Title: LYOPHILIZED MONOCLONAL ANTIBODY COMPOSITIONS

Abstract: The invention concerns an improved solid pharmaceutical composition comprising a lyophilized monoclonal antibody composition that exhibits enhanced stability in the dry form and a high glass transition temperature. In accordance with the invention, a lyophilized monoclonal antibody composition is provided comprising a therapeutically active monoclonal antibody or fragment thereof in admixture with a stabilizing excipient comprised of a combination of a disaccharide and a hydroxyethyl starch. Preferably, the monoclonal antibody is abciximab.
LYOPHILIZED MONOCLONAL ANTIBODY COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority of United States provisional application serial number 60/342,063, filed Nov. 9, 2001.

FIELD OF THE INVENTION

The present invention relates to the field of pharmaceutical compositions and the manufacture of lyophilized monoclonal antibody and antibody fragment compositions. More particularly, the invention relates to a lyophilized composition of abciximab and infliximab. Abciximab is the Fab fragment of the chimeric human-murine monoclonal antibody 7E3 which inhibits platelet aggregation in humans. Infliximab is the chimeric human-murine monoclonal antibody against tumor necrosis factor. The composition exhibits high glass transition temperatures and improved stability.

BACKGROUND OF THE INVENTION

An essential feature in the development of a protein therapeutic is to design a formulation that is stable during shipment and storage before administration to a patient. In most cases, an aqueous formulation is the easiest and most economical to handle for the manufacturer and easiest to administer to the patient. Accordingly, most preparations are prepared and stored in a liquid state. However, because many protein compositions are subject to chemical degradation (by deamidation or oxidation) and/or physical degradation (aggregation and precipitation) in the aqueous liquid state, precise control of storage conditions such as refrigeration and minimization of agitation is necessary to retain stability. Such conditions are often seen as a drawback to commercial application of the product.

It is known in the art that lyophilizing a product which is relatively unstable in aqueous solution can result in a product that is stabilized and therefore has a longer shelf life than an aqueous solution. (See “Remington’s Pharmaceutical Sciences”, 15th Ed. Mack Publishing Co., Easton, Pa., pp 1483-1485). In the dried solid, degradative reactions can be avoided or slowed sufficiently such that the protein therapeutic remains stable for months or years at ambient temperatures. Accordingly, the technique known as lyophilization is often
employed for injectable pharmaceuticals which exhibit poor stability in aqueous solution. This process involves freeze-drying, whereby ice is sublimed from frozen solutions leaving only the solid, dried components of the original liquid. The process has numerous advantages in that the aqueous solution can be processed and filled into dosage containers in a liquid state, dried at low temperatures thereby eliminating adverse thermal effects, and stored in the dried state where it may be more stable. In addition, the lyophilized product is ordinarily rapidly soluble and is easily reconstituted prior to administration to a patient.

However, in order to achieve a lyophilized product having the desired characteristics, appropriate care must be taken to choose the conditions and excipients for use in the lyophilization process. Without appropriate stabilizing excipients, most protein preparations are at least partially denatured by the freezing and dehydration stresses encountered during lyophilization.

Additionally, excipients should be chosen that assure stability during long term storage in the dried solid. To assure long term stability of the protein in the dried solid, the glass transition temperature (Tg') of the amorphous phase in the product, which contains the protein, must exceed the planned storage temperature. Since water is a plasticizer of the amorphous phase, low residual moisture is needed to insure that Tg' is greater than the highest temperature encountered during shipping and storage. The Tg' of the liquid state prior to lyophilization is a critical factor in lyophilization, and is determined by excipients in the liquid state and container configuration. In the case where a dual chamber syringe such as a Lyo-Ject syringe is to be used, a composition with a high Tg' is necessary to be compatible with the uniqueness of the syringe design.

A monoclonal antibody is a protein molecule produced by fusing a chosen B cell line with an immortal myeloma cell line to produce hybridomas, immortal cells that secrete only the selected antibody type of the selected B cell clone. Structurally, each antibody is formed by the interaction of two identical "heavy" chains and two identical light chains, all of which combine to form a Y shape (the heavy chains span the entire Y, and the light chains the two arms only. An antibody according to the present invention includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin
molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, or any combination thereof, and the like. An antibody fragment as used in the present invention includes the fragment of the immunoglobulin molecule known as the Fab containing the CDR antigen binding site, generated by cleavage of the antibody with the protease papain which cuts at the “hinge” region of the Y shaped antibody molecule producing two Fab fragments. As monoclonal antibodies are protein molecules, they are generally formulated as aqueous solutions for injection. As such, they have limited shelf life and must be stored under controlled conditions as discussed above.

The monoclonal antibody abciximab (REOPRO®), is the Fab fragment of the chimeric human-murine monoclonal antibody 7E3. Abciximab binds to the glycoprotein (GP) IIb/IIIa receptor of human platelets and inhibits platelet aggregation. Abciximab also binds to the vitronectin (αvβ3) receptor found on platelets and vessel wall endothelial and smooth muscle cells. It is indicated as an adjunct to percutaneous coronary intervention for the prevention of cardiac ischemic complications. Abciximab is sold commercially as a clear, colorless, sterile solution for intravenous use and must be stored under refrigerated conditions at 2 to 8°C. A detailed description of abciximab is disclosed in US patent 5,770,198 hereby incorporated by reference into the present application.

The monoclonal antibody infliximab (REMICADE®) is a chimeric IgG1k monoclonal antibody with an approximate molecular weight of 149,100 daltons. It is composed of human constant and murine variable regions. Infliximab binds specifically to human tumor necrosis factor alpha (TNF(α)) with an association constant of $10^{10} \text{ M}^{-1}$. Infliximab is produced by a recombinant cell line cultured by continuous perfusion and is purified by a series of steps that includes measures to inactivate and remove viruses. REMICADE is currently supplied as a sterile, white, lyophilized powder for intravenous infusion. Following reconstitution with 10 mL of Sterile Water for Injection, USP, the resulting pH is approximately 7.2. Each single-use vial contains 100 mg
infliximab, 500 mg sucrose, 0.5 mg polysorbate 80, 2.2 mg monobasic sodium phosphate, monohydrate, and 6.1 mg dibasic sodium phosphate, dihydrate. No preservatives are present. A detailed description of infliximab is disclosed in US patent 5,656,272 hereby incorporated by reference into the present application.

Prior to administration to a patient, a lyophilized product has to be reconstituted with sterile water. A convenient way to do this is to deliver the product in a dual chamber syringe such as a Lyo-Ject® syringe. The Lyo-Ject is a dual-chamber prefilled syringe with in situ lyophilized drug. The lyophilized drug and diluent are contained in dual chambers in the same syringe and can be easily reconstituted prior to injection. However, due to the uniqueness of the syringe design, the inventors have found that it is necessary to develop a formulation with high glass transition temperature (Tg') As stated, Tg' is a critical factor in lyophilization and should be greater than -26°C to be compatible with the dual chamber syringe.

Thus, the object of the present invention is to provide a monoclonal antibody composition with enhanced stability by the application of lyophilization to the aqueous solution. Another object is to provide a lyophilized monoclonal antibody product which exhibits the high Tg' to be compatible with a dual chamber syringe.

**SUMMARY OF THE INVENTION**

The invention concerns an improved solid pharmaceutical composition comprising a lyophilized monoclonal antibody composition that exhibits enhanced stability in the dry form and a high glass transition temperature. In accordance with the invention, a lyophilized monoclonal antibody composition is provided comprising a therapeutically active monoclonal antibody or fragment thereof in admixture with a stabilizing excipient comprised of a combination of a disaccharide and a hydroxyethyl starch.

Preferably, the monoclonal antibody is abciximab. A facile process for the production of the solid composition is also provided by the present invention, comprising the steps of:

(a) filling a container with monoclonal antibody to a antibody content;
(b) lyophilizing the suspension to a residual water content of 2% w/v or less by rapidly freezing the liquid solution in the container to about -40\(^{\circ}\) C. or below and reducing chamber pressure at appropriate shelf temperature to complete sublimation of ice; and

(c) aseptically sealing the container which contains the lyophilized monoclonal antibody solid composition.

**DETAILED DESCRIPTION**

The therapeutically active component of this invention is a monoclonal antibody as described above. Preferably, the monoclonal antibody is abciximab as disclosed in U.S. Pat. Nos. 5,770,198 and 5,976,532, hereby incorporated by reference into the present application.


Methods for the production of the abciximab monoclonal antibody polypeptides are described in U.S. Pat. No. 5,770,198, hereby incorporated by reference into the present application. Methods for the production of the infliximab monoclonal antibody are described in U.S. Pat. No. 5,656,272 hereby incorporated by reference into the present application.

In accordance with the present invention, the lyophilized monoclonal antibody composition is manufactured from the aqueous solution containing approximately 5 mg/ml polypeptide. Appropriately sized vials, preferably 2-10 ml,
are filled with the aqueous solution with a fill volume of 2 to 5 ml per vial. The vials are then frozen in the lyophilization chamber, preferably at a gradual rate of 0.5°-1° C./min, for approximately 2 hours to a temperature of ≤ -40° C. or until completely frozen. After cooling the lyophilization chamber pressure is reduced to about 100 microns Hg or less. The shelf temperature is then raised to -20° C. - +20° C. and held until sublimation of ice is substantially complete. The shelf temperature is then gradually raised, preferably at a rate of 0.5° C./min to 30° C and held for about 2 hours or more. Chamber pressure is then raised to atmospheric pressure and the vials are aseptically sealed.

The lyophilized dry composition prepared by the method of the instant invention exhibits enhanced stability and can be stored at room temperature for 6 months or greater depending on product specifications. The sealed vials are intended for use as single dose formulations following reconstitution with appropriate volumes of Sterile Water for Injection. It is intended that the filled vials will allow rapid dispersion of the solid composition upon reconstitution with water in situ giving an appropriate sterile solution of the desired monoclonal antibody concentration for administration. The lyophilized product is generally a white powder which undergoes reconstitution in about 1-5 minutes by swirling the vial.

The vials utilized should be capable of maintaining a sterile environment by being hermetically sealed by means of a stopper and overseal. The vials should be of an appropriate size, considering the volume of solution to be held upon reconstitution of the lyophilized composition; and should be made of appropriate material, generally Type I glass. The stopper means employed, preferably sterile rubber closures or an equivalent, should provide the appropriate seal but allow entry for the purpose of introducing the diluent for reconstitution.

The lyophilized monoclonal antibody composition of the invention exhibits enhanced stability in the dry form and a high glass transition temperature and is obtained by lyophilizing the monoclonal antibody in the presence of a stabilizing excipient comprised of a combination of a disaccharide and a hydroxyethyl starch.
The hydroxyethyl starch (HES) used in the invention is obtained from native starch after partial hydrolysis and substitution of the hydroxy groups by hydroxyethyl groups (see E. Nitsch, Chemie der Hydroxyethstarke (HES), *Beitr. Anaesth. Intesivmed.,* 26 (1988) 15-26). This procedure renders HES soluble in water allowing it to be used for a variety of pharmacological uses. HES is a known stabilizing agent in the freeze drying of biological material and comes in various commercially available forms depending on the degree of molar substitution. Preferably the HES has an average molecular weight of 10,000 – 200,000, preferably 20,000-60,000.

The disaccharide is selected from known disaccharides, such as sucrose, trehalose, maltose and lactose, with the non-reducing disaccharides such as sucrose being preferred.

In accordance with the invention, a combination of HES and disaccharide is employed in the lyophilizing process. By adjusting the ratio of disaccharide and HES, the desired Tg' and stabilizing effect can be customized for the monoclonal antibody preparation employed. The combination of HES and disaccharide is employed at an appropriate ratio to provide a Tg' of greater than -26°C and sufficient stabilizing effect. Preferably, where the monoclonal antibody is abciximab, the ratio of sucrose to HES is 6:4 but a range of 10:1 to 4:6 can be employed. The disaccharide and HES should preferably be contained in the aqueous monoclonal antibody solution in an amount of not less than 1 mg, more preferably 3 mg to 65 mg, per milliliter of said solution. The combination of disaccharide and HES provides sufficient stability, minimizes aggregation, shortens the lyophilization cycle time and minimizes the formation of visible particles. Monoclonal antibody preparation formulated in accordance with the invention exhibits long term stability on storage of 6 months or greater at room temperature.

The lyophilized monoclonal antibody product of the present invention is generally prepared as follows:

1. To an aqueous solution containing monoclonal antibody in an appropriate concentration, there is added the disaccharide HES in an amount sufficient to make a concentration of not less than 1 mg/ml, preferably 5 to 65 mg/ml. The above mentioned optional excipients may also be added. The therapeutic
antibody product is then lyophilized in accordance with the above mentioned procedure.

It is contemplated that other ingredients may be included in the formulation of the product of the present invention. These may include buffers to affect the pH of the solution, wetting or emulsifying agents, antimicrobial agents, preservatives, surfactants, isotonizing agents and the like. Also, bulking agents such as sodium bicarbonate, lactose, maltose, mannitol, sorbitol, or dextrose may be included to improve the characteristics of the freeze-dried cake. Many variations of the above, along with other suitable vehicles will suggest themselves to those skilled in the art in light of the foregoing detailed description. All such obvious variations are contemplated to be within the scope of the invention.

The lyophilized monoclonal antibody composition of the present invention is preferably formulated for intravenous administration when reconstituted as a solution.

The following examples describe in detail methods for preparation of a solid composition of the present invention. It will be apparent to one skilled in the art that many modifications, both of methods and materials may be practiced without departing from the purpose and intent of this disclosure. From the foregoing description and the following examples, it is believed that one skilled in the art is able to use the invention to the fullest extent.

EXAMPLE 1

Abciximab monoclonal antibody composition containing approximately 2 mg/ml total antibody is prepared in accordance with the procedures outlined in Coller et al., U.S. Pat. No. in US patent 5,770,198.

To an aqueous solution containing abciximab in a concentration of 5 mg/ml, there is added sucrose HES in an amount sufficient to make a concentration of not less than 2 mg/ml, preferably 5 to 65 mg/ml and the resultant solution is then sterile filtered. The above mentioned optional excipients may also be added. The therapeutic antibody product is then lyophilized in accordance with the following procedure:
5 ml vials containing 2 ml drug product are filled and placed into the lyophilization chamber. Shelf temperature is lowered at 0.5°-1° C./min. to -40° C. and held below -40° C. for 2 hours. Chamber pressure is then reduced to 100 microns Hg, the shelf is ramped to 0° C. at 0.5° C./min. and held for 48 hours. Shelf temperature is then raised to approximately +30° C. at 0.5° C./min. and held for 12 hours. The chamber is then brought to atmospheric pressure with dry nitrogen and the vials are stoppered and removed from the chamber and sealed.

Prior to use in therapy, the vial contents are reconstituted with 5 ml of Sterile Water for Injection, physiological saline or injectable glucose solution to yield the original concentration of solids in the vial.

EXAMPLE 2

Lyophilized abciximab monoclonal antibody composition prepared in accordance with Example 1 using various combinations of sucrose and HES were tested for glass transition temperature (Tg') and the results are set forth in Table 1:

<table>
<thead>
<tr>
<th>Sucrose %</th>
<th>HES%</th>
<th>Tg' (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>-30°C</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>-28°C</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-26°C</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-25°C</td>
</tr>
</tbody>
</table>

An examination of the data demonstrates that the lyophilized composition of the present invention exhibits superior Tg' and is thus suitable for use in a dual chamber syringe. The product also exhibits long term stability.
Claims

We claim:

1. A monoclonal antibody composition lyophilized in the presence of a mixture of disaccharide and hydroxyethyl starch.

2. The composition according to claim 1, wherein the monoclonal antibody is selected from abciximab and infliximab.

3. The composition according to claim 1, wherein the monoclonal antibody is abciximab.

4. The composition according to claim 1, wherein the disaccharide is selected from sucrose, maltose and lactose.

5. The composition according to claim 1, wherein the disaccharide is sucrose.

6. The composition of claim 3 wherein the abciximab is in a concentration of 2 mg/ml as an aqueous solution.

7. The composition of claim 1 wherein the HES has an average molecular weight of 10,000 – 200,000.

8. The composition of claim 1 wherein the HES has an average molecular weight of 20,000-60,000.

9. The composition of claim 1 having a glass transition temperature of greater than -26°C.

10. The composition of claim 1 wherein the disaccharide/HES is in a weight ratio of 10:1 to 4:6.
11. A lyophilized monoclonal antibody solid composition containing a mixture of a disaccharide and HES wherein said lyophilized composition is prepared by the process comprising the steps of:
   (a) filling a container with monoclonal antibody to a desired antibody content;
   (b) lyophilizing the solution to a residual water content of 5% w/v or less by rapidly freezing the suspension in the container to about -40° C. or below and reducing chamber pressure at appropriate shelf temperature to complete sublimation of ice; and
   (c) aseptically sealing the container which contains the lyophilized monoclonal antibody solid composition.

12. A pharmaceutical composition comprising the lyophilizate composition according to claim 1 reconstituted with sterile water for administration.

13. A single dose formulation comprising the lyophilized composition in accordance with claim 1 in a single dose vial container means of sufficient size to allow reconstitution with water to give an intended volume of solution of desired monoclonal antibody composition for administration.

14. The single dose formulation of claim 4 wherein the lyophilized composition comprises approximately 5mg/ml antibody.

15. The single dose formulation of claim 5 wherein said composition is reconstituted with 4 ml of water to provide solution for administration.

16. A method of producing a monoclonal antibody composition which comprises adding disaccharide and HES to an aqueous monoclonal antibody solution, freezing the resulting solution to produce the frozen composition and drying the frozen composition under reduced pressure to produce a lyophilized composition.

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