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(54) Title: LYOSPHERES COMPRISING GONADOTROPIN

(57) Abstract

The invention relates to lyospheres comprising gonadotropin, the preparation thereof, as well as pharmaceutical preparations containing the same.

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LYOSPHERES COMPRISING GONADOTROPIN

The invention relates to lyospheres comprising gonadotropin, the preparation thereof, as well as pharmaceutical preparations comprising the same.

Lyospheres are freeze-dried droplets. Spherical freeze-dried particles are known from Price et al. (US 3,655,838). These spherical beads, contain material for immunological reactions. Lyospheres comprising biological active materials are known, for instance from USP 3,932,943, and also from many other patents. Known advantages of lyospheres are the uniformity of the particles, the easy to handle products, the faster freeze-dry process, less degradation during the freeze-dry process, and the improved dissolution properties.

Surprisingly, it has now been found that lyospheres comprising gonadotropin have an improved shelf-life in comparison to conventionally freeze-dried gonadotropins. Moreover, as additional advantages it has been found that the recovery of the gonadotropin after freeze-drying of the lyospheres is better than the recovery after conventional freeze-drying, and that the analysis properties are improved.

The invention therefore, relates to a lyosphere which comprise a gonadotropin. Preferably said gonadotropin is HCG (human chorionic gonadotropin), FSH (follicle stimulating hormone), or LH (luteinizing hormone), or a combination thereof. Said gonadotropins may may obtained by isolation from natural sources or by recombinant techniques. With most preference, said gonadotropin is rec-FSH.
The lyospheres may also comprise pharmaceutically acceptable auxiliaries, such as fillers, stabilizers,

and surfactants. Usual auxiliaries are for example sucrose, mannose, trehalose, dextran, Tween, polyvinyl pyrrolidone, sodium citrate and the like.

The lyospheres can be prepared by freeze-drying droplets of an aqueous gonadotropin solution, which optionally also comprises the auxiliaries. To obtain droplets of the required size, the solution can be sprayed into a cold bath (for example as disclosed in DT 2,140,747, EP 81913, or US 3,928,566), into liquid nitrogen (for example as disclosed in J5 9169,504), or onto a refrigerated drum (for example as disclosed in USP 3892876) or a refrigerated plate (for example as disclosed in US 4,501,719). Various other methods are well-known in the art.

The lyospheres of the invention can be processed into a pharmaceutical preparation. The term pharmaceutical preparation means a vial or syringe, or any other means in which the lyospheres can be introduced, and as such can be used by physicians. When the lyosphere comprises FSH or rec-FSH as the gonadotropin, pharmaceutical preparations comprise 75, 100, 150, 300, and preferably 50 IU of the FSH. Preferably each lyosphere comprises a fixed amount of gonadotropin, for instance 50 IU. Determination of the amount of IU's present in the pharmaceutical preparation can then simply achieved by counting the number of lyospheres. When the lyosphere contains hCG or rec-CG as the gonadotropin, pharmaceutical preparations comprise preferably 1, 2.5, 5, 10, or 5000 IU of the gonadotropin.

The lyospsheres of the invention may be used for medical applications where gonadotropins are required. The lyospheres containing FSH or rec-FSH are particularly useful for application in IVF (in vitro fertilisation).

The invention is further illustrated by the following examples.

Example 1

Lyospheres were prepared as follows. The ingredients (gonadotropin and the expedients sucrose or trehalose, polysorbate 20, and possibly sodium citrate) were dissolved in water and diluted to the desired final concentration. The pH was adjusted to 7 using hydrochloric acid and/or sodium hydroxide. After filtration through a disposable 0.2 µm Durapore membrane filter, 100 µl droplets were formed and frozen, for instance in liquid nitrogen. The frozen droplets were collected and freeze-dried at -50 °C in a manner known per se. The freeze-dried droplets (lyospheres) were transferred into an ampoule or vial.

Example 2

The recovery of freeze-drying of lyospheres and conventional freeze-drying was compared by EIA (enzyme immuno assay) using aqueous formulations containing 10 U of rec-HCG.

Result:

formulation	content of HCG in U after freeze-drying	
	lyospheres	conventional
A	10	7.4
B	9.9	7.2

Formulations per ml:

A: 50 mg of sucrose, 0.04 mg of Tween 20, 0.92 mg of sodium citrate, 10 U of rec HCG.

B: 50 mg of sucrose, 0.20 mg of Tween 20, 0.92 mg of sodium citrate, 10 U of rec HCG.

Example 3

A 500 µl solution containing 75 IU of rec-FSH, 25 mg of sucrose, 7.35 mg of sodium citrate and 0.1 mg of polysorbate 20 was conventionally freeze-dried and compared with a 100 µl solution containing 75 IU of rec-FSH, 25 mg of sucrose, 7.35 mg of sodium citrate and 0.02 mg of polysorbate 20 which was freeze-dried as a lyosphere. Both freeze-dried products were stored for 6 months at 4, 25, 30, 40, and 50 °C, and for 12 months at 4, 25 and 30 °C. After 6 and 12 months the *in vitro* activity was determined according to the method of Mannaerts et al. in Roland et al. (ed.): Neuroendocrinology of reproduction, 1987, p.49-58, and expressed as percentage of the activity of the sample stored at 4 °C:

temp in °C	lyosphere		conventional	
	activity in %			
	6	12	6	12 months
4	100	100	100	100
25	-	96	102	73
30	118	85	97	77
40	118	-	76	-
50	100	-	50	-

Example 4

A 500 µl solution containing 5 IU of rec-HCG, 25 mg of sucrose, 0.46 mg of sodium citrate and 0.1 mg of polysorbate 20 was conventionally freeze-dried and compared with a 100 µl solution containing 5 IU of HCG, 25 mg of sucrose, 0.46 mg of sodium citrate and 0.02 mg of polysorbate 20 which was freeze-dried as a lyosphere. Both freeze-dried products were stored for 2 months at -18 and 50 °C. After 2 months the activity was determined by EIA and expressed as percentage of the activity of the sample stored at -18 °C:

temp in °C	lyosphere	conventional
	activity in %	
-18	100	100
50	84	59

Example 5

A 500 µl solution containing 5 IU of urinary HCG, 25 mg of sucrose, 0.46 mg of sodium citrate and 0.1 mg of polysorbate 20 was conventionally freeze-dried and compared with a 100 µl solution containing 5 IU of urinary HCG, 25 mg of sucrose, 0.46 mg of sodium citrate and 0.02 mg of polysorbate 20 which was freeze-dried as a lyosphere. Both freeze-dried products were stored for 2 or 6 months at -18, 8, 30, and 50 °C. After 2 or 6 months the activity was determined by EIA and expressed as percentage of the activity of the sample stored at -18 °C:

temp in °C	lyosphere		conventional
month	2	6	2
	activity in %		
-18	100	100	100
8	100	100	-
30	100	93	82
50	82	78	25

Claims:

1. A lyosphere, characterized in that the lyosphere comprises a gonadotropin.

2. The lyosphere of claim 1, wherein the gonadotropin is HCG, FSH, or LH, or a combination thereof.

3. The lyosphere of claim 1 or 2, wherein the gonadotropin is mixed with pharmaceutically acceptable auxiliaries.

4. The lyosphere any one of claims 1-3 for use in IVF.

5. The preparation of the lyosphere of any one of claims 1-3, characterized in that the gonadotropin is freeze-dried as a droplet.

6. A pharmaceutical preparation comprising the lyospheres of any one of claims 1-3.

7. The pharmaceutical preparation of claim 6, wherein the lyospheres comprises 50 IU of FSH or rec-FSH.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01303

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K9/14 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 448 146 (AKZO N.V.) 25 September 1991 see claims 1-10 ---	1-7
Y	US,A,3 932 943 (A. R. BRIGGS ET AL.) 20 January 1976 cited in the application see claims 1-8 ---	1-7
Y	GB,A,2 160 528 (KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) 24 December 1985 see page 1, line 13 - line 19 see claims 1-13 --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 081 913 (JOHN WYETH & BROTHER LIMITED) 22 June 1983 cited in the application see claims 1-11	1-7
A	WO,A,90 13285 (ENZYTECH INC.) 15 November 1990 see page 4, line 19 - line 29 see claims 1-5,11	1-7

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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