SG9610030-0
RTMP'S COPY

THIS SHEET HAS BEEN REPLACED

The present invention relates to an aqueous solution of interferon alpha which is suitable for parenteral administration. The manufacture of interferon solutions involves a number of problems which are caused by the sensitivity of the active ingredient against physical and chemical influences and which hitherto could not be solved satisfactorily. Like other proteins interferon in aqueous solutions is subject to chemical degradation mechanisms such as proteolysis, oxidation, disulfide exchange, oligomerisation, deamidation and beta-elimination, and physical mechanisms such as aggregation, precipitation and adsorption. Interferon solutions therefore contain additives which are to counteract these effects. For instance, human serum albumin (HSA) is used in commercial preparations as a stabilisator which, however, is problematic in view of the danger of viral contamination and formation of aggregates which in turn may cause antibody formation. Therefore, interferon solutions have already been proposed which avoid the use of HSA and which contain other auxiliary agents, inter alia, non-ionic detergents (cf the International Patent Application WO 89/04177 and Japanese Patent Publication 61-277633). It is further known that the maintainance of particular pH values is important for the stability of interferon solutions. For instance, a pH range of 4.0-6.0 is mentioned in patent application WO 89/04177. Finally, as in other injection solutions further excipients can be required, e.g., agents for adjusting an isotonic solution, and preserving agents.

Since interferon is highly active and is present in minimal concentration in pharmaceutical preparations, the stability of interferon preparations and guaranteeing a constant concentration of the active ingredient is of particular importance. It has been found that in order to guarantee optimal utilization properties the excipients of an interferon solution must be selected carefully from a multitude of potentially suitable agents and be harmonized with each other. For example, the adsorption of interferon-alpha 2a on glass surfaces has a maximum at pH 5-6 so that this pH would in principle seem unfavourable. On the other hand, covalent degradation reactions proceed through a minimum at this pH. Commercial HSA-stabilized solutions have pH 7. The utilization properties of interferon

This Sheet Has Been Replaced

solutions are influenced by a number of non-correlating factors in an unpredictable manner.

It has now been found that aqueous HSA-free interferon-alpha solutions containing

(a) an interferon-alpha;
(b) a non-ionic detergent;
(c) a buffer for adjusting pH 4.5-5.5;
(d) benzyl alcohol; and, optionally,
(e) an isotonizing agent;

exhibit optimal utilization properties, i.e. storage stability and bioavailability of the declared amount of active ingredient.

For use in the present invention, any interferon-alpha can be used, e.g., interferon-alpha as disclosed in European Patent No. 43980 (referred to therein as mature human leukocyte interferon-A, see also J. Pharm. Biomed. Analysis Vol. 7, No. 2, 233-238 (1989)).

The interferon alpha used in this invention may be conjugated to a polymer such as a polyalkylene glycol (substituted or unsubstituted), for example polyethylene glycol, to form PEG-interferon alpha. Conjugation may be accomplished by means of various linkers known in the art, in particular by linkers such as those disclosed in European patent publication EP-A-0510356 and A-0593868. The molecular weight of the polymer, which is preferably polyethylene glycol, may range from 300 to 30,000 daltons, and one or more, preferably one to three, polymers may be conjugated to the interferon alpha. A preferred interferon-alpha conjugate is formed using interferon alpha 2a.

A preferred interferon-alpha for use in the present invention is interferon-alpha 2a and pegylated (PEG) interferon-alpha 2a. Preferably, the solutions in accordance with the present invention contain 10^6 - 10^8, particularly 1-36 x 10^6 International Units (IU) interferon-alpha per ml.

Examples of non-ionic detergents for use in the preparations in accordance with the invention are Polysorbates, such as e.g. Polysorbate 20

or Polysorbate 80 (polyoxyethylene(20)sorbitan monooleate). The amount of detergent in the solutions in accordance with the invention is about 0.01 - 0.5 mg/ml, preferably 0.05 - 0.2 mg/ml. Preferred buffer substances are ammonium acetate and sodium lactate. The concentration of these buffer substances is suitably about 10 - 15 mmolar. Preferably, the interferon solutions in accordance with this invention are adjusted to pH 5.0 ± 0.1. Benzyl alcohol is contained in the solutions in accordance with this invention in an amount of about 8 - 20 mg/ml, particularly 10 mg/ml. As isotonizing agents there come into consideration in particular sodium chloride, mannitol, glycerol and amino acids, particularly arginine, lysine, histidine and methionine, as well as ethanolamine. Sodium chloride or mannitol are preferred. The amount af these auxiliary agents which is required for achieving isotonicity depends on the composition of the solution and can be determined with ordinary skill.

The invention is further illustrated by the Examples which follow.

Example 1

Preparation of PEG-IFN alpha 2a

PEGylation: IFN-alpha 2a was dialyzed twice against 10 liters of a buffer consisting of 5 mM sodium acetate pH 5.0 containing 120 mM NaCl. One gram of material (7.26 mg/ml) was PEGylated using a 3:1 molar ratio of solid PEG reagent alpha-methyl-omega-[2-[[(3-methyl-2-pyridinyloxy)-carbonyl]amino]ethoxy]poly(oxy-1,2-ethanediyl) SRU 110. The pH of the solution was adjusted by adding one-tenth volume of 100 mM sodium borate pH 10.7. Following a one hour incubation at room temperature, the reaction was quenched by addition of 1 M glycine to a final concentration of 20 mM glycine. One twentieth volume of 1 M sodium acetate, pH 4.0 was added to achieve a final pH of 5.0-6.0. The protein solution was diluted fourfold with buffer consisting of 40 mM ammonium acetate pH 4.5.

Purification: The diluted PEGylation mixture was loaded onto a 333 ml CM-cellulose column equilibrated with 40 mM ammonium acetate pH 4.5 at a flowrate of 19 ml/min. PEGylated interferon was eluted with a 0-250 mM NaCl gradient over 8 column volumes. Fractions containing PEG-IFN were pooled according to the results of SDS-PAGE. The final pool contained

291 mg at 0.831 mg/ml. Pooled material was concentrated to 3.96 mg/ml via an Amicon stirred cell ultrafiltration unit using a YM10 (MW cutoff 10000) membrane.

Concentrated material (238 mg) was loaded onto a 6.3 L S-200 gel filtration column equilibrated with 40 mM ammonium acetate and 125 mM NaCl. The flowrate was 20 ml/min. Fractions were collected and analyzed via SDS-PAGE. The S-200 column pool contained 480 ml at 0.48 mg/ml. An aliquot of the S-200 column pool was concentrated to 8.7 mg/ml using an Amicon stirred cell. This material was used to prepare the formulations of Examples 4 and 5.

Example 2

Interferon Solution

Ingredient	amount per ml
Interferon-alpha 2a	1 - 36 x 10^6 IU
Ammonium acetate	0.77 mg
Sodium chloride	7.21 mg
Benzyl alcohol	10.0 mg
Polysorbate 80	0.2 mg
Acetic acid ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

Manufacturing procedure:

The formulations were prepared under aseptic conditions in a laminar flow bench in 50 ml sterile polypropylene tubes with srew cap. The excipients were dissolved in water for injection, the pH was adjusted and the solutions were gassed with nitrogen. Then, Interferon bulk solution was added under gentle stirring, followed by an adjustment of the pH, if necessary, and the adjustment to the final volume by addition of water for injection. The solutions were sterile filtered into a fresh polypropylene tube using a low protein binding 0.2 µm filter and filled into 2 ml vials of glass type I. The vials were flushed with nitrogen and closed with a butyl rubber stopper, which was laminated with an inert film of fluoropolyethylene.

Example 3

Interferon Solution

Ingredient	amount per ml
Interferon-alpha 2a	1 - 36 x 10^6 IU
Ammonium acetate	0.77 mg
Glycerol	20.0 mg
Benzyl alcohol	10.0 mg
Polysorbate 80	0.2 mg
Acetic acid ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

Manufacturing procedure: as in Example 2.

Example 4

Interferon Solution

Ingredient	Amount per ml
PEG-Interferon-alpha 2a	1 - 18 x 10^6 IU
Ammonium acetate	1.0 mg
Sodium chloride	5.0 mg
Benzyl alcohol	10.0 mg
Polysorbate 80	0.05 mg
Acetic acid ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

Manufacturing procedure: as in Example 2.

Example 5

Interferon Solution

Ingredient	Amount per ml
PEG-Interferon-alpha 2a	1 - 18 x 10^6 IU
Ammonium acetate	1.0 mg
Sodium chloride	3.0 mg
Mannitol	30.0 mg
Benzyl alcohol	10.0 mg
Polysorbate 80	0.05 mg
Acetic acid ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

Manufacturing procedure: as in Example 2.

For comparison purposes, the interferon-alpha 2a solutions of Example 2 with 3 x 10^6 IU IFN-alpha-2a (A/3), 6 x 10^6 IU IFN-alpha-2a (A/6), 9 x 10^6 IU IFN-alpha-2a (A/9), 18 x 10^6 IU IFN-alpha-2a (A/18) and 36 x 10^6 IU IFN-alpha-2a (A/36) and corresponding solutions without benzyl alcohol (B/3-36) were prepared according to the manufacturing procedure given in Example 2 and stored in the dark at 5, 25 and 35°C. The contents of interferon-alpha-2a in the vials was determined after 3 months of storage. Samples were filtered through a 0.45 µm filter and analyzed by reverse phase HPLC for the remaining main component of interferon-alpha-2a. The HPLC method has a standard deviation of about 5 %. The results of the storage trial is set out in Table 1.

Table 1

Solution/ x 10^6 IU IFN alpha 2a per ml	Contents of the main component of IFN alpha 2a in % after 3 months at		
	5°C	25°C	35°C
A/3	93.8	60.7	43.5
A/6	91.2	73.9	54.6
A/9	94.1	80.3	61.6
A/18	94.1	84.5	69.0
A/36	91.9	88.5	71.1
B/3	81.0	41.0	8.2
B/6	88.9	55.1	8.5
B/9	89.1	63.3	25.8
B/18	92.2	62.8	26.8
B/36	95.2	72.8	41.2

The better storage stability of the solution A is particularly evident at increased storage temperature.

In analogy, a solution of pegylated IFN of Example 4 with 3 x 10^6 IU pegylated interferon-alpha-2a (C/3) and a corresponding solution without benzyl alcohol (D/3) were prepared and stored for 24 months at 5 and 25 °C. The results of the storage trial are shown in Table 2.

Table 2

Solution/ x 10^6 PEG IU IFN alpha 2a per ml	Contents of the main component of PEG IFN alpha 2a in % after 24 months at	
	5°C	25°C
C/3	79.6	55.8
D/3	60.1	5.8

The better storage stability of solutions which were prepared with the addition of benzyl alcohol is evident from these trials also.

Example 6

As mentioned above, HSA-free solutions of interferons are known from the International Patent Application WO 89-04177 and the Japanese patent publication 61-277633. The stability of the solutions according to the invention was compared with the stability of interferon-alpha-2a solutions which were prepared in analogy to these known solutions. Solutions of the following composition were prepared:

Solution X

Interferon-alpha 2a	3 - 36 x 10^6 IU
Ammonium acetate	0.77 mg
Sodium chloride	8.77 mg
Polysorbate 80	0.3 mg
Acetic acid ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

Solution Y

Interferon-alpha 2a	3 - 36 x 10^6 IU
Succinic acid	0.27 mg
Di-Sodium succinate	0.73 mg
D-Mannitol	40.0 mg
Polysorbate 80	0.1 mg
HCl 0.1 N ad pH 5.0 ± 0.1	q.s.
NaOH 0.1 N ad pH 5.0 ± 0.1	q.s.
Water for injection	ad 1.0 ml

The solutions X and Y correspond to solutions described in the above documents, whith 3,6,9,18 and 36 x 10^6 IU interferon-alpha-2a being used instead of interferon-beta or interferon-gamma. The results obtained after 3 months at various storage temperatures are set out in Table 3 hereinafter

Table 3

Solution/ x 10^6 IU IFN alpha 2a	Contents of the main component of IFN alpha 2a in % after 3 months at		
	5°C	25°C	35°C
X/3	72.5	13.7	0.0
X/6	72.8	3.1	0.0
X/9	83.7	42.6	1.7
X/18	86.5	50.5	2.6
X/36	88.0	54.1	4.5
Y/3	67.1	32.7	0.0
Y/6	80.0	53.5	0.0
Y/9	84.9	63.4	4.3
Y/18	89.0	59.5	13.7
Y/36	90.7	60.0	18.9

From these data it is evident that when applying the technology described in the above-mentioned documents of the state of the art to interferon-alpha-2a no acceptable storage stability can be achieved.



Claims

1. An aqueous interferon solution containing

(a) an interferon-alpha;
(b) a non-ionic detergent;
(c) a buffer for adjusting pH 4.5-5.5;
(d) benzyl alcohol; and, optionally,
(e) an isotonizing agent.

2. An interferon solution according to claim 1, wherein the amount of interferon-alpha is 10^6 - 10^8 IU per ml; the amount of non-ionic detergent is about 0.01 - 0.5 mg per ml; the concentration of buffer is about 10 - 15 mmolar; and the amount of benzyl alcohol is about 8 - 20 mg per ml.

3. An interferon solution according to claim 1 or 2 containing

(a) interferon-alpha-2a or PEG-interferon-alpha-2a;
(b) polyoxyethylene(20)sorbitan monooleate;
(c) ammonium acetate or sodium lactate;
(d) benzyl alcohol; and
(e) sodium chloride, mannitol, glycerol, arginine, lysine, histidine, methionine or ethanolamine.

4. An interferon solution according to claim 1 or 2 containing per ml

(a) 1-36 x 10^6 IU of interferon-alpha-2a;
(b) 0.2 mg of polyoxyethylene(20)sorbitan monooleate;
(c) 10 mM of ammonium acetate or sodium lactate;
(d) 10 mg of benzyl alcohol; and
(e) sodium chloride in an amount sufficient to provide an isotonic solution.

5. An interferon solution according to claim 1 or 2 containing per ml

(a) 1-36 x 10^6 IU of PEG-interferon-alpha-2a;
(b) 0.05 mg of polyoxyethylene(20)sorbitan monooleate;
(c) 13 mM of ammonium acetate;
(d) 10 mg of benzyl alcohol; and

* C L M S *

(e) sodium chloride or mannitol in an amount sufficient to provide an isotonic solution.

6. An interferon solution according to any one of claims 1-5 having a pH of 5.0 ± 0.1.

7. The invention as described hereinbefore, especially with reference to Examples 2-5.

***