FORMULATIONS OF DEOXYCHOLIC ACID AND SALTS THEREOF

FIELD OF THE INVENTION

[0001] This invention relates to aqueous pharmaceutical formulations containing very low concentrations of a salt of deoxycholic acid ("DCA"), wherein the formulation is maintained at a pH such that precipitation of DCA is substantially inhibited. In a preferred embodiment, the pharmaceutical composition is buffered to maintain a physiologically acceptable pH such that the composition is suitable for injection.

BACKGROUND

[0002] Recently published literature reports that the aqueous solutions of DCA has fat removing properties when injected into fatty deposits *in vivo* (*See*, WO 2005/117900 and WO 2005/112942, US2005/0261258; US2005/0267080; US2006/127468; and US2006/0154906). DCA injected into fat tissue degrades fat cells *via* a cytolytic mechanism to provide the desired aesthetic results.

[0003] Notwithstanding the benefits of aqueous formulations of DCA, it has been found that at low concentrations of DCA (i.e., less than or about 2% w/v;) in aqueous solutions which optionally contain benzyl alcohol, forms a precipitate after storage over a period of time. Surprisingly, it has been found that the lower the concentration DCA, the higher is the rate of precipitation notwithstanding any significant change in the pH of the solution. This precipitation at very low concentrations is a problem for commercialization as a precipitate is counter-indicated for subcutaneous injections of DCA.

[0004] In each treatment regimen, the current clinical trials of aqueous formulations of DCA employ multiple injections of small amounts of the aqueous formulation into different sites defining the fat deposit to be treated.

[0005] As is apparent, aqueous formulations of DCA used in such treatments overlap with the problems arising from precipitation of the DCA. That is to say that an initially clear aqueous solution of DCA when stored for a period of time, will form a precipitate at commercially relevant concentrations of DCA notwithstanding the fact that the pH of these solutions are between about 7.50 and about 8.0 which are substantially above the pKa of deoxycholic acid.

[0006] Thus, there is a need for stabilizing the low concentration aqueous solutions of deoxycholic acid or a salt thereof against precipitation during a shelf life of at least 2 months.

SUMMARY OF THE INVENTION

[0007] It has been surprisingly found that aqueous solutions of a salt of deoxycholic acid at concentrations of from about 0.4% w/v to less than about 2% w/v can be stabilized by adjusting the pH of the solution to above about 8 and preferably from a physiologically acceptable pH from about 8.1 to about 8.5. The pH is preferably maintained within this range by using buffers.

[0008] Accordingly, in one of its composition aspects, this invention is directed to aqueous formulations consisting essentially of a salt of deoxycholic acid at a concentration of from about 0.4% w/v to less than about 2% w/v and optionally a preservative effective amount of benzyl alcohol which formulations are stabilized against precipitation by adjusting the pH of the initially formed clear solution to a pH of from about 8.1 to about 8.5. In another embodiment, this invention is directed to an aqueous formulation consisting essentially of a salt of deoxycholic acid at a concentration of from about 0.5% w/v to about 1% w/v and optionally a preservative effective amount of benzyl alcohol which formulations are stabilized against precipitation by adjusting the pH of the initially formed clear solution to a pH of from about 8.1 to about 8.5.

[0009] In another embodiment, this invention is directed to an aqueous formulation consisting essentially of:

a sterile aqueous solution buffered to a pH of about 8.3;
about 0.5% w/v or about 1% w/v of a salt of deoxycholic acid;
optionally a preservative effective amount of benzyl alcohol; and
about 1% w/v of sodium chloride,

wherein the composition is stable against precipitation.

[0010] Also disclosed herein is a method for stabilizing an aqueous composition of a salt of deoxycholic acid against precipitation during storage wherein the concentration of the deoxycholic acid salt is an amount effective to lyse fat cells provided that the amount of the deoxycholic acid salt ranges from about 0.4% w/v to less than 2% w/v, which method comprises:

forming an aqueous solution of a deoxycholic acid salt at an initial pH above its pKa; adjusting the pH of the aqueous solution to a pH of from about 8.1 to about 8.5; and optionally including a sufficient amount of buffer to maintain the pH at from about 8.1 to about 8.5.

[0011] Also disclosed herein is a method to lyse a fat cell comprising administering to said cell a composition according to this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1 illustrates (67 X enhancement) that an aqueous composition of water and 0.9 % w/v benzyl alcohol and no sodium deoxycholate contains only a trace of precipitate, which is presumed to be bioprene tubing particles.

[0013] Fig. 2 illustrates (67 X enhancement) that an aqueous composition of water, 0.5 % w/v sodium deoxycholate and 0.9 % w/v benzyl alcohol contains significant amounts of precipitate which is presumed to be deoxycholate crystals.

[0014] Fig. 3 illustrates (67 X enhancement) that an aqueous composition of water, 1 % w/v sodium deoxycholate and 0.9 % w/v benzyl alcohol contains significant amounts of precipitate, albeit less than that of 0.5 % w/v sodium deoxycholate. As before, the precipitate is presumed to be deoxycholate crystals.

[0015] Fig. 4 illustrates (67 X enhancement) that an aqueous composition of water, 2 % w/v sodium deoxycholate and 0.9 % w/v benzyl alcohol contains significant amounts of precipitate but substantially less than that observed in Figs. 2 and 3.

DETAILED DESCRIPTION

[0016] As used herein, certain terms have the following defined meanings.

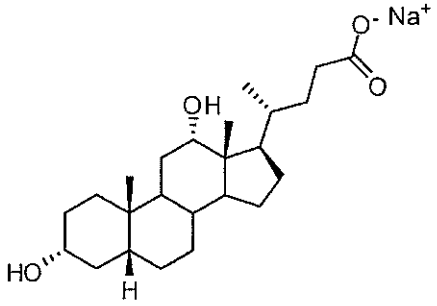
[0017] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (–) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". The term "about" also includes the exact value "X" in addition to minor increments of "X" such as "X + 0.1" or "X – 0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0018] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude others.

[0019] "Consisting essentially of" when used to define compositions and methods, shall mean excluding any active ingredients. An "active ingredient" is a substance intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or any function of the human body. Thus, for example, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like but would exclude enzymes such as phosphatases, and proteins. Non-limiting examples of such proteins are heparin, albumin, and the like

[0020] "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0021] As used herein, the term "salt of deoxycholic acid" or "a salt thereof" refers to pharmaceutically acceptable salts of (4R)-4-((3R,5R,10S,12S,13R,17R)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate having an alkali metal or an ammonium ion as the cation. Preferred are alkali metal salts, with sodium salts being more preferred.



[0022] Sodium deoxycholate or sodium (4*R*)-4-((3*R*,5*R*,10*S*,12*S*,13*R*,17*R*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)pentanoate can be prepared according to the methods disclosed in PCT/US2010/061150 titled "Methods for the Purification of Deoxycholic Acid," filed on December 17, 2010.

[0023] As used herein, the term "aqueous pharmaceutical formulation" refers to a composition of a deoxycholic acid or a salt thereof in water suitable for administration to a patient preferably via subcutaneous injection from a syringe.

[0024] As used herein, the term "buffer" refers to an aqueous solution comprising a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. A buffer has the property that the pH of the solution changes very little when a small amount of acid or base is added to it. Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications. Examples of suitable buffers include phosphate buffers and those known in the literature (*see*, for example, Troy, D.B., ed. (2005) Remington: The Science and Practice of Pharmacy, 21st ed., Lippincott Williams & Wilkins).

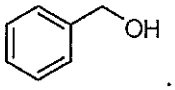
[0025] As used herein, the term "base" refers to various typically water-soluble compounds, molecules or ions that in solution have a pH greater than 7. Such compounds, molecules or ions are able to take up a proton from an acid or are able to give up an unshared pair of electrons to an acid. Examples of suitable bases include metal carbonates and bicarbonates, for example sodium carbonate, calcium carbonate, magnesium carbonate, zinc carbonate, sodium bicarbonate and the like; and metal hydroxides, for example sodium hydroxide, potassium hydroxide, and the like, such as those known in the literature (*see*, for example, Troy, D.B., ed. (2005) Remington: The Science and Practice of Pharmacy, 21st ed., Lippincott Williams & Wilkins).

[0026] As used herein, the term "metal carbonates" refers to the metal salt of CO_3^{2-}. For example, sodium carbonate, calcium carbonate, magnesium carbonate, zinc carbonate, and the like.

[0027] As used herein, the term "metal bicarbonates" refers to the metal salt of HCO_3^-. For example, sodium bicarbonate, and the like.

[0028] As used herein, the term "metal hydroxides" refers to the metal salt of ^-OH. For example, sodium hydroxide, potassium hydroxide, and the like.

[0029] As used herein, the terms "sterile water" or "water for injection" refer to a sterile, nonpyrogenic preparation of water for injection which contains no bacteriostat, antimicrobial agent or added buffer. In general, the osmolar concentration of additives totals at least 112 mOsm/liter (two-fifths of the normal osmolarity of the extracellular fluid -280 mOsm/liter).

[0030] As used herein, the term "benzyl alcohol" refers to the compound 

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[0031] As used herein, the term "precipitation" refers to the formation of a solid in a solution and is readily differentiated from gel formation.

[0032] As used herein, the term "solution" refers to a substantially homogeneous mixture comprising two or more substances dissolved in a solvent.

[0033] As used herein, the terms "substantially inhibit precipitation" and "inhibits precipitation" means to inhibit most or all visible precipitation so as to maintain homogeneity for a period of time ranging from at least 1 month to at least 1 year.

[0034] As used herein, the term "relative standard deviation for homogeneity" or "H_E" refers to the value obtained by dividing the standard deviation of the homogeneity by the absolute value of the mean. An H_E less than 10 indicates very good homogeneity.

Formulations

[0035] Knowledge about the chemical and physical stability of a drug composition in the desired media for delivery is valuable. In the longer term, the stability of the composition will dictate the shelf life of the marketed product. It is preferable that the active ingredient in a pharmaceutical composition be at the required concentration when administered to a patient.

[0036] In the discussion below, sodium deoxycholate is recited for illustrative purposes only and it is understood that other pharmaceutically acceptable salts of deoxycholic acid can be used interchangeably with the sodium salt.

[0037] Current clinical methods for the administration of a sodium deoxycholate to a patient to dissolve fat include the administration via subcutaneous injections of a low concentration (i.e., <2% w/v) of an aqueous solution of the salt of deoxycholic acid where the amount of the salt of deoxycholic acid is sufficient to lyse fat cells (about 0.4% w/v and higher). At such concentrations, as it has been shown that the low concentration is beneficial for the effective and safe removal of fat deposits in the body. However, it has been observed that a precipitate forms at such relatively low concentrations of sodium deoxycholate in aqueous media. This precipitation results in a limited shelf life of aqueous solutions of

sodium deoxycholate, even at cold temperatures (3-5 °C). In one embodiment, the sodium salt can be replaced by another alkali metal salt.

[0038] This instability of aqueous solutions of sodium deoxycholate can be circumvented by the preparation of an aqueous solution of sodium deoxycholate at a concentration of about 5% to about 16% w/v, and having the practitioner dilute the pharmaceutical composition of the sodium deoxycholate solution just prior to use. Whereas this dilution method is effective to allow for both storage stability and effective patient dosing, it is not ideal as a method for routine use especially if a sterile injectable solution of no more than about 2 mL is required. Moreover, current clinical plans include up to 50 injections per treatment session.

[0039] It has been found that aqueous formulations of sodium deoxycholate at concentrations ranging from about 0.4% w/v to less than about 2% w/v can be stabilized by adjusting the pH of the solution. This invention is directed to an aqueous formulation consisting essentially of a salt of deoxycholic acid at a concentration ranging from about 0.4% w/v to less than about 2% w/v and optionally a pharmaceutically acceptable excipient such as a preservative effective amount of benzyl alcohol and/or a pH adjusting buffer, wherein said formulation is maintained at a pH of about 8.1 to about 8.5.

[0040] In another embodiment, the aqueous formulation is lyophilized to provide for a stable composition which is ready to be reconstituted by addition of the appropriate amount of water. In this embodiment, this invention comprises lyophilized compositions as described above which optionally further contain a lyophilization aid.

[0041] In one embodiment, the aqueous formulation contains about 0.5% w/v of a salt of deoxycholic acid. In another embodiment, the aqueous formulation contains about 1% w/v of a salt of deoxycholic acid.

[0042] In a further embodiment, the water employed in the aqueous formulation is sterile water. In still a further embodiment, the preservative effective amount of benzyl alcohol is about 0.9% w/v benzyl alcohol and the pH of the formulation is about 8.3. In one embodiment, said salt is an alkali metal salt. In another embodiment, said salt is a sodium salt.

[0043] In one embodiment, the pharmaceutical formulations disclosed herein are suitable for injection into a human. The method of injection can be any type of injection, such as subcutaneous injection, as well as other forms of injection.

[0044] In one preferred aspect of this invention, the precipitation of the salt of deoxycholic acid in the aqueous formulation is inhibited for a period of at least about six months. In another aspect, the precipitation is inhibited for a period of at least about one year. In yet another aspect, the precipitation is inhibited for a period of at least about two years.

[0045] It is contemplated that when stored at various temperatures, for example at ambient or cold temperatures, the formulation can have an increased shelf life. In certain embodiments, the composition is stored at a temperature of from about 17 °C to about 27 °C. In some embodiments, the temperature of the formulation is increased to a temperature of about 25 °C to about 37 °C. In other embodiments, the formulation is stored at a temperature of from about 2 °C to about 8 °C.

[0046] In certain embodiments, the pH of the formulation ranges from about 8.1 to about 8.5. In one embodiment, the pH of the composition is about 8.1, or alternatively, about 8.2, or alternatively, about 8.3, or alternatively, about 8.4, or alternatively, about 8.5. In a preferred embodiment, the pH of the formulation is about 8.3.

[0047] In one embodiment, the pH is established by the use of a base. It is contemplated that any base can be used to increase the pH of the composition provided that it does not react with the sodium deoxycholate and will not cause harm to the patient. In some embodiments, the base is selected from the group consisting of metal carbonates, metal bicarbonates, metal hydroxides, or a mixture thereof. Examples of bases include, but are not limited to, a base selected from the group consisting of sodium carbonate, calcium carbonate, magnesium carbonate, zinc carbonate, sodium bicarbonate, sodium hydroxide and potassium hydroxide or a mixture thereof. In one embodiment, the base is sodium hydroxide.

[0048] In certain cases, the pH of the composition may be maintained at the desired pH during storage with the use of a buffer. Various buffers are known in the art and it is contemplated that any buffer having buffering capacity at the desired pH can be used in the formulations disclosed herein. In one embodiment, the buffer is a phosphate buffer. The amount of phosphate in the composition can be determined to provide a desired pH and salt

concentration. In one embodiment, the composition comprises about 10 mM phosphate buffer. In a preferred embodiment, the composition comprises about 10 mM dibasic sodium phosphate buffer.

[0049] In some embodiments, the composition comprises at least one excipient to aid in achieving a composition with desired properties, such as increased solubility, preservability or to provide an isotonic solution. Such excipients are known in the art. In one embodiment, the composition comprises about 1% w/v sodium chloride. In another embodiment, the composition comprises about 0.9% w/v benzyl alcohol. In some embodiments, the composition comprises about 0.9% w/v benzyl alcohol and about 1% w/v sodium chloride.

[0050] In some embodiments, the pH of the composition is established by use of a base and optionally maintained by use of a buffer.

[0051] In a preferred embodiment, this invention provides a stabilized composition comprising:

a phosphate buffer of a pH of about 8.3;
about 0.5% w/v or about 1% w/v of sodium deoxycholate;
a preservative effective amount of benzyl alcohol; and
about 1% w/v of sodium chloride,

wherein the composition is stabilized against precipitation.

[0052] In a further embodiment, the phosphate buffer is 10 mM dibasic sodium phosphate buffer.

[0053] In one embodiment, the preservative effective amount of benzyl alcohol is about 0.9% w/v.

[0054] The formulations disclosed herein comprise from about 0.4% w/v to less than about 2% w/v of a salt of deoxycholic acid in water maintained at a pH sufficient to substantially inhibit precipitation of the salt of deoxycholic acid. The amount of precipitation or homogeneity of the composition can be measured using various methods. For example, it can be measured quantitatively using light scattering by illuminating the composition with a spectrophotometer. Or alternatively, the homogeneity can be measured qualitatively by observing the visual clarity of the solution with the eye. In some embodiments, the

composition has a relative standard deviation for homogeneity of less than about 5%. Alternatively, the composition has a relative standard deviation for homogeneity of less than about 4%, or alternatively, about 3%, or alternatively, about 2%, or alternatively, about 1%.

[0055] In another embodiment, this invention is directed to a composition consisting essentially of:

a sterile aqueous solution buffered to a pH of about 8.3;
about 0.5% w/v or 1% w/v of sodium deoxycholate;
about 0.9% w/v benzyl alcohol; and
about 1% w/v of sodium chloride,

wherein the composition is stable against precipitation.

[0056] In another embodiment, this invention is directed to a composition consisting of:

an aqueous solution buffered to a pH of about 8.3;
about 0.5% w/v or about 1% w/v of sodium deoxycholate;
about 0.9% w/v benzyl alcohol; and
about 1% w/v of sodium chloride,

wherein the composition is stable against precipitation.

[0057] In some embodiments, the solutions herein do not include lipids, phospholipids, or phosphatidylcholine. In some embodiments, the solutions herein include up to 5% w/w, w/v, or v/v lipids, specifically phospholipids, or more specifically phosphatidylcholine. Preferably, the amount of lipids used is less than that of sodium deoxycholate or another salt of deoxycholic acid .

[0058] In some embodiments, the aqueous pharmaceutical composition of the invention can further comprise a second therapeutic agent selected from the group consisting of: anti-microbial agents, vasoconstrictors, anti-thrombotic agents, anti-coagulation agents, suds-depressants, anti-inflammatory agents, analgesics, dispersion agents, anti-dispersion agents, penetration enhancers, steroids, tranquilizers, muscle relaxants, and anti-diarrhea agents. In some embodiments, a solution is in a container that contains up to 500 mL of solution. Such container can be a syringe or syringe-loadable container.

[0059] In some embodiments, the formulations further comprise a molecule known to cause fat to die by an orthogonal mechanism. Such molecules include neuropeptide Y (NPY)

antagonists including, but not limited to, NPY receptor antagonists, such as BIBP-3226 (Amgen), BIBO-3304 (Boehringer Ingleheim), BMS-192548 and AR-H040922 (Bristol-Myers Squibb), LY-357897 (Eli Lilly), 1229U91 and GW438014S (GlaxoSmithKline), JNJ-5207787 (Johnson & Johnson), Lu-AA-44608 (Lundbeck), MK-0557 (Merck NPY), NGD-95-1 (Neurgogen), NLX-E201 (Neurologix), CGP-71683 (Novartis), PD-160170 (Pfizer), SR-120819A, BIIE0246, and S.A.0204 (Sanofi Aventis), S-2367 (Shiongli), dihydropyridine and dihydropyridine derivatives that are NPY receptor antagonists, bicyclic compounds that are NPY receptor antagonists, carbazole NPY receptor antagonists, and tricyclic compounds that are NPY receptor antagonists (*See*, e.g., WO 2006/133160 and U.S. 6,313,128). Also contemplated are fat selective pro-apoptotic peptides such as the CKGGRAKDC peptide that homes to white fat vasculature (*See*, Kolonin M.G. *et al.*, Nat. Med., 2004, 10(6): 625-32).

[0060] Another aspect of the invention relates to mixing adipo-ablative bile acids, such as, deoxycholic acid (DCA) with agents that kill fat cells. In one aspect, this invention contemplates a means to enhance the aesthetic effects of deoxycholate injections by mixing into the deoxycholate injectate a molecule that causes fat to die by an orthogonal mechanism. Examples of such candidate molecules include, but are not limited to, neuropeptide Y (NPY) antagonists and fat selective pro-apoptotic peptides. Since fat cell killing may be required to mediate the desired effects, the effects of an agent with fat killing ability can be enhanced via the addition of a molecule with potent fat cell killing effects. Additionally, molecules that require access to the vasculature to kill (such as certain pro-apoptotic peptides that bind to proteins expressed on the luminal side of capillaries) can gain access to these proteins because deoxycholate may cause vascular leakage. Thus, such agents can be synergistic with deoxycholate potentially creating a more potent means to mediate body contouring in fewer therapeutic sessions.

[0061] Examples of NPY antagonists include, but are not limited to, NPY receptor antagonists, such as BIBP-3226 (Amgen), BIBO-3304 (Boehringer Ingleheim), BMS-192548 and AR-H040922 (Bristol-Myers Squibb), LY-357897 (Eli Lilly), 1229U91 and GW438014S (GlaxoSmithKline), JNJ-5207787 (Johnson & Johnson), Lu-AA-44608 (Lundbeck), MK-0557 (Merck NPY), NGD-95-1 (Neurgogen), NLX-E201 (Neurologix), CGP-71683 (Novartis), PD-160170 (Pfizer), SR-120819A, BIIE0246, and S.A.0204 (Sanofi Aventis), S-2367 (Shiongli), dihydropyridine and dihydropyridine derivatives that are NPY receptor antagonists, bicyclic compounds that are NPY receptor antagonists, carbazole NPY receptor

antagonists, and tricyclic compounds that are NPY receptor antagonists. *See, e.g.,* WO 2006/133160 and U.S. 6,313,128.

[0062] Exemplary fat selective pro-apoptotic peptides includes, but is not limited to, CKGGRAKDC peptide that homes to white fat vasculature. *See,* Kolonin M.G. *et al.*, Nat. Med. June 10(6):625-32 (2004).

[0063] Sodium deoxycholate or sodium (4*R*)-4-((3*R*,5*R*,10*S*,12*S*,13*R*,17*R*)-3,12-dihydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[a]phenanthren-17-yl)pentanoate can be prepared according to the methods disclosed in PCT/US2010/061150 titled "Methods for the Purification of Deoxycholic Acid," filed on December 17, 2010. Other salts of deoxycholic acid can be prepared likewise by the skilled artisan.

Methods

[0064] Disclosed herein are methods for stabilizing an aqueous formulation of deoxycholic acid salt against precipitation during storage wherein the concentration of or a salt thereof is an amount effective to lyse fat cells provided that the amount of salt ranges from about 0.4% w/v to about less than 2% w/v, which method comprises:

forming an aqueous solution of the salt of deoxycholic acid at an initial pH above its pKa;

adjusting the pH of the aqueous solution to a pH of from about 8.1 to about 8.5; and optionally including a sufficient amount of buffer to maintain the pH at from about 8.1 to about 8.5.

[0065] In one aspect of this invention, methods disclosed herein substantially stabilize the formulation of deoxycholic acid salt against precipitation over a period of time preferably for at least about six months. In another aspect, the methods stabilize the formulation of deoxycholic acid salt against precipitation for a period of at least about one year. In yet another aspect, the methods stabilize the formulation of deoxycholic acid salt against precipitation for a period of at least about two years.

[0066] It has been found that the pH of the solution can inhibit the precipitation of deoxycholic acid or a salt thereof at concentrations of from about 0.4% w/v to less than about 2% w/v in water to allow deoxycholic acid or a salt thereof, to be maintained in solution. In one embodiment, the pH is established by the use of a base. It is contemplated that any base

can be used to increase the pH of the composition provided that it does not react with deoxycholic acid or a salt thereof. In some embodiments, the base is selected from the group consisting of metal carbonates, metal bicarbonates, and metal hydroxides, or a mixture thereof. Examples of bases include, but are not limited to, a base selected from the group consisting of sodium carbonate, calcium carbonate, magnesium carbonate, zinc carbonate, sodium bicarbonate, sodium hydroxide and potassium hydroxide or a mixture thereof. In one embodiment, the base is sodium hydroxide.

[0067] In certain embodiments, the pH ranges from about 8.1 to about 8.5. In one embodiment, the pH of the composition is about 8.1, or alternatively, about 8.2, or alternatively, about 8.3, or alternatively, about 8.4, or alternatively, about 8.5. In a preferred embodiment, the pH of the aqueous solution is about 8.3.

[0068] In certain cases, the pH of the composition may need to be maintained with the use of a buffer. Various buffers are know in the art and it is contemplated that any buffer having buffering capacity at the desired pH can be used in the formulations disclosed herein. In one embodiment, the buffer is a phosphate buffer. The amount of phosphate required to provide a desired pH and salt concentration can be calculated using methods well known in the art. In one embodiment, the composition comprises about 10 mM phosphate buffer. In another embodiment, the phosphate buffer is 10 mM dibasic sodium phosphate buffer.

[0069] In certain cases, the pH is established by use of a base and optionally maintained by use of a buffer.

[0070] In one embodiment, the methods disclosed herein provide formulations which are suitable for injection into a human. The method of injection can be any type of injection, such as subcutaneous injection, as well as other forms of injection. Therefore, in some embodiments, the aqueous solution comprises sterile water or water for injection (WFI).

[0071] In one aspect, it may be that one or more excipients are used to maintain the solubility, or increase the preservability of deoxycholic acid salt present in the formulation. In one embodiment, the method comprises adding about 1% w/v benzyl alcohol. In some embodiments, the formulation also comprises at least one excipient to aid in achieving an isotonic solution. Such excipients are known in the art. In one embodiment, the method comprises adding about 1% w/v sodium chloride. In some embodiments, the method

comprises adding both 1% w/v benzyl alcohol and 1% w/v sodium chloride. In some embodiments, the method comprises adding both 0.9% w/v benzyl alcohol and 0.9% w/v sodium chloride. Using the methods disclosed herein, an aqueous solution comprising less than about 2% w/v of deoxycholic acid salt is maintained at a pH sufficient to substantially inhibit precipitation of deoxycholic acid salt. The amount of precipitation or homogeneity of the composition can be measured using various methods. For example, it can be measured quantitatively by measuring the light scattering via illumination by a spectrophotometer. Or alternatively, the homogeneity can be measured qualitatively by simply observing the visual clarity of the solution with the eye. In some embodiments, the method provides a pharmaceutical composition having a relative standard deviation for homogeneity of less than about 5%. Alternatively, the relative standard deviation for homogeneity of less than about 4%, or alternatively, about 3%, or alternatively, about 2%, or alternatively, about 1%.

[0072] The storage temperature can assist in maintaining the solubility of deoxycholic acid salt in the formulation. In certain embodiments, the storage temperature is from about 17 °C to about 27 °C. In some embodiments, the storage temperature is about 25 °C to about 37 °C. In other embodiments, the storage temperature is from about 2 °C to about 8 °C.

[0073] It is contemplated that the concentration of the salt of deoxycholic acid in the formulation is about 0.5% w/v, or alternatively about 0.7% w/v, or alternatively about 1% w/v, or alternatively about 1.2% w/v, or alternatively about 1.4% w/v, or alternatively less than about 2% w/v. In a preferred embodiment, the salt of deoxycholic acid is sodium deoxycholate. In another preferred embodiment, the composition comprises 0.5% w/v of sodium deoxycholate. In another preferred embodiment, the composition comprises 1% w/v of sodium deoxycholate.

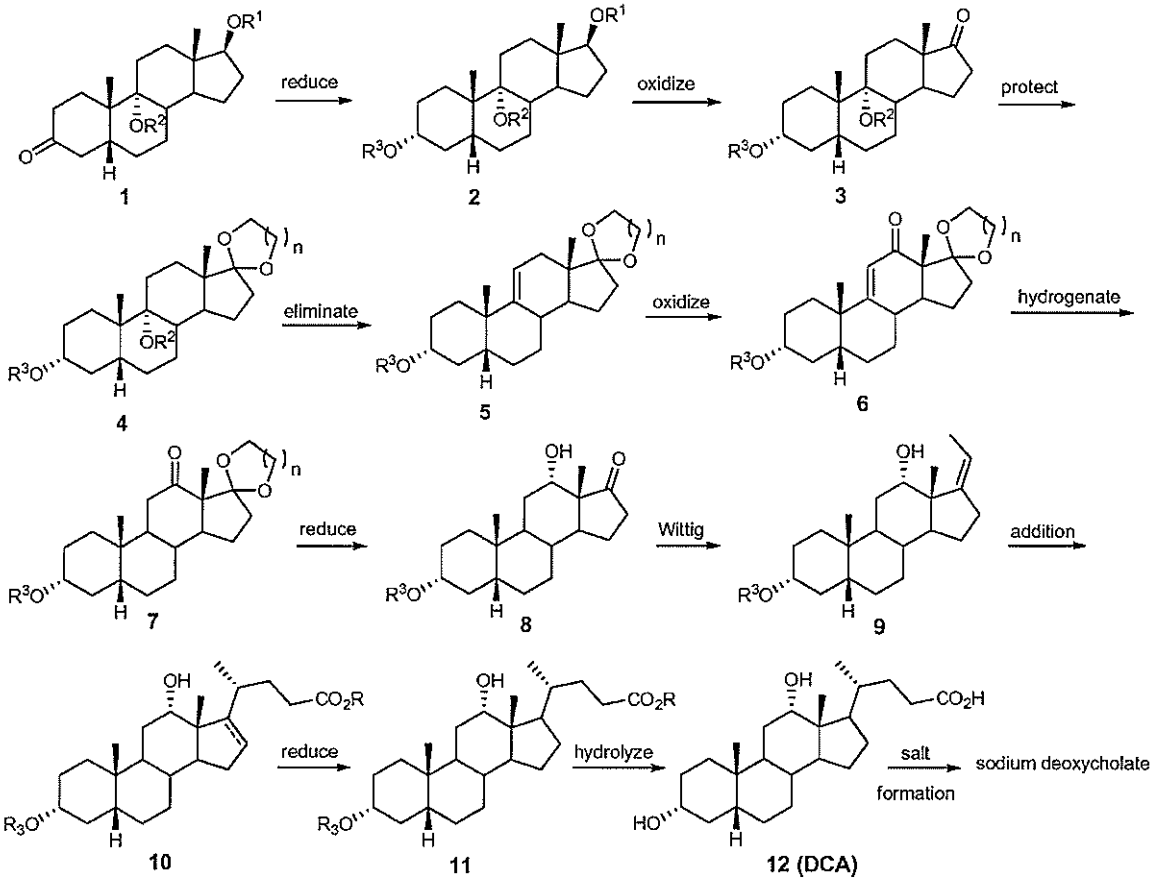
[0074] In one embodiment, the aqueous formulation is split into a plurality of individual solutions which are separately administered to the fat cells. For example, the aqueous formulation is split into 5, 10, 15, 20, 25 or 30 separate solutions and, in some cases, up to 50 separate solutions.

[0075] In a preferred embodiment, the salt of deoxycholic acid is sodium deoxycholate. As the methods of this invention include subcutaneous injections, there is also provided a syringe comprising a chamber, a plunger and an injection needle wherein the chamber comprises a

formulation of this invention. Preferably, the chamber is sufficient to hold at least 2 mL and preferably no more than 4 mL of the formulation.

[0076] In another embodiment, this invention provides a synthesis of DCA from protected commercially available 9-α,17-β-dihydroxy-5-α-androstan-3-one as shown in scheme 1 below.

Scheme 1: Synthesis of DCA



[0077] The 9-α,17-β hydroxyl groups of commercially available 9-α,17-β-dihydroxy-5-α-androstan-3-one are differentially protected with hydroxyl protecting groups which can be removed under conditions where one of the hydroxyl groups is regenerated while the other hydroxyl group remains protected. Such differential protection is referred to as orthogonal protection and uses well known reagents and reaction conditions. In one example, one hydroxy group is protected as an acetyl group, whereas the other hydroxy group is protected as a benzyl group. Each group can be selectively removed under reaction conditions that retain the other hydroxyl protecting group intact.

[0078] It is contemplated that the relatively sterically protected 9-α-hydroxyl group may not need to be protected as the reactions contemplated prior to elimination of that group are likely inhibited at this position due to steric hindrance. Regardless, protection of this hydroxyl group adequately insures that the group remains intact until elimination of the hydroxyl group via dehydration is desired.

[0079] The 3-one group of orthogonally protected 9-α,17-β-dihydroxy-5-α-androstan-3-one, compound **1**, is reduced with conventional reducing agent such as sodium borohydride to provide the 3-α-hydroxy derivative which is then protected with yet another orthogonal protecting group to provide compound **2**.

[0080] The hydroxyl protecting group at the 17-position of compound **2** is then selectively removed and the hydroxyl group so regenerated is then oxidized with a suitable oxidation reagent such as CrO_3 to provide the 17-keto derivative, compound **3**. The 17-keto group in compound **3** is protected as a ketal under standard ketalization conditions such as reaction with 1,2-dihydroxyethane or 1,3-dihydroxypropane to give compound **4** (which illustrates ketal formation with the 1,2-dihydroxyethane for illustrative purposes only).

[0081] Deprotection of the 9-α-hydroxyl as necessary is followed by dehydration of that hydroxyl group under conditions such as acid-catalyzed elimination provides the 9,10-unsaturated derivative, compound **5**. Generation of a 12-keto group is accomplished by allylic oxidation of compound **5** with oxidation reagents such as chromic acid or TBHP (tert-butyl hydroperoxide) and NaOCl to provide compound **6**. See, for example, U.S. Patent Application Serial No. 61/348,686. Alternatively, the allylic oxidation is accomplished by using about 2 to 5 equivalents of TBHP and about 0.3 to 0.5 equivalents of CuI as a catalyst. The reaction is carried out in a solvent such as acetonitrile at 40 °C for about 40-55 hours. The slow portionwise addition of TBHP results in more efficient oxidation. The product formed contains a mixture of compound **6** and the corresponding allylic alcohol. The product mixture is then oxidized with PCC to give compound **6**.

[0082] Hydrogenation of compound **6** under standard conditions such as 10% Pd/C and H_2 provides compound **7**. Reduction of the 12-keto group in compound **7** with reagents such as $LiAl(OBu^t)_3H$ provides the 12-hydroxy derivative, compound **8**. Olefination of compound **8** under standard Wittig conditions such as using ethyltriphenylphosphonium bromide in presence of a base such as potassium *tert*-butoxide provides compound **9**. Addition of an alkyl acrylate such as methyl acrylate in presence of a Lewis acid provides compound **10**,

wherein R is an alkyl group such as methyl. Reduction of the double bond in compound **10** again proceeds under standard hydrogenation conditions such as Pd/C and H_2 to provide compound **11**. Deprotection of the 3-OR_3 followed by hydrolysis with a base such as LiOH provides DCA, compound **12**.

[0083] Compound **12** (crude DCA) was further purified with methanol wash and recrystallized from ethanol. It was diluted with 2 mol% MeOH in CH_2Cl_2 (25 vol) and heated to 35–37 °C for 1 hour. The slurry was allowed to cool to 28–30 °C and filtered. The filter cake was washed with CH_2Cl_2 (5 vol) and dried under vacuum at 40 °C to afford DCA.

[0084] DCA was dissolved in 10% DI water/ EtOH (12 vol), polish filtered over celite and washed with 10% DI water/ EtOH (3 vol). The resulting 15 volume filtrate was added to DI water (30 vol) and a thin white slurry was afforded. The slurry was held for 24 hours, filtered, washed with DI water (20 vol) and dried under vacuum at 40 °C to afford DCA.

[0085] Conversion of DCA to a pharmaceutically acceptable salt such as sodium deoxycholate proceeds via conventional conditions. Alternatively, conversion of a pharmaceutically acceptable salt of DCA such as sodium deoxycholate to DCA also proceeds via conventional conditions.

[0086] In another embodiment, this invention provides a stabilized formulation comprising:

> a buffered aqueous solution having a pH of about 8.1 to about 8.5 and further comprising about 0.5% of sodium deoxycholate and about 0.9% of benzyl alcohol,

wherein the formulation is stabilized against precipitation, and the sodium deoxycholate is prepared according to scheme 1.

[0087] In another embodiment, this invention provides a stabilized formulation comprising:

> a buffered aqueous solution having a pH of about 8.1 to about 8.5 and further comprising about 1% of sodium deoxycholate and about 0.9% of benzyl alcohol,

wherein the formulation is stabilized against precipitation, and the sodium deoxycholate is prepared according to scheme 1.

Examples

[0088] In the examples and elsewhere in the specification, abbreviations have the following meanings:

Mg	=	Milligram
mL	=	Milliliter
Mm	=	Millimeter
mM	=	Millimolar
T	=	Time
UV	=	Ultraviolet
v/v	=	Volume /Volume
w/v	=	Weight/Volume (g/mL)
w/w	=	Weight/ Weight
WFI	=	Water for Injection
mOsm	=	Milliosmole

[0089] The invention is further understood by reference to the following examples, which are intended to be purely exemplary of the invention.

Example 1

Concentration dependent precipitation from a solution of sodium deoxycholate

[0090] Solutions of sodium deoxycholate at different concentration were evaluated for precipitate formation after 1 week of storage. The results are depicted in Figs. 1-4, and demonstrate that at about 0.5% and at about 1 % (w/v) concentration of sodium deoxycholate in an aqueous solution containing only water and 0.9 % w/v benzyl alcohol, a significant amount of precipitate is formed such that it would inhibit use of the solution as a composition for subcutaneous injections. By visual inspection of Figs. 1-4, the amount of precipitation can be rated as tabulated below.

Table 1

% w/v Sodium Deoxycholate	Precipitation Rating	Comment
0	1	Precipitation substantially invisible to the naked eye
0.5	10	Significant amounts of precipitate visible to the naked eye
1.0	7	Significant amounts of precipitate visible to the naked eye but less than that for 0.5% w/v
2.0	2	Precipitation visible to the naked eye but present in substantially smaller amounts compared to the 0.5 and 1% solutions above

[0091] The precipitation rating estimates that "0" refers a clear solution and that "10" refers to a mixture exhibiting substantial precipitation readily visible to the naked eye.

[0092] Such an observation demonstrates that in the concentration ranges tested, the precipitation phenomena was substantially affected by deoxycholate concentration. To ascertain pH's effect on precipitation, the pH of the solutions were measured, as provided in Table 2, which demonstrates that the pH of the solutions were substantially the same, especially for the 1% and the 2% solutions. The inverse aqueous solubility of sodium deoxycholate, where a more dilute solution (0.5% or 1%) provides more precipitation than a more concentrated solution (2%), is a surprising observation and also evidences that the precipitation phenomena is not directly related to pH, because the pH of the solutions were substantially the same again, especially for the 1% and 2% solutions.

Table 2

% w/v Sodium Deoxycholate	Reading #	Temperature/ °C	pH
0	1	24.0	7.75
0	2	24.1	7.58
0.5	1	24.7	7.77
0.5	2	24.5	7.71
1	1	24.6	7.93
1	2	24.5	7.97
2	1	24.9	8.07
2	2	24.7	8.06

[0093] Accordingly, this invention provides that the surprising precipitation from dilute, 0.4% to less than 2% (w/v), salt of deoxycholic acid solutions are inhibited, to the extent that such solutions are useful for subcutaneous injections, by increasing the solution pH.

Example 2

Sodium deoxycholate (API) formulations with and without benzyl alcohol

1. A composition of sodium deoxycholate (0.5% and 1%) was prepared comprising sodium phosphate (10 mM), sodium chloride (75-90 mM), benzyl alcohol (0.9%), deoxycholic acid, pH 8.3.

2. An isotonic composition of sodium deoxycholate without benzyl alcohol was prepared using the free acid form, namely, deoxycholic acid, as follows.

a. Preparation of 100 mL isotonic batches at 10 mg/mL

[0094] 1.0 g of the deoxycholic acid (DCA) added to the solution only after a basic solution was made with 70 mL water, 142 mg anhydrous dibasic sodium phosphate and 267 μL 10M NaOH. It took about 20 minutes for the API to go into solution. The pH of the solution was 11.1. The rapid addition of HCl was known to cause some precipitation, so 225 μL of 1M HCl was slowly added to bring the solution to pH 8.3. The solution was allowed to mix for an additional 15 minutes. After bringing the volume up to 100 mL with water, the osmolality was found to be 51 mOsm. Addition of 859 mg of NaCl brought the osmolality up to 305 mOsm.

[0095] The solution so prepared could optionally be lyophilized to provide for a lyophilized product which could be reconstituted by addition of the appropriate amount of sterile water. Accordingly, this invention also provides for lyophilized products of the solutions disclosed herein.

b. Preparation of 1000 mL isotonic batches at 10 mg/mL

[0096] The results from section a (above) did not scale up perfectly when multiplied ten fold. To 900 mL of water, 1.4g anhydrous dibasic sodium phosphate, 8.6 g NaCl, and 2.7 mL 10 M NaOH were added. 10.0 g of DCA was then added and allowed to mix to clarity for 30 minutes. The pH of the solution was 10.4. 1.5 mL 1 M HCl was slowly added and allowed to mix for 5 minutes. The final pH was 8.1. An additional 20 μL of 10M NaOH had to be added to bring the pH to 8.3. After bringing the volume up to 1000 mL with water, the osmolality was 314 mOsm.

[0097] Based on observations of the pH change during the addition of 1 M HCl, it was determined that for 1000 mL batches at 10 mg/mL API, just 1.0 mL of 1M HCl should be immediately added and then slowly titrated with small volumes of the acid. The suggested order of addition for 1000 mL of 10 mg/mL API is outlined in Table 3.

c. Preparation of 100 mL isotonic batches at 5 mg/mL

[0098] 0.50 g of deoxycholic acid (DCA) was added to the solution only after a basic solution was made with 70 mL water, 142 mg anhydrous dibasic sodium phosphate and 134 μL 10M NaOH. It took about 20 minutes for the API to go into solution. The pH was 10.7. The rapid addition of HCl was known to cause some precipitation, so 115 μL of 1 M HCl was

slowly added to bring the solution to pH 8.3. The solution was allowed to mix for an additional 15 minutes. After bringing the volume up to 100 mL with water, the osmolality was found to be 39 mOsm. Addition of 859 mg of NaCl brought the osmolality up to 294 mOsm.

d. Preparation of l000 mL isotonic batches at 5 mg/mL

[0099] The results from section c (above) did not scale up perfectly when multiplied ten fold. To 900 mL of water, 1.4g anhydrous dibasic sodium phosphate, 8.6 g NaCl, and 1.3 mL 10M NaOH were added. 5.0 g of DCA was then added and allowed to mix to clarity for 30 minutes. The pH was 8.6. After adding just 350 μL 1M HCl, the pH dropped to 8.0. An additional 25 μL of 10M NaOH had to be added to bring the pH to 8.4. After bringing the volume up to 1000 mL with water, the osmolality was 305 mOsm. Based on observations of the pH change during the addition of 1 M HCl, it was determined that for 1000 mL batches at 5 mg/mL API, that the solution should be slowly titrated with small volumes of 1M HCl. The suggested order of addition for 1000 mL of 5 mg/mL is outlined in Table 3.

Table 3. Order of addition (left to right) for isotonic 1000 mL benzyl alcohol free formulation

API Concentration	Dibasic Anhydrous Sodium Phosphate	10M NaOH	DCA	NaCl	1M HCl	pH
10 mg/mL	1.4 g	2.7 mL	10.0g	8.6 g	1.0 mL + incremental addition to final pH	8.3
5 mg/mL	1.4 g	1.3 mL	5.0 g	8.6 g	incremental addition to final pH	8.3

CLAIMS

1. A method for stabilizing an aqueous solution of a salt of deoxycholic acid against precipitation during storage, comprising forming an aqueous solution of a deoxycholic acid salt at an initial pH above the pKa of the deoxycholic acid salt and adjusting the pH of the aqueous solution to be from about 8.1 to about 8.5, wherein the aqueous solution comprises from about 0.4% w/v to about 2% w/v deoxycholic acid salt and at least one pharmaceutically acceptable excipient.

2. The method of claim 1, further comprising including a sufficient amount of a buffer to maintain the pH.

3. The method of claim 1 or 2, wherein precipitation of the deoxycholic acid salt is substantially inhibited for a period of at least six months.

4. The method of claim 3, wherein precipitation of the deoxycholic acid salt is substantially inhibited for a period of at least six months at a temperature of from about 17 °C to about 27 °C, from about 25 °C to about 37 °C, or from about 2 °C to about 8 °C.

5. The method of any preceding claim, wherein the pH is adjusted to be between about 8.2 and about 8.5.

6. The method of any preceding claim, wherein the pH is adjusted to about 8.3.

7. The method of any preceding claim, wherein the deoxycholic acid salt is present in an amount from about 0.5% w/v to about 1% w/v.

8. The method of any preceding claim, wherein the deoxycholic acid salt is present in an amount of about 1% w/v.

9. The method of any preceding claim, wherein the excipient is a solvent, a buffer, a preservative, a lyophilization aid, or any combination thereof.

10. The method of claim 9, wherein the excipient is a solvent, such as sterile water.

11. The method of claim 9, wherein the excipient is a preservative, such as benzyl alcohol.

12. The method of claim 11, wherein the preservative is about 0.9% benzyl alcohol.

13. The method of any preceding claim, wherein the solution is subcutaneously injectable.

14. A method for stabilizing an aqueous solution of a salt of deoxycholic acid against precipitation during storage, comprising forming an aqueous solution of a deoxycholic acid salt at an initial pH above the pKa of the deoxycholic acid salt and adjusting the pH of the aqueous solution to be about 8.3, wherein the aqueous solution comprises about 0.5% w/v of the salt of deoxycholic acid, about 0.9% w/v of benzyl alcohol, and about 1% w/v of sodium chloride.

15. A method for stabilizing an aqueous solution of a salt of deoxycholic acid against precipitation during storage, comprising forming an aqueous solution of a deoxycholic acid salt at an initial pH above the pKa of the deoxycholic acid salt and adjusting the pH of the aqueous solution to be about 8.3, wherein the aqueous solution comprises about 1% w/v of the salt of deoxycholic acid, about 0.9% w/v of benzyl alcohol, and about 1% w/v of sodium chloride.

16. The method of any preceding claim, wherein the salt is a sodium salt.

17. An aqueous solution of a salt of deoxycholic acid as stabilized by any preceding claim.