Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2116192 C 2005/05/10

(11)(21) 2 116 192

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 1994/02/22

(41) Mise à la disp. pub./Open to Public Insp.: 1994/08/24

(45) Date de délivrance/Issue Date: 2005/05/10

(30) Priorité/Priority: 1993/02/23 (57826/1993) JP

(51) Cl.Int.^{6}/Int.Cl.^{6} A61K 38/19, A61K 47/16

(72) Inventeurs/Inventors:
HAYASAKA, HITOSHI, JP;
KAWASHIMA, NOBUYUKI, JP;
UEDA, MASATSUGU, JP;
KUMAZAWA, EITARO, JP

(73) Propriétaire/Owner:
DAIICHI PHARMACEUTICAL CO., LTD., JP

(74) Agent: DENNISON ASSOCIATES

(54) Titre : PREPARATIONS PHARMACEUTIQUES DE TCF A CONCENTRATION ELEVEE
(54) Title: HIGHLY CONCENTRATED TCF PHARMACEUTICAL PREPARATIONS

(57) Abrégé/Abstract:
This invention provides pharmaceutical preparations containing highly concentrated tumor cytotoxic factor (TCF). The pharmaceutical prparations of the present invention contain highly concentrated TCF and a basic amino acid or their salts, or an organic or inorganic salt as a solubizer. The resultant compositions described in the present invention dissolve TCF at high concentrations of 10 mg/ml or over under about neutral and isotonic conditions. The resultant compositions are stable and are suitable for injection preparations.

Abstract

This invention provides pharmaceutical preparations containing highly concentrated tumor cytotoxic factor (TCF). The pharmaceutical prparations of the present invention contain highly concentrated TCF and a basic amino acid or their salts, or an organic or inorganic salt as a solubizer. The resultant compositions described in the present invention dissolve TCF at high concentrations of 10 mg/ml or over under about neutral and isotonic conditions. The resultant compositions are stable and are suitable for injection preparations.

Title of the Invention

Highly Concentrated TCF Pharmaceutical Preparations

Field of the Invention

This invention relates to pharmaceutical preparations having tumor cytotoxic factor activity.

Background of the Invention

Tumor cytotoxic factor, hereinafter abbreviated as TCF, is an another name of TCF-II found in a cultured supernatant of human fibroblast cells and disclosed in WO 90/10651. TCF is a glycoprotein consisting of heterodimer having molecular weight of about 76-80 kDa in unreduced state, and α subunit having molecular weight of about 52-56 kDa and β or β' subunit having molecular weight of about 30-36 kDa in reduced state.

TCF exhibits various biological activities such as the activities of hepatocyte growth factor; HGF, scatter factor; SF, proliferation factor of renal tubular epithelial cells, repair factor for damaged tissues and proliferation factor of vascular endothelial cells, in addition to TCF activity. TCF is a cytokine belonging to a member of HGF family. TCF is expected to be developed as pharmaceuticals for the treatment of diseases of liver and kidneys, wounds and tumors due to its various kinds of physiological activity.

However, the solubility in water of TCF is very low. Thus aqueous preparations such as injections with high concentrations satisfying

medical use are hardly obtained. Therefore, one of the most serious subject to be solved for its application to the clinical use is preparing such a concentrated solution of TCF. TCF has rapid metabolic turnover in vivo and high dosage is expected on the clinical use. The clinical dosage of TCF is expected to be 1-10 mg/day for adult patients. To make sure of the quality of final products, such as injections, the production processes require to dissolve TCF at high concentrations and to mix it with additives such as stabilizers under low temperatures. Furthermore, a highly concentrated TCF solution is demanded for medical treatment, that is the solution of neutral pH and has isotonicity for injections. No such method to prepare highly concentrated TCF solution has been developed yet. For example, an isotonic saline solution, containing 0.15 M sodium chloride usually used for injectins, dissolves TCF less than 5 mg/ml and the TCF solution is unstable. The solubility of TCF decreases and TCF becomes insoluble with the progress of time at room temperature. Furthermore, solubility of TCF in saline markedly decreases to about 1 mg/ml at 5 ℃ or lower.

Therefore, it is an important subject to establish a method for preparing a neutral, isotonic and highly concentrated TCF solution at low temperatures. Above mentioned WO 90/10651 discloses preparations containing a protein, a sugar, an amino acid and so forth as an adsorption preventive agent or a stabilizer. However, neither highly concentrated TCF solution of the present invention is disclosed nor suggested.

Thus, the object of the present invention is to provide a highly concentrated and isotonic TCF injection solutions for medical treatment.

Brief Description of the Drawings

Fig. 1 shows a stability on storage of the TCF solution prepared by adding sodium chloride as an dissolution adjubant.

Fig. 2 shows a stability on storage of the TCF solution prepared by adding L-arginine hydrochloride and sodium chloride as dissolution adjuvants and D-mannitol as a stabilizer.

Fig. 3 shows a stability on storage of the TCF solution prepared by adding sodium chloride as an dissolution adjuvant, and human serum albumin (HSA) and D-mannitol as stabilizers.

Summary of the Invention

The inventors have been investigating to overcome the difficulty in dissolving TCF at high concentrations and found the following characteristic feature of solubility of TCF and accomplished the present invention.

(1) TCF shows temperature dependent solubility in water.

(2) TCF exhibits higher solubility at lower pH regions under a pH range of 5-8.

(3) Addition of a salt such as sodium chloride, preferably at concentrations of 0.3 M or over, provides markedly increased solubility of TCF.

(4) Addition of a basic amino acid, preferably 1.0-4.0 % of arginine

or lysine, markedly elevates the solubility under neutral pH and isotonicity of about 300 mOsm.

One object of the present invention is to provide preparatins of TCF solution with improved solubility to satisfy the use for medical treatments.

Other object of the present invention is to provide highly concentrated TCF solution containing one or more solubilizing agents selected from the group consisting of basic amino acids or their salts, and these amino acids together with pharmacologically acceptable organic or inorganic salts.

Further object of the present invention is to provide highly concentrated TCF injections with neutral pH and isotonicity containig one or more solubilizing agents selected from the group consisting of basic amino acids or their salts, and these amino acids together with pharmacologically acceptable organic or inorganic salts.

Detailed Description of the Invention

The TCF pharmaceuticals of the present invention contain 5 mg/ml or over TCF together with a basic amino acid and/or inorganic or organic salt as solubilizing agent(s) at concentrations to give isotonic solution. The pharmaceuticals of the present invention must be homogenous mixtures of TCF and the solubilizing agent(s) in the case of dissolving them before use. To prepare these pharmaceuticals, the TCF solution at the desired concentration must be prepared and divided in vials or ampoules, occasionally lyophilyzed, and sealed. Highly

concentrated TCF solutions are essential to prepare these pharmaceuticals. However, the solubility of TCF in water is very low. Acidic condition of pH 6 or less, or ionic strength of 0.3 M or over of sodium chloride is required to increase the solubility of TCF. But acidic injections cause patients pain on injection and are unpreferable. Also, a higher concentration of sodium chloride is unpreferable because of raising osmotic pressure of the injections.

To maintain isotonicity, one may prepare 0.15 M sodium chloride solution having osmotic pressure of about 300 mOsm. However, this solution dissolves only about 1 mg/ml of TCF at low temperature. To dissolve TCF up to about 10 mg/ml, 0.3 M or over sodium chloride solution is required, but its osmotic pressure becomes 600 mOsm or over. Therefore, multiple solubilizing agents are required to obtain the solution dissolving TCF at 10 mg/ml or over on the isotonic condition.

As solubilizing agents, basic amino acids and organic or inorganic salts and both of them can be used. Arginine or lysine is preferable as a basic amino acid and their salts also be used. Solutions containing these amino acids or their salts at concentrations of 3-4 % of as free amino acid are almost isotonic. These solutions can dissolve TCF at concentrations of 10-20 mg/ml. Furthermore, these basic amino acids or their salts may be combined with one or more organic and inorganic salts. For examples, 1.5-1.75 % of the amino acid solution with pharmacologically acceptable organic and inorganic salts can be isotonic. Sodium citrate or sodium lactate may be

exemplified as organic salts. Sodium chloride, disodium hydrogenphosphate or sodium hydrogencarbonate may be exemplified as inorganic salts, and sodium chloride is preferable. TCF dissolves at concentration of 5-10 mg/ml by using the basic amino acid with the salt. This TCF solution can be used as injections after being sterilized, divided in vials or ampoules and sealed. Also the solution may be freeze-dried to give lyophilized pharmaceuticals. Lyophilized pharmaceuticals may be preparaed by dissolving TCF at twofold concentration into a solution containing a basic amino acid and a salt at two times concentration (e.g. 7 % or higher basic amino acid or a combination of 4 % of basic amino acid and 0.15 M sodium chloride) and lyophilizing, as TCF easily dissolves at a high concentration in a solution of a basic amino acid and a salt. This procedure may reduce time and energy required for lyophilization. The lyophilized preparations may be re-dissoleved in twofold distilled water for injection to give isotonic intramuscular or intravenous injections. The TCF pharmaceuticals or injections of the present invention include these lyophilized pharmaceuticals.

As TCF is easily adsorbed to glass or synthetic resins, addition of a surfactant, an adsorption preventive agent or a stabilizer may be helpful to prevent TCF from adsorping. Tween* 20, Tween 80 and Tween 100 can be exemplified as surfactat. Human serum albumin, gelatin, sorbitol, mannitol and xylitol disclosed in WO 90/10651 may be exemplified as adsorption preventive agents and stabilizers.

The TCF pharmaceutical preparations of the present invention can be

*-trademark

stored for a long period of time maintaining sufficient amount of TCF for the treatment of diseases which require higly concencentrated solution of TCF.

The present invention will be explained by the following examples and reference examples. The present invention, however, is not restricted by these examples.

【Examples】

The present examples show the preparation of highly concentrated pharmaceuticals of TCF.

TCF used for examples, reference examples and experiments is recombinant TCF (r-TCF) produced by genetically engineered Namalwa cells by application of recombinant DNA technique according to the method disclosed in WO 92/1053. Solutions containing TCF were prepared with 10 mM phosphate buffer containing 0.01 % Tween 80 as an adsorption preventive agent.

(1) A preparation of injections containing TCF at high concentration

① TCF solution was prepared by dissolving TCF to 20 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 0.01 % of Tween 80 and 0.3 M of sodium chloride.

② An aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 2.33 % L-arginine hydrochloride and 0.01 % Tween* 80 was prepared. This solution and the TCF soliution prepared in ① were mixed at a ratio of 3:1. After the solutions were mixed well, the mixed solution was sterilized with a filter having 0.22 μm pores and

*-trademark

divided 1 ml each in ampoules and sealed. The prepared solution showed neutral pH and isotonicity and contained 5 mg/ml of TCF, 0.075 M of sodium chloride and 1.75 % L-arginine hydrochloride. Therefore this solution is most preferable for injections as pharmaceuticals. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

(2) A preparation of injections containing TCF at high concentration

TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 3.5 % of DL-arginine hydrochloride and 0.01 % of Tween 80. This TCF solution was sterilized with the filter and divided 1 ml each to vials and sealed. The solution, showed neutral pH and isotonicity and contained 10 mg/ml of TCF, is most preferable for injections. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

(3) A preparation of injections containing TCF at high concentration

TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 3.0 % of L-lysine hydrochloride and 0.01 % of Tween*80. This TCF solution was sterilized with the filter and divided 1 ml each to vials and sealed. The solution, shows neutral pH and isotonicity and contains 10 mg/ml of TCF, is most preferable for injections. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

*-trademark

(4) A preparation of lyophilized injections containing TCF at high concentration

TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 7.0 % of DL-arginine hydrochloride and 0.02 % of Tween* 80. This TCF solution was sterilized with the filter, divided 1 ml each to vials, lyophilized and sealed. The lyophilized preparation was re-dissolved in 2 ml of distilled water for injection before use to give 5 mg/ml solution of TCF which showed neutral pH and isotonicity.

[Experiment 1]

The solubility test of TCF is explained by the following test experiments. The present test gave findings concerning the profile of TCF solubility required for preparaing pharmaceuticals of TCF.

(1) Evaluation of solubility of TCF

TCF was weighed in polypropylene tubes, and solutions of various pHs containing various concentrations of sodium chloride and/or an amino acid were added into the tubes. To dissolve TCF, the tubes were immediately placed at a constant temperature and stirred for 30 min. according to the method described in the XIIth Pharmacopoeia of Japan (JP XII), General notices 24. The tubes were ultracentrifuged at 30,000 x g for 30 min. at a constant temperature to separate unsolved TCF. The concentration of TCF in the obtained saturated TCF solutions was measured by Lowry-Folin's method to determine solubility of TCF.

(2) Effect of pH on solubility of TCF

*-trademark

Solutions of various pHs containing 0.15 M sodium chloride or not were prepared. Solubilities of TCF in these solutions were determined at 5℃ and 20℃ according to the method shown in experiment (1) and the results are shown in Table 1. The results showed that the solubilitiies of TCF were increased depending on the decline of pH at pH 7 or lower.

【Table 1】

pH	0 M NaCl	0.15 M NaCl	
	20 ℃	5 ℃	20 ℃
5.5	1.9	5.6	15.0
6.0	1.0	2.8	12.4
6.5	0.6	1.7	6.5
7.0	0.4	1.2	4.9
7.5	———	———	4.6
8.0	———	———	4.8

* Solubility is expressed as mg/ml.

(3) Effect of concentration of sodium chloride on solubility of TCF

Solutions of various concentration of sodium chloride at pH 6, pH 6.5 and pH 7 were prepared. Solubilities of TCF in these solutions were determined at 20 ℃ according to the method shown in experiment (1) and the results are shown in Table 2. The results showed the remarkable increse of solubility of TCF when the concentration of sodium chloride was raised from 0.15 M to 0.3 M. However, the increase of concentration of sodium chloride from 0.3 M to 1.2 M made a slight raise of the solubility of TCF.

【Table 2】

Concentration of sodium chloride (M)	pH		
	6.0	6.5	7.0
0	1.0	0.6	0.4
0.15	12.4	6.5	4.9
0.3	53.7	51.4	49.4
1.2	62.1	57.5	53.9

* Solubility is expressed as mg/ml.

(4) Effect of temperature on solubility of TCF

Solutions containing 0.15 M or 0.3 M sodium chloride at pH 6, pH 6.5 and pH 7 were prepared. Solubilities of TCF in these solitions were determined at different temperatures according to the method shown in experiment (1) and the results are shown in Table 3. The results showed the remarkable increase of solubility of TCF in temperature dependent manner.

【Table 3】

Temperature	0.15 M NaCl			0.3 M NaCl		
(℃)	pH 6.0	pH 6.5	pH 7.0	pH 6.0	pH 6.5	pH 7.0
5	2.8	1.7	1.2	50.6	39.2	37.0
20	12.4	6.5	4.9	53.7	51.4	49.4
40	32.9	31.1	30.0	60.7	59.7	58.7

* Solubility is expressed as mg/ml.

(5) Effect of solubilizer on solubilitiy of TCF

In consideration of physiological conditions to use TCF for pharmaceuticals, solutions of neutral pH of 6.8-7.2 for dissolving TCF were prepared using various amino acids as solubizers and sodium chloride to adjust osmotic pressure to about 300 mOsm. The solubility of TCF was determined at 5 ℃ according to the method shown in

experiment (1) and the results are shown in Table 4.

【Table 4】

Amino acid	Concentration	0 M NaCl	0.075 M NaCl	0.15M NaCl
———	———	———	———	1.2
Gly	2 %	1.0	———	———
	1 %	———	3.0	———
L-Ala	2.5 %	1.5	———	———
	1.25 %	———	3.2	———
L-Ser	3 %	1.0	———	———
	1.5 %	———	1.9	———
L-Met	4 %	1.2	———	———
	2 %	———	2.0	———
L-Pro	3 %	1.4	———	———
	1.5 %	———	3.0	———
L-Asp・Na・H_2O	3 %	5.3	———	———
	1.5 %	———	3.4	———
L-Glu・Na・H_2O	3 %	4.7	———	———
	1.5 %	———	3.3	———
L-Arg・HCl	3.5 %	18.4	———	———
	1.75 %	———	7.6	———
D-Arg・HCl	3.5 %	21.7	———	———
	1.75 %	———	10.3	———
DL-Arg・HCl	3.5 %	21.7	———	———
	1.75 %	———	8.5	———
L-Lys・HCl	3 %	10.4	———	———
	1.5 %	———	6.9	———
L-His	4 %	3.5	———	———
	2 %	———	3.2	———

* Solubility is expressed as mg/ml.

Neutral amino acids such as glycine at concentration of 1-4 % gave slight increase of solubility and acidic amino acids such as sodium

L-aspartate monohydrate at concentration of 1-5 % incresed the solubility about 3-4 times than that in the solution with no amino acid. But significant solubilizing effect of these amino acids were not found.

On the contrary, basic ammino acids such as L-arginine at concentration of 1.75-3.5 % significantly increased solubility of TCF.

The solubilities were 5-15 times higher than that of no addition of amino acid. Furthermore, L-, D- and DL-forms of arginine gave same results. L-lysine at concentration of 1.5-3 % also gave increase of solubility. However, L-histidine at concentration of 2-4 % showed only about 3 times higher solubilities than that of no addition of amino acid.

The present invention can provide TCF pharmaceuticals of 10 mg/ml or more concentrations using basic amino acids, sodium chloride and so forth as a solubilizing agent. On the contrary, TCF dissolves at a concentration of about 1 mg/ml in neutral and isotonic solution without the solubilizing agent.

【Experiment 2】

Basic amino acids used in the present invention are effective to improve the stability of TCF during storage. The effect is explained by the following test experiments which show the effects of various additives on the stability of TCF during storage.

Human serum albumin (HSA) and D-mannitol were added to the solutions containing sodium chloride and/or L-arginine hydrochloride. TCF was

dissolved at concentrations of 1 mg/ml in these solutions at room temperature. The prepared TCF solutions were sterilized with a filter having pore size of 0.22 μm and divided in polypropylene tubes. The tubes were kept at 5 ℃ or 20℃ for 1, 4 and 7 days. The tubes were ultracentrifuged by the method described in Experiment (1). The concentration of TCF in the supernatant was measured by the method of Lowry-Folin and enzyme-linked immunosorbent assay (ELISA)

and

residual TCF was caluculated. The effects of the additives on the solubility of TCF were evaluated.

The results are shown in Fig. 1, 2 and 3. The values are expressed as percentage to the initial amount of TCF. As shown in the figures, L-arginine hydrochloride increased the stability of the pharmaceuticals of the present invention.

Claims

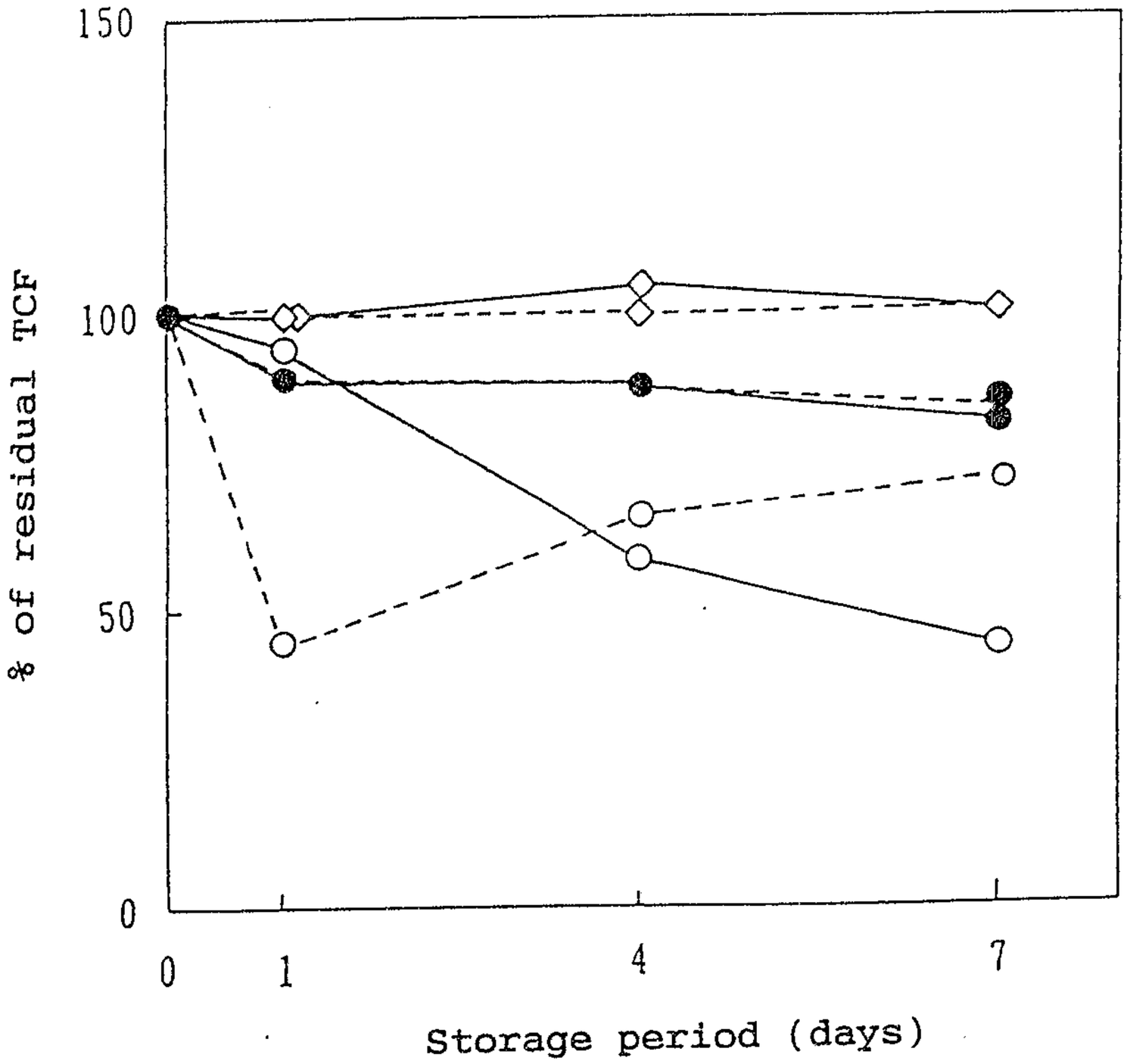
1. An isotonic tumor cytotoxic factor (TCF) pharmaceutical preparation having a neutral pH comprising:
at least 5 mg/ml TCF, and
one or more solubilizers selected from a group consisting of basic amino acids and their salts, optionally with pharmacologically acceptable organic or inorganic salts.

2. An isotonic tumor cytotoxic factor (TCF) injection preparation having a neutral pH comprising:
at least 5 mg/ml TCF, and
one or more solubilizers selected from a group consisting of basic amino acids and their salts, optionally with pharmacologically acceptable organic or inorganic salts.

3. An isotonic TCF injection preparation according to Claim 2 wherein the preparation comprises at least 10 mg/ml TCF.

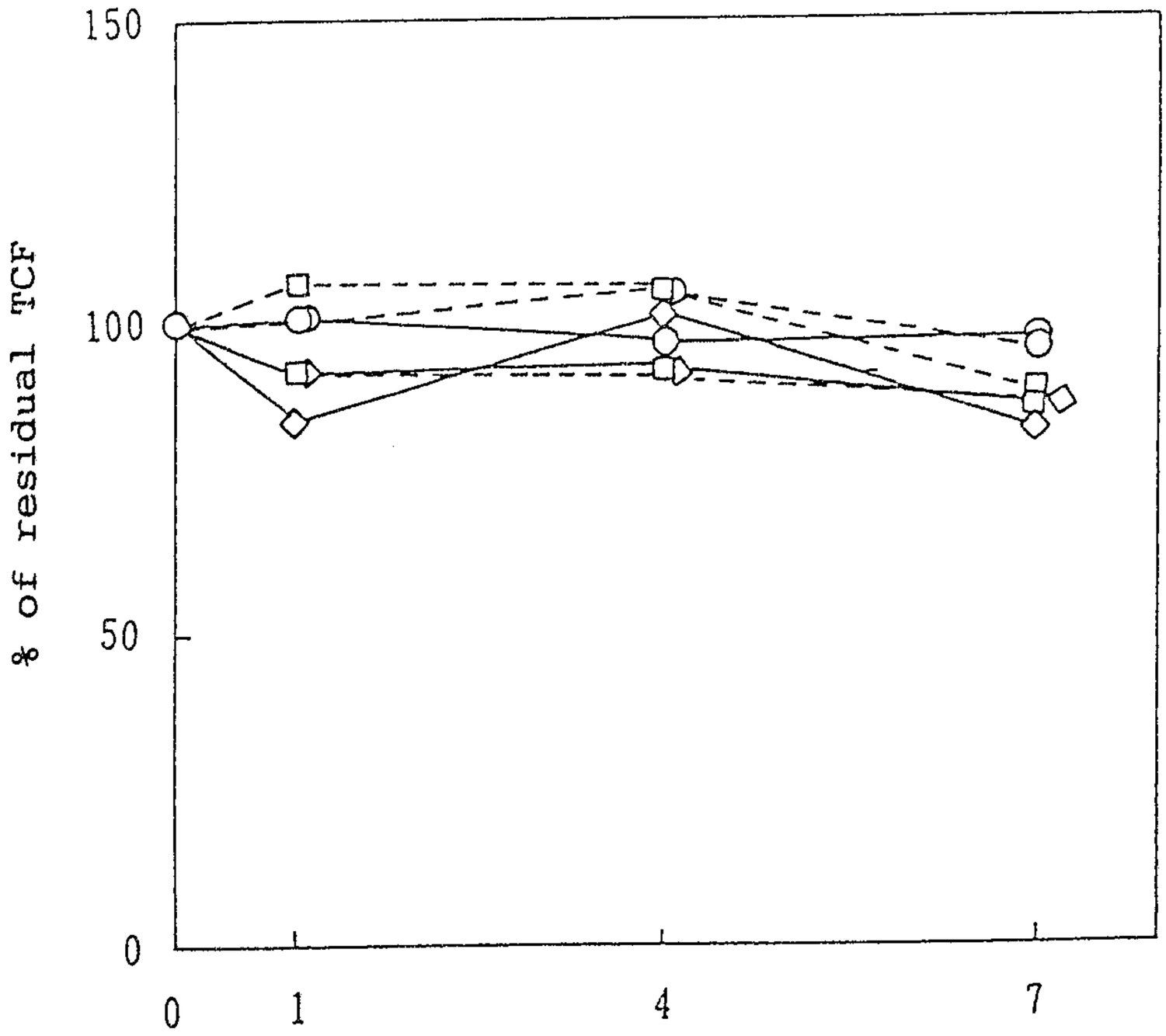
4. An isotonic TCF injection preparation according to Claim 2 wherein said solubilizing basic amino acid is lysine or arginine.

Fig. 1



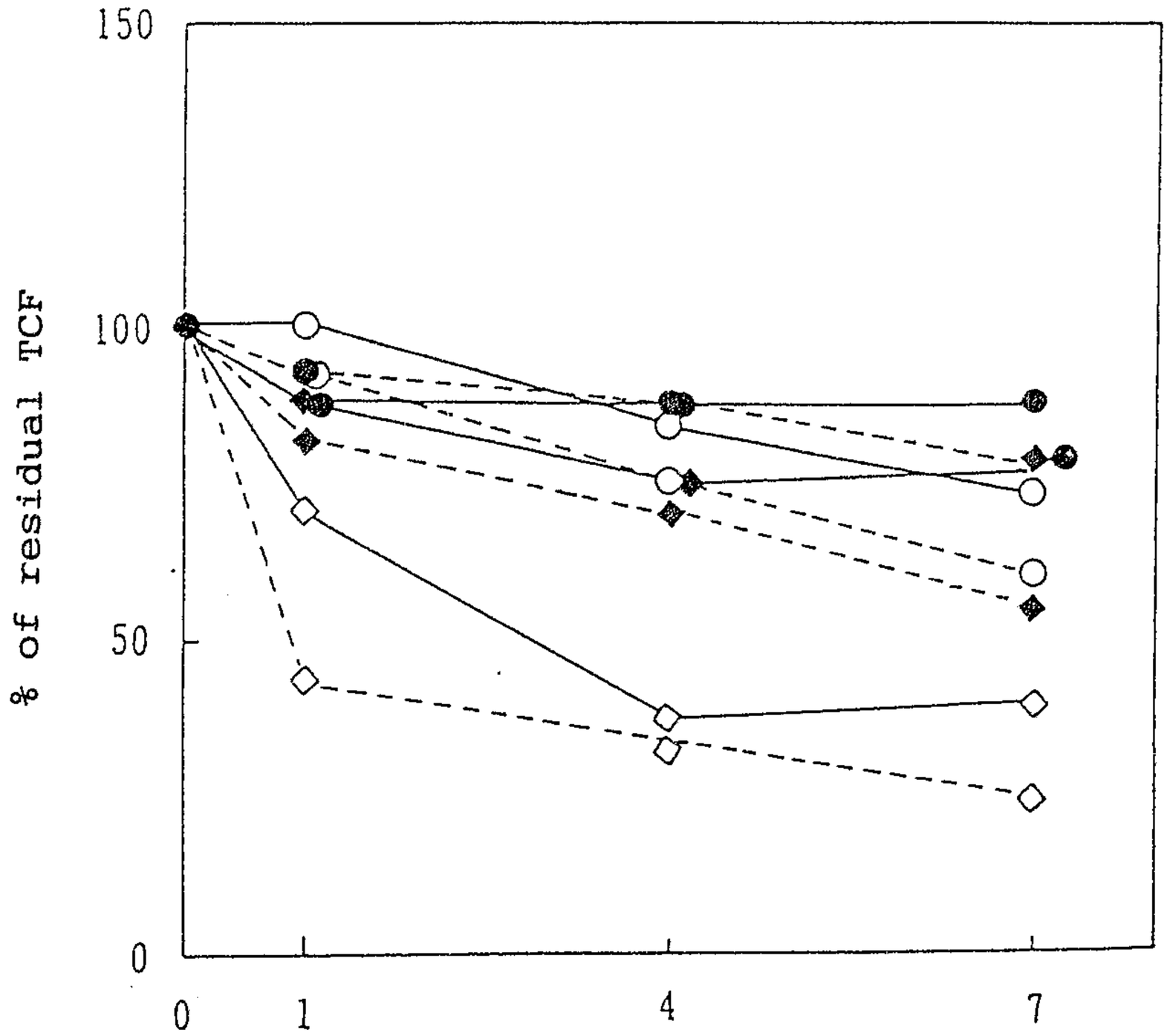
---: 5 °C
——: 20 °C
○: 0.15 M NaCl, pH 7.0
◇: 0.3 M NaCl, pH 7.0
●: 0.15 M NaCl, pH 6.0

Fig. 2



---: 5 °C
——: 20 °C
○ : 3.5 % L-arginine HCL
: 0 M NaCl
: 0 % D-mannitol, pH 7.0
◇ : 1.75 % L-arginine HCL
: 0.075 M NaCl
: 0 % D-mannitol, pH 7.0
□ : 1.75 % L-arginine HCL
: 0 M NaCl
: 2.5 % D-mannitol, pH 7.0

Fig. 3



---: 5 °C
——: 20 °C
○: 0.5 % HSA
: 0.15 M NaCl
: 0 % D-mannitol, pH 7.0
◇: 0.5 % HSA
: 0.075 M NaCl
: 2.5 % D-mannitol, pH 7.0
●: 0.5 % HSA
: 0.15 M NaCl
: 0 % D-mannitol, pH 6.0
◆: 0.5 % HSA
: 0.075 M NaCl
: 2.5 % D-mannitol, pH 6.0