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Methods and Compositions for Stabilizing Acetylcholine Compositions

This invention relates generally to pharmaceutical acetylcholine products. In a preferred embodiment, the invention relates to ophthalmic acetylcholine products.

Anterior segment surgery of the eye is relatively common today. These surgical procedures include cataract surgery, penetrating keratoplasty and iridectomy.

Post-surgical treatments are also common. A typical treatment which follows anterior segment surgery involves an immediate application of a pharmaceutical agent which increases the rate of miosis (*i.e.*, constriction of the iris). A preferred miosis-inducing pharmaceutical is acetylcholine, and the chloride salt is the preferred salt. Acetylcholine is also called 2-(acetyloxy)-N,N,N-trimethyl-ethanamium; chloride; acecoline; or arterocoline.

An outstanding product for increasing the miosis rate is sold under the trademark Miochol®-E, which is an intraocular acetylcholine solution marketed by CIBA Vision Corporation, Duluth, Georgia. Since aqueous acetylcholine solutions are unstable, the Miochol®-E product is packaged in a vial having two compartments or chambers which are separated from one another by a rubber stopper. The lower chamber contains 20 mg of acetylcholine chloride and 56 mg of mannitol. The upper chamber contains 2 ml of a diluent of sodium chloride, potassium chloride, magnesium chloride hexahydrate, calcium chloride dihydrate and sterile water for injection.

In use, an eye care professional turns a plunger-stopper mechanism a quarter turn and presses to cause the rubber stopper to dislodge and allow diluent from the upper chamber to pass into the lower chamber, thereby mixing the two solutions. The solution is then administered via injection into the anterior chamber (e.g., seconds after a lens has been inserted during cataract surgery), either before or after securing one or more sutures.

An object of the invention is to increase the stability of an acetylcholine pharmaceutical product.

An object of the invention is to improve the pH consistency of an acetylcholine pharmaceutical product without causing substantial degradation of the acetylcholine.

Another object of the invention is to provide an improved acetylcholine ophthalmic product.

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This and other objects and advantages are achieved through the innovative stabilized product which includes two-compartment acetylcholine product. In one compartment, acetylcholine is stored. In a second compartment, a buffered diluent solution is stored. Upon mixing the buffered diluent, a buffered acetylcholine product is prepared for injection.

Another embodiment of the invention is a method of providing an acetylcholine product which has a substantially consistent final pH without substantial degradation of the acetylcholine. The method involves storing an unbuffered acetylcholine separate from a diluent solution which has been buffered, and mixing prior to use.

FIGURE 1 is a side sectional view of a two-compartment acetylcholine pharmaceutical storage container.

While acetylcholine ophthalmic products have been found to be very effective in post-surgical miosis-inducing treatments, the susceptibility of acetylcholine to degradation or decomposition causes significant manufacturing difficulties. Acetylcholine is similar to a number of other choline esters and related esters of carboxylic acids in that it can easily undergo acid and alkaline catalyzed hydrolyses. Accordingly, the current commercial process of manufacturing acetylcholine involves lyophilization (freeze-drying) and storage of the lyophilized acetylcholine in a separate compartment addresses some of the decomposition concerns.

However, acetylcholine is still susceptible to some decomposition. Due to its hygroscopic nature, minute amounts of water may be adsorbed subsquent to lyophilization and prior to packaging, and this water can catalyze decomposition. While this amount of degradation may not be sufficient to cause efficacy problems in the final product, any decomposition of acetylcholine generates acetic acid. Even minor amounts of degradation causes a substantial shift in final product pH, sometimes sufficient to lower the pH below 5.0. Since the product is injected into the eye, the pH must be maintained at an ophthalmically acceptable level. Accordingly, there exists a need to improve the pH stability or consistency of aqueous acetylcholine products, and the present invention addresses this need.

Acetylcholine is susceptible to decomposition as the pH varies from neutral, either in an acidic or a basic range. Thus, buffering of the acetylcholine composition in its storage chamber would presumably be a method of inhibiting acetylcholine decomposition and

associate lower of pH from the acetic acid product. However, quite unexpectedly, it was found that addition of buffer to the acetylcholine chamber actually increased the decomposition rate. While large amounts of buffer could presumably control the final product pH, the buffer cause unacceptable levels of drug decomposition.

In order to remedy the problem, buffer was added to the diluent storage chamber. In this way, only minor decomposition occurs in the acetylcholine-containing chamber. While the pH shifts in the acetylcholine chamber, once the buffered diluent is mixed with the acetylcholine, there is sufficient buffering capacity to bring the pH to an ophthalmically acceptable level. The final solution is utilized shortly after mixing with buffered diluent, so there is not sufficient time for acetylcholine to decompose substantially due to the presence of the buffer.

Hence, in one embodiment, the invention is a pharmaceutical product in a container which includes two chambers. A first chamber retains an aqueous diluent solution comprising at least one buffer. A second chamber retains a pharmaceutically active agent comprising acetylcholine. Upon mixing the solution from the first chamber with the contents of the second chamber, the buffer is present in sufficient quantity to buffer the pH of the mixed solution to an ophthalmically acceptable level. Preferably, the buffer is present in sufficent quantity to buffer the solution above a pH of about 5.0. A preferred upper limit on pH is about 8.2. The acetylcholine chamber is preferably substantially free or entirely free of added buffer.

In another embodiment, the invention is a method of buffering a pharmaceutical product including acetylcholine, comprising the steps of providing a buffered diluent solution in a first container; and providing a substantially unbuffered active solution comprising acetylcholine in a second container. The contents of the containers are mixed prior to use to form a solution having an ophthalmically aceptable pH. Preferably, the buffer is present in sufficient quantity to buffer the pH of the mixed solution above about 5.0.

FIG. 1 illustrates a two-chamber product configuration which is an exemple of a useful product configuration in accordance with the present invention. Ophthalmic product **10** includes container **12** which has first chamber **14** and second chamber **16**, positioned such that first chamber **14** is directly over second chamber **16** when container **12** is in a resting position. The chambers are separated by separation means **18**, such as a rubber stopper positioned in a narrowed region of container **12**. Flexible sealing means **20** provides a

removable seal to first chamber **14**. First chamber **14** holds a buffered diluent solution. Second chamber **16** holds acetylcholine.

In use, an eye care practitioner may twist and depress sealing means **20** thereby generating pressure which dislodges separation means **18** and allowing diluent solution from first chamber **14** to mix with acetylcholine in second chamber **16**.

The first or upper chamber preferably includes salts as tonicity adjustors and for other purposes. A preferred composition of upper chamber includes sodium chloride, potassium chloride, magnesium chloride hexahydrate, calcium chloride dihydrate, at least one buffer and sterile water for injection. The concentration of chloride salts is preferably in the ranges of about 0.005% to 0.10% of each of potassium chloride, magnesium chloride hexahydrate, and calcium chloride dihydrate, based on total final product weight. Particularly preferred concentrations are in the ranges of about 0.04% potassium chloride, about 0.03% magnesium chloride hexahydrate, and about 0.01% calcium chloride dihydrate.

The quantity of diluent in the upper chamber may range somewhat, but is preferably from about 1 to 5 milliliters. A preferred quantity of diluent is about 2 ml.

The buffer may be selected from the group of buffers which can achieve an ophthalmically acceptable pH without generating any other substantial detriment to the composition. Buffers may generally be selected from the group consisting of acetates, borates, phosphates, carbonate, citrates and mixtures thereof. Acetates are a preferred group because of the generation of acetic acid when acetylcholine decomposes. A particularly preferred acetate is sodium acetate, and a widely available form is the trihydrate.

The buffer concentration depends upon the buffer selected and the goal of achieving a final product pH which is ophthalmically acceptable. If sodium acetate is chosen as the buffer, the sodium acetate concentration may range from about 0.05 to 0.5, preferably about 0.10 to 0.30 and more preferably about 0.13 to 0.17 weight percent.

The second or lower chamber will include acetylcholine, but may include other additives. For example, the lower chamber preferably includes a tonicity adjustor such as mannitol. However, it is preferred to avoid the presence of buffer with acetylcholine until a time nearing the actual use of the final mixed product, in order to minimize the decomposition

rate of acetylcholine. Thus, the lower chamber is preferably substantially or completely free of added buffer.

In one embodiment, the lower chamber includes lyophilized acetylcholine and a tonicity adjustor. Preferably, the lower chamber includes about 0.5 to 5% (5 to 50 mg) of lyophilized acetylcholine and about 1 to 10% (10 to 100 milligrams) of mannitol, based on weight percentages of final product solution. In a preferred embodiment, the lower chamber includes about 2% (20 mg) of lyophilized acetylcholine and about 5.6% (56 mg) of mannitol.

Typically, the mannitol is mixed with the acetylcholine prior to the lyophilization process. Accordingly, the lower chamber preferably includes a cake of lyophilized acetylcholine and mannitol.

The final product characteristics are dictated, at least in part, by ophthalmic compatibility. Thus, the pH and osmolality are those which are ophthalmically compatible. A pH of about 5.0 to 8.2 is a preferred range, while a pH of about 5.5 to 8.0 is more preferred. A preferred osmolality range is about 275 to 330, more preferably about 290 to 310.

The invention has been described in detail, with reference to certain preferred embodiments, in order to enable the reader to practice the invention without undue experimentation. However, a person having ordinary skill in the art will readily recognize that many of the components and parameters may be varied or modified to a certain extent without departing from the scope and spirit of the invention. Furthermore, titles, headings, definitions or the like are provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention. Accordingly, the intellectual property rights to this invention are defined only by the following claims and appropriate extensions and equivalents thereof.

**CLAIMS**

1. A pharmaceutical product, comprising a container which includes:

a first chamber, which retains an aqueous diluent solution consisting of:

(a) a salt selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride hexahydrate, and calcium chloride dihydrate;

(b) at least one buffer;

(c) sterile water for injection; and

a second chamber, which retains a pharmaceutically active agent comprising acetylcholine,

wherein, upon mixing the solution from the first chamber with the contents of the second chamber, the buffer is present in sufficient quantity to buffer the pH of the mixed solution to an ophthalmically acceptable level.

2. A pharmaceutical product of claim 1, wherein the pH of the mixed solution is above 5.0.

3. A pharmaceutical product of claim 1, wherein the buffer is selected from the group consisting of acetates, borates, phosphates, carbonate, citrates and mixtures thereof.

4. A pharmaceutical product of claim 3, wherein the buffer includes at least one acetate.

5. A pharmaceutical product of claim 4, wherein the buffer includes sodium acetate.

6. A pharmaceutical product of claim 5, wherein the sodium acetate is present at a concentration of 0.05 to 0.50 weight percent, based on total component weight percentages in the final product which is a mixture of the first chamber and the second chamber.

7. A pharmaceutical product of claim 1, wherein said diluent solution in the first chamber consists of:

potassium chloride; magnesium chloride hexahydrate; calcium chloride dihydrate; sodium acetate; and sterile water for injection.

8. A pharmaceutical product of claim 7, wherein the first chamber comprises 1 to 5 milliliters of a solution consisting of:

0.005 to 0.10% potassium chloride;

0.005 to 0.10% magnesium chloride hexahydrate;

0.005 to 0.10% calcium chloride dihydrate;
0.10 to 0.20% sodium acetate; and
sterile water for injection,
wherein concentrations are based on total component weight percentages in the final product which is a mixture of the first chamber and the second chamber, and
wherein concentrations are based on total component weight percentages in the final product which is a mixture of the first chamber and the second chamber.

9. A pharmaceutical product of claim 1, wherein the second chamber comprises: lyophilized acetylcholine; and a tonicity adjusting agent.

10. A pharmaceutical product of claim 9, wherein the tonicity adjusting agent includes mannitol.

11. A pharmaceutical product of claim 10, wherein the second chamber comprises:
0.5% to 5% lyophilized acetylcholine; and
1% to 10% mg of mannitol
wherein concentrations are based on total component weight percentages in the final product which is a mixture of the first chamber and the second chamber.

12. Use of a pharmaceutical product of claim 1 in the preparation of a medicament for the treatment of the eye following cataract surgery, penetrating keratoplasty, or iridectomy.

13. A pharmaceutical product of claim 1, wherein the second chamber is free of buffer.

14. An ophthalmic pharmaceutical product, comprising:
a first chamber, which retains an aqueous diluent solution consisting of:
0.005 to 0.10% potassium chloride;
0.005 to 0.10% magnesium chloride hexahydrate;
0.005 to 0.10% calcium chloride dihydrate;
0.05 to 0.50% sodium acetate; and
sterile water for injection, and
a second chamber, which retains a pharmaceutically active agent, comprising:
0.5 to 5% lyophilized acetylcholine; and
1 to 10% mannitol;

wherein, upon mixing the solution from the first chamber with the contents of the second container, buffer is present in sufficient quantity to buffer the pH of the mixed solution above 5.0, and

wherein concentrations are based on total component weight percentages in the final product which is a mixture of the first chamber and the second chamber.

15. A method of stabilizing a pharmaceutical product including acetylcholine, comprising the steps of:

providing in a first container an aqueous diluent solution consisting of:

(a) a salt selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride hexahydrate, and calcium chloride dihydrate;

(b) at least one buffer;

(c) sterile water for injection; and

providing an unbuffered active solution comprising acetylcholine in a second container;

wherein, upon mixing the solution from the first chamber with the contents of the second container, buffer is present in sufficient quantity to buffer the pH of the mixed solution an an ophthalmically acceptable pH.

16. A method of claim 15, wherein the first container is affixed to the second container.

17. A method of claim 15, wherein the pH of the mixed solution is above 5.0.

18. A method of claim 15, wherein the buffer is an acetate.

19. A method of claim 18, wherein the buffer is sodium acetate and the concentration is 0.05 to 0.50 weight percent.