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(54) **STABLE FREEZE-DRIED PHARMACEUTICAL FORMULATION**

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(58) **Field of Search** 424/489, 450

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(57) **ABSTRACT**

The subject of the invention is a freeze-dried formulation consisting of an amorphous phase and a crystalline phase, which is pharmaceutically acceptable, comprising at least one nonprotein active ingredient, characterized in that it contains mannitol and alanine in a ratio R of between 0.1 and 1, R representing the mass of mannitol to the mass of alanine.

13 Claims, 2 Drawing Sheets

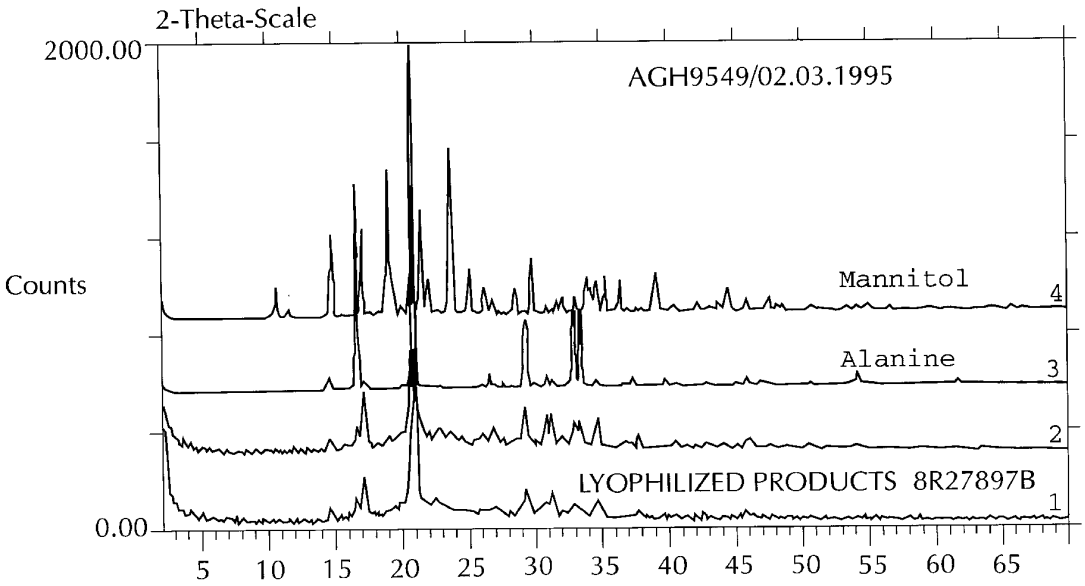


FIG. 1

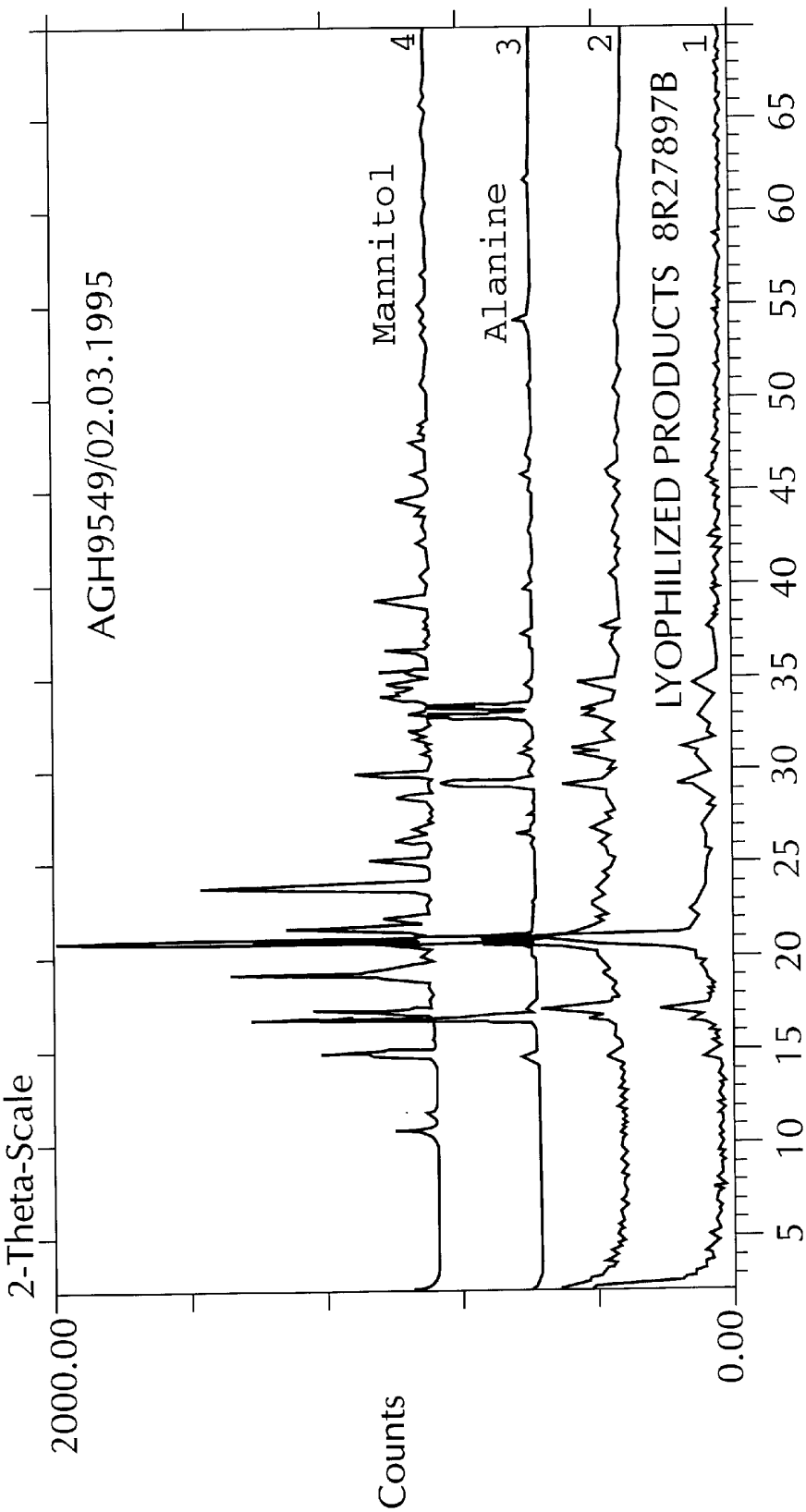
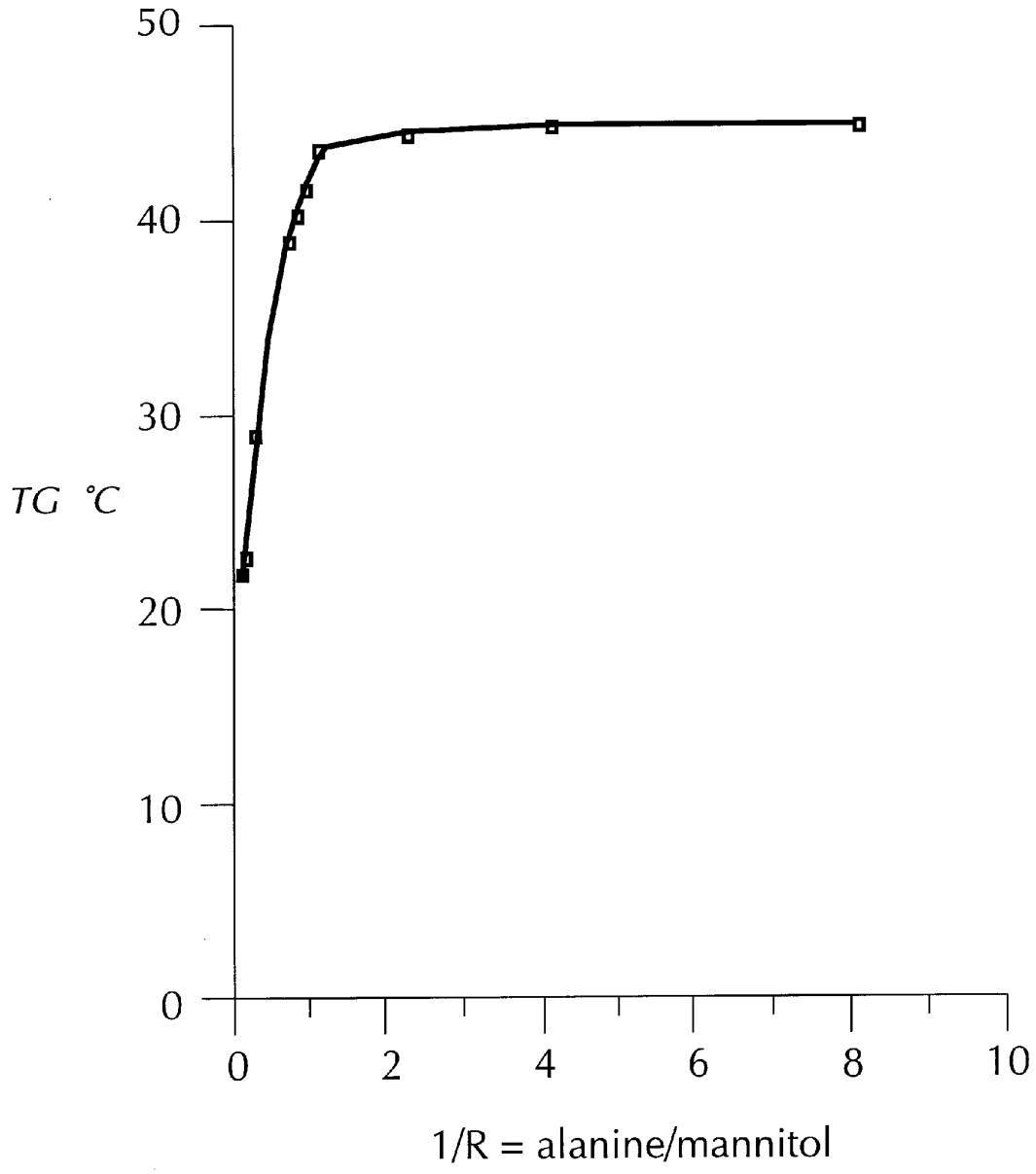


FIG. 2



STABLE FREEZE-DRIED PHARMACEUTICAL FORMULATION

This application is a 371 of PCT/FR 96/01706 filed Oct. 30, 1996.

The present invention relates to a pharmaceutical formulation provided in the form of a freeze-dried product and containing at least one active ingredient of nonprotein nature. More particularly, the invention relates to such a formulation, stable at temperatures which may be as high as 25° C. to 40° C., which may be either reconstituted in liquid form by addition of a solvent for its administration via the parenteral or oral route, or directly administered via the oral route, to man or to animals.

The active ingredient contained in the formulation according to the invention may be alone or else combined with another active ingredient of protein or nonprotein nature.

It is known that freeze-drying may have a considerable effect on the degradation of the pharmaceutical active ingredients in a formulation, as well as a strong impact on their stability in freeze-dried form. The various variables which affect these parameters are mainly the pH, the quantity of salts present, the type and quantity of excipients in the formulation, the type of cryoprotection chosen, as well as the temperatures, pressure and time chosen for the freezing, sublimation and drying operations. These different variables influence the physical state of the freeze-dried product obtained, namely: vitreous amorphous, soft amorphous, crystalline or a combination of these states.

For the preservation of the freeze-dried products, amino acids, preferably glycine, and polyols, preferably mannitol, are often used; but the literature, which is highly abundant on the subject, gives no information on the solution to the general problem of obtaining a stable pharmaceutical formulation which takes into account the different parameters involved in the operations for formulating and freeze-drying a nonprotein active ingredient in combination with an amino acid and a polyol.

More particularly, the literature teaches that the presence of an amino acid, of a polyol, for example mannitol, of a crystalline phase or of an amorphous phase may have, besides certain advantages, disadvantages which lead, in the case of freeze-dried products containing particularly sensitive active ingredients, to relatively short shelf lives and/or storage temperatures for these freeze-dried products which are less than 8° C. It would, however, be particularly advantageous, especially for an ambulatory treatment, to be able to obtain a formulation which is stable at room temperature until it is reconstituted and to thereby avoid its storage in a refrigerator before or during treatment.

The role of the polyol and of amino acid has been studied separately in the case of the human growth hormone (hGH), but their synergistic effect is still poorly elucidated (Pikal M. J., Dellermann K. M., Roy M. L., Riggin M. N., The effects of formulation variables on the stability of freeze-dried Human Growth Hormone, Pharm. research., 1991, 8, No. 4, 427–436).

The advantages and disadvantages linked to the presence of amino acids, of mannitol, of a crystalline phase or of an amorphous phase are listed below.

Advantages linked to the presence of amino acids.

It has been demonstrated that the presence of glycine in a freeze-dried product induced crystallization of the molecules present in solution during the freezing stage of the freeze-drying (Korey D. J., Schwartz J. B., Effects of excipients on the cristallization of pharmaceutical compounds during lyophylization, J. Parenteral Sci. Tech., 1989, 43, 2, 80–83). This crystallization of the active ingredient makes it possible to enhance its stability.

Alanine, in crystallized form, has the advantage of preventing the collapse of the freeze-dried product during sublimation and drying and or allowing the production of a freeze-dried product with a greater specific surface area and therefore allows a more rapid drying (Pikal M. J., Freeze-drying of proteins, Biopharm., 26–30 October 1990).

Disadvantages linked to the presence of amino acids.

The addition of an amino acid to a sugar or to a polyol in a solution to be freeze-dried generally has the effect of decreasing the glass transition temperature of the sugar (te Booy M. P. W. M., de Ruiter R. A., de Meere A. L. J., Evaluation of the physical stability of freeze-dried sucrose containing formulations by differential scanning calorimetry, Pharm. Research., 1992, 9, 109–114). Now, a decrease in the glass transition temperature is generally synonymous with a lower stability of a freeze-dried product (Franks F., Freeze-drying; from empiricism to predictability, Cryo-letters, 1990, 11, 93–110).

Advantages linked to the presence of mannitol.

The presence of mannitol in the composition of a freeze-dried product is generally justified as freeze-drying ballast, that is to say that it makes it possible both to maintain the solid and rigid structure of the volume of the freeze-dried product corresponding to the volume of solution to be freeze-dried, but its presence also makes it possible to adjust the isotonicity of the reconstituted solution to be injected. When mannitol is the predominant excipient in the composition of a freeze-dried product, it is most often in crystalline form (Lyophilized formulations recombinant tumor necrosis factor, Hora M. S., Rana R. K., Smith F. W., Pharm. Res., 1992, 9 (1), 33–36).

Disadvantages linked to the presence of mannitol.

It has been reported that the degree of hydrolysis of methylprednisolone sodium succinate, in freeze-dried form, was greater in the presence of mannitol than in the presence of lactose, and that this level increased with the quantity of mannitol present in the freeze-dried product. This has been explained by the fact that the crystallization of mannitol during freeze-drying changes the distribution of water in the matrix of the freeze-dried product. The increase in the quantity of water present in the microenvironment of the active ingredient resulting therefrom enhances the hydrolysis of the active ingredient and reduces its stability (The effect of bulking agent on the solid state stability of freeze dried methylprednisolone sodium succinate, Herman B. D., Sinclair B. D., Milton N., Nail S. L., Pharma. Res., 1994, 11 (10), 1467–1473).

Advantages linked to the presence of a crystalline phase.

The presence of a crystallized solute in a frozen solution is a means of stabilizing the proteins during drying (Carpenter J. F. & Crowe J. H., Modes of stabilization of a protein by organic solutes during dessiccation, Cryobiology, 1988, 25, 459–470). Furthermore, the crystallization, during freezing, of the predominant excipients present in a solution to be freeze-dried makes the secondary sublimation and drying operations more effective by increasing the specific surface area for exchange between the atmosphere in the freeze-drying vessel and the solid to be sublimed. This increase in the specific surface area of the crystalline forms compared with the amorphous forms facilitates heat exchanges during freeze-drying. The consequence of this increased efficiency in the freeze-drying is the production of freeze-dried forms whose residual water content is lower, which means an increased stability of the freeze-dried

product at higher temperatures (Korey D. J., Schwartz. J. B., Effects of excipients on the cristallization of pharmaceutical compounds during lyophylization, J. Parenteral Sci. Tech., 1989, 43, 2, 80–83).

Disadvantages linked to the presence of a crystalline phase.

In general, the crystallized substances have less rapid dissolution rates than the amorphous substances. Indeed, more energy is required to detach a molecule from an organized lattice of a crystalline arrangement than to detach it from a disorganized assembly of an amorphous state. Sometimes, the dissolution rate becomes insufficient to allow a sufficiently rapid absorption of these substances, which may lead to a decrease in their activity, especially in the case of molecules which are not very stable in solution. Likewise, the perfect regularity of crystals being an ideal case, the heterogeneity of the crystalline phase and the polymorphism which are obtained for the same substance and between associated substances induce different rates of dissolution for the same substance and between each of the substances, which may result in unreproducible therapeutic effects (Galénica 2, Biopharmacie 2nd edition, 1982, technique and documentation).

In addition, it has been demonstrated that the loss of activity of a freeze-dried protein was directly linked to the degree of crystallinity of the cryoprotective molecule (Izutsu K. L., Yoshioka S., Terao T., Decreased protein-stabilizing effects of cryoprotectants due to crystallization, Pharm. Research. 1993, 10, No. 8, 1232–1237; Izutsu K. I., Yoshioka S., Kojima S., Increased stabilizing effects of amphiphilic excipients on freeze drying of lactate dehydrogenase (LDH) by dispersion into sugar matrixes, Pharm. Res., 1995, 12 (6), 838–843). In the formulation of medicines containing proteins, the crystallization of the excipients should be avoided according to: (Hermansky M., Pesak M., Lyophilization of drugs, VI Amorphous and Cristalline forms Cesk. Farm., 1993, 42, (2), 95–98).

Advantages linked to the presence of an amorphous phase.

Based on the same line of thinking, the amorphous form dissolves more rapidly than the crystallized form and does not exhibit the disadvantages linked to the heterogeneity and to the polymorphism of the crystallized substances.

Moreover, the presence of additives in the amorphous state stabilizes the activity of certain enzymes proportionally to the concentration of the additive according to Izutsu K. L., Yoshioka S., Terao T., Decreased protein-stabilizing effects of cryoprotectants due to crystallization, Pharm. Research., 1993, 10, No. 8, 1232–1237.

The cryoprotective effect of the excipients is attributed to the amorphous state of the glycine in the freeze-dried product obtained (Pikal M. J., Dellermann K. M., Roy M. L. Riggin M. N., The effects of formulation variables on the stability of freeze-dried Human Growth Hormone, Pharm. Research., 1991, 8, No. 4, 427–436).

Disadvantages linked to the presence of an amorphous phase.

In the presence of a solid amorphous phase alone, the freeze-dried product collapses at temperatures greater than the glass transition temperature during freezing. Within a soft amorphous phase, the chemical degradation reactions have much more rapid kinetics than within a crystalline phase (Solid state stability and preformulation study of a new parenteral cephalosporin antibiotics (E1040), Ashizawa K., Uchikawa K., Hattori T., Ishibashi Y., Miyake Y., Sato T., Yakugaku Zasshi, 1990, 110 (3), 191–201).

Furthermore, the higher rate of dissolution of the amorphous substances is sometimes accompanied by a greater instability, the conversion of a form generally occurring from the amorphous state to the crystallized state (Galénica 2, Biopharmacie 2nd edition, 1982, technique et documentation).

In conclusion, the scientific literature on the subject of the effect of excipients on the stabilization of pharmaceutical active ingredients gives contradictory information on their properties and furthermore does not make it possible to obtain some information on the subject of the relationships between the structure of a freeze-dried product and its stability. Likewise, the role of the polyols and of the amino acids, alone or in combination, is not described according to a set of generalizable properties, but has been observed with contradictory results according to the active principles studied and the quantities of excipients used.

It has now been found that a synergistic effect exists between mannitol and alanine on the stabilization of freeze-dried pharmaceutical active ingredients. It has in particular been demonstrated that this synergistic effect exists only in a narrow range of relative concentrations of each of these two excipients.

The discovery of a surprising synergistic effect resulting from the coexistence of an amorphous phase and a crystalline phase which has the consequence of stabilizing the freeze-dried pharmaceutical active ingredient forms the basis of the present invention. The present invention therefore describes the production of this effect for specific mannitol/alanine ratios.

Thus, the present invention relates to a freeze-dried pharmaceutical formulation consisting of an amorphous phase and a crystalline phase, comprising an effective quantity of at least one nonprotein pharmaceutical active ingredient, mannitol and alanine, the latter two excipients being in a mass ratio R of between 0.1 and 1, R being the ratio of the mass of the mannitol to the mass of the alanine. The active ingredient included in the said formulation remains stable at temperatures which may range from 25° C. to 40° C. in freeze-dried form. Where appropriate, the dissolution of the freeze-dried product obtained is rapid and complete. The freeze-dried product does not have a collapsed appearance and its water content is compatible with maintaining the stability of the active ingredient.

It has been demonstrated that, for R of between 0.1 and 1:

- the freeze-dried product consists of an amorphous phase and a crystalline phase,
- the amorphous phase predominantly consists of mannitol and active ingredient,
- the crystalline phase predominantly consists of alanine

Although the invention is not limited to a specific theory which explains the stabilization obtained by combining one or more nonprotein active ingredients, mannitol and alanine in the indicated ratios, the following hypothesis can be made:

- the amorphous phase, demonstrated by differential scanning calorimetry, cryoprotects the pharmaceutical active ingredient during freezing, the active ingredient itself being dispersed in this amorphous form, and the crystalline phase, demonstrated by X-ray diffractometry, fixes the structure of the freeze-dried product and avoids its collapse.

According to another of these features, the subject of the present invention is the production of stable freeze-dried products containing a pharmaceutical active ingredient cryoprotected by an amorphous solid phase consisting completely or partially of mannitol, this amorphous phase coexisting within the freeze-dried product obtained after

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