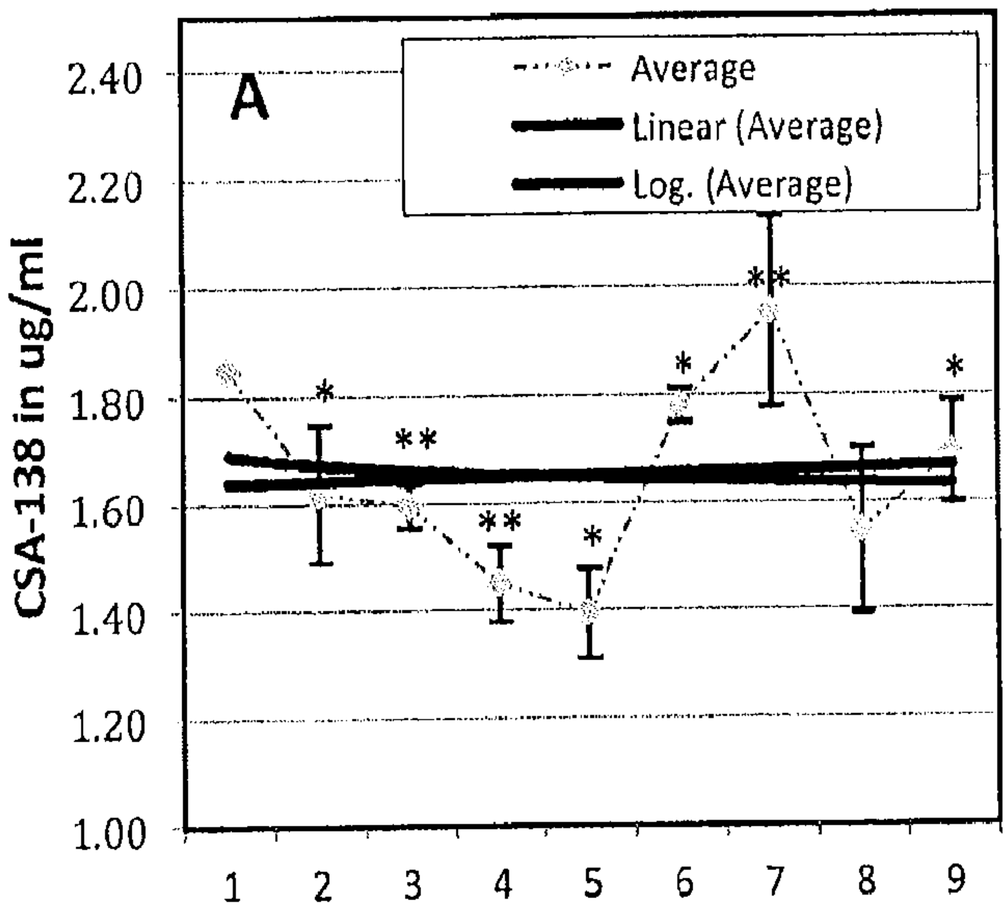



Figure 5




Figures 6

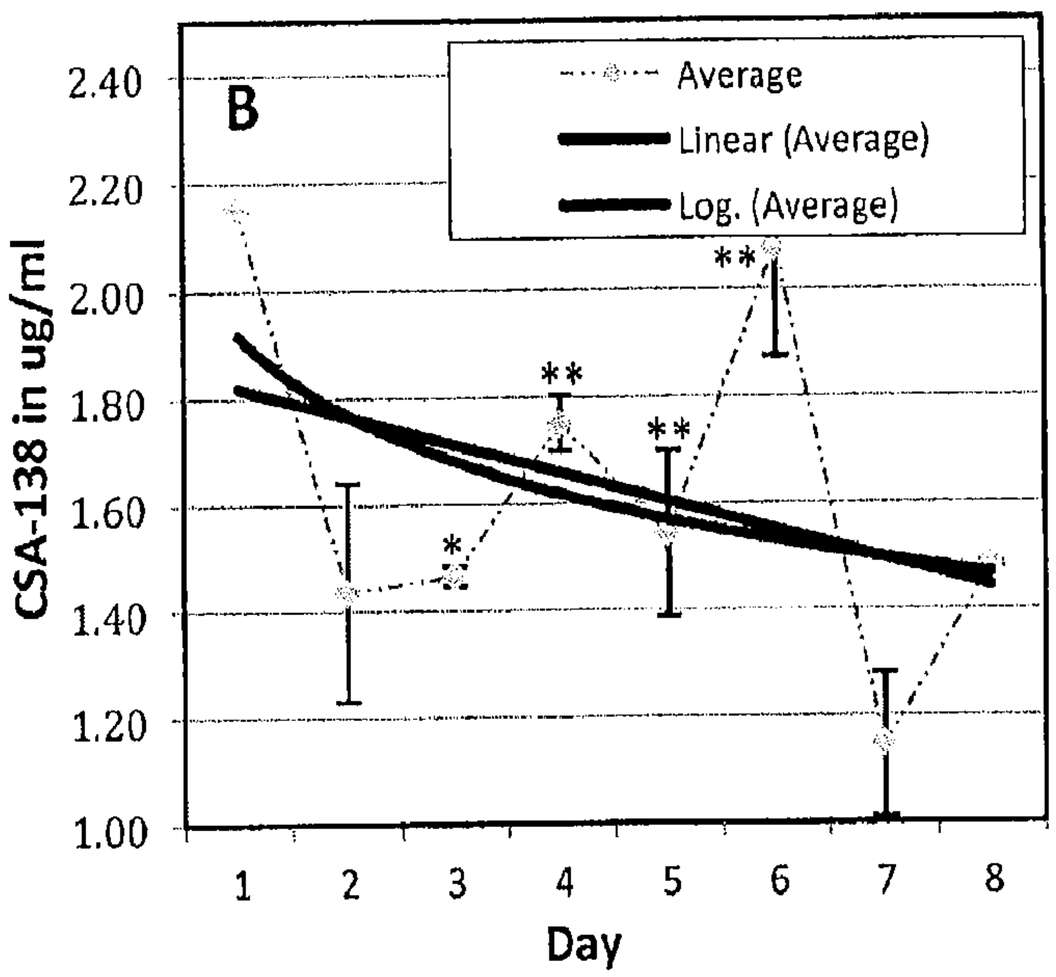



Figure 7