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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 05-091313-00003-0000

Fecha: 2007-11-29 15:48:49 Dep. 2020 NUECREACIONE
Tm: 11 PATENTE/II Eje: 379 FASENACIONAL/II
Act: 036 PLICION Folios: 2**Clarke, Modet & Cº**

COLOMBIA

SEÑORES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES
E.S.D

REF EXPEDIENTE No.: 05091313
PATENTE: FORMULACIONES DE INMUNOGLOBULINA Y
MÉTODO DE PREPARACIÓN DE LA MISMA
SOLICITANTE : ELAN PHARMACEUTICALS, INC.

ASUNTO: SOLICITUD ESTUDIO DE FONDO

DILIA RODRÍGUEZ D'ALEMAN, en mi condición de apoderada de la Sociedad solicitante, por el presente escrito atentamente solicito a Usted que habiéndose surtido la publicación de la presente solicitud, en la Gaceta de la Propiedad Industrial No.576 de fecha Mayo 30 de 2007 y se proceda con el estudio de Fondo.

Para tal fin me permito anexar la tasa correspondiente.

Agradezco continuar con el presente trámite.

Señor Jefe

DILIA RODRÍGUEZ D'ALEMAN
C.C. 41.654.882 Bogotá

p-663

PBS. In addition, 0.02%, as a final concentration, of a neutral gelatin was added thereto, and further 0.5 or 1%, as a final concentration, of mannitol was added thereto. The resulting monoclonal antibody solution was then put in polypropylene cryotubes under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

The results obtained are shown in Table 6, as a relative activity based on the activity of the sample before frozen of being 10. The antigen-binding activity was well recovered in all the freeze-dried products, each containing a different concentration of mannitol.

Table 6

Concentration of Mannitol (%)	0.5	1.0
Antigen-Binding Activity	10	10

Example 7:

1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 0.1 M phosphate buffer (pH 7.0) containing a neutral gelatin (0.01%), sodium citrate (0.02 M), mannitol (0.5%) and sodium chloride (0.05 M). The resulting monoclonal antibody solution was then put in 10 ml-volume glass vials (Nishi Glass Co.) under a sterilized condition, each in an amount of 1 ml, and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 8:

1 mg/ml, as a final concentration, of the same monoclonal antibody as that used in Example 1 was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%). The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in Example 1. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 9:

Cells of human-human hybridoma MP5121 (FERM BP-2270) producing a human IgM reactive to Group A serotype *Pseudomonas aeruginosa*, which had been produced by cell fusion, were cultured, and the monoclonal antibody was purified from the culture supernatant in the same manner as in Example 1. The monoclonal antibody was dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), to have a final concentration thereof of being 1 mg/ml. The resulting monoclonal antibody solution was then put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured in the same manner as in Example 1, provided that the antigen LPS was extracted from Group A serotype *Pseudomonas aeruginosa* (ATCC 27577). As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity as that of the samples before frozen.

Example 10:

Monoclonal antibodies were purified from culture supernatants of cells of human IgM-producing human-human hybridoma MP5097, MP5139, MP5144 and MP5156 (FERM BP-2268, 2272, 2289, 2339, respectively), all of which had been produced by cell fusion. These monoclonal antibodies had reactivity with *Pseudomonas aeruginosa* and were reactive to Groups B, E, G and I serotypes *Pseudomonas aeruginosa*, respectively. Five kinds of monoclonal antibodies

comprising 4 kinds of these monoclonal antibodies and the monoclonal antibody used in Example 9 were dissolved in 0.1 M phosphate buffer (pH 7.0) containing sodium citrate (0.02 M), sodium chloride (0.05 M) and mannitol (0.5%), each in an amount, as a final concentration, of 5 mg/ml. These monoclonal antibody solutions were put in glass vials and frozen therein at -80°C. These were freeze-dried in vacuo. The same amount, as that before freeze-drying, of a distilled water for injection was added to each of the freeze-dried products so that each product was dissolved. The antigen-binding activity of each of the monoclonal antibodies in the resulting solutions was measured in the same manner as in Example 1, provided that as the antigen, to each antibody, used were LPSs as extracted from ATCC 27577 (to Group A serotype), ATCC 27578 (to Group B serotype), ATCC 27581 (to Group E serotype), ATCC 27584 (to Group G serotype) and ATCC 27586 (to Group I serotype), respectively. As a result, the freeze-dried monoclonal antibody products were found to have the same antigen-binding activity to the five *Pseudomonas aeruginosa* LPSs of different serotypes, respectively, as that of the samples before frozen.

ADVANTAGE OF THE INVENTION

In accordance with the present invention characterized by addition of gelatin or carboxylic acid or its salt to a monoclonal antibody-containing solution to be freeze-dried, denaturation of a monoclonal antibody during freeze-drying can be well prevented so that a monoclonal antibody-containing freeze-dried preparation having a stable antigen-binding activity can be provided. The present invention may be applied to a monoclonal antibody of any globulin class, including IgG, IgM, IgA and IgE. Especially, it can be sufficiently applied to unstable IgM. The present invention may be well applied to any of human-derived, mouse-derived and rat-derived monoclonal antibodies. The number of the kinds of the monoclonal antibodies to be contained in the freeze-dried preparation of the present invention may be one or more.

The monoclonal antibody-containing freeze-dried preparation of the present invention may be used, like other immunoglobulin preparations, as an adjuvant for immunotherapy for prophylaxis and treatment of bacterial infectious diseases and viral infectious diseases.

Claims

1. A preparation which is prepared by freeze-drying a solution comprising a monoclonal antibody and carboxylic acid or its salt and having pH of from 6.4 to 6.7.
2. The preparation as claimed in claim 1, in which the carboxylic acid is citric acid.
3. The preparation as claimed in claim 1 or claim 2, in which the monoclonal antibody is a human-derived one.
4. The preparation as claimed in any one of claims 1 to 3, in which the monoclonal antibody is one having a globulin class of IgM.
5. The preparation as claimed in any one of claims 1 to 4, which further contains at least one of monosaccharide, disaccharide, sugar alcohol and amino acid.
6. The preparation as claimed in any one of claims 1 to 5, which contains plural monoclonal antibodies.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 07 - 89,727
FECHA : NOVIEMBRE 1 DE 2007

Mo. 05-091313-00003-0000
Dep. 2020 NUECREACION
Lve: 379 FASENACIONAL!!
Folios: 2

***** CONSIGNACION

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
CLARKE NGDET Y CO. COLOMBIA	CONSIGNACION	BANCO POPULAR	050-00110-6	71030245	01/11/2007	32,804,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL
1	50000-01-01	SOLICITUDES	
		85 EXAMEN DE PATENTABILIDAD DE INVEN	404,000.00
		TOTAL:	404,000.00

SON: CUATROCIENTOS CUATRO MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 05-91313

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **576** DE

FECHA **31 DE MAYO DE 2007** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **31 DE AGOSTO DE 2007**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020

Bogotá, D.C., 30 de noviembre de 2008

Apoderado: Dilia D'Aleman

ASUNTO	Radicación	05-91313
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	16169
	Folios	

Patente de Invención



Vía PCT (fase nacional)



Cap. I



Cap. II ☐

No. Sol. Internacional

PCT/US2004/003873

Fecha de Presentación Internal.

WO2004/071439

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:

Folios: 37 a 76

(como se radicó inicialmente)

Reivindicaciones:

Folios: 77 a 86

(como se radicó inicialmente)

Prioridad:

Folios: 1, US 60/455,818, 10 de febrero de 2003

2. Objeto de la invención

Título de la solicitud: Formulación de Inmunoglobulina y Método de Preparación de la Misma.

El objeto de la presente solicitud de patente de invención consiste en formulaciones que comprenden inmunoglobulina, buffer fosfato y cloruro de sodio.

Reivindicaciones 1-26, 29, 30, 31: composiciones que comprenden inmunoglobulinas.

Reivindicaciones 32- 40: Procedimiento de mezcla para preparar las composiciones.

Reivindicación 27, 28, 42: método de tratamiento;

Reivindicación 41 Un artículo que contiene la formulación;

Reivindicaciones 43 y 44 uso.

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Se catalogó la invención, según la Clasificación Internacional de Patentes (IPC.)
A61K39/00, 39/38, 39/395.

De la solicitud de Patente

Reivindicaciones (Artículo 30 D 486 de la C.A.N.)

La reivindicación 41 reclama un "artículo" el cual no está técnicamente definido, porque define contenedor por contenido y si es un "artículo novedoso" para contener la composición es de otro campo tecnológico.

Por lo tanto esa reivindicación no cumple con el requisito de definición, claridad, concisión y sustento por la descripción de acuerdo al art. 30 de la Decisión 486.y/o art.22 Tratado PCT.

Excepción a la patentabilidad (Artículo 20 D486 literal d) de la C.A.N.)

-Las reivindicaciones 27, 28 y 42 reclaman un método de tratamiento para el cuerpo humano o animal, por lo tanto esta comprendido en el artículo 20 D 486, por lo tanto no es patentable.

Reivindicaciones relativas a un uso o a un segundo uso (Artículos 14 y 21 D 486 de la C.A.N.)

-Las reivindicaciones 43 y 44 reclaman el uso de composiciones farmacéuticas, de acuerdo con la legislación Andina el uso no se considera para protección por patente, como se lee en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina "... Los Países Miembros otorgarán patentes para las invenciones sean de **productos o procedimientos** en todos los campos de la tecnología..."; aquí no se hace alusión al uso. Demás los usos o segundos usos no son patentables, de conformidad con la sentencia del TJAC 89-AI-2000. Los usos en general no son patentables.

Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el estado de la técnica es la fecha de presentación de la solicitud de la cual se reclama prioridad 10 de febrero de 2003.

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

Nº	Documento	Título	Fecha de publicación*
D1	EP0841067	Freeze-dried preparation containing monoclonal antibody	13-may-1992
D2	W09822136	Stable lyophilized pharmaceutical substances from monoclonal o polyclonal	28-may-1998

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		antibodies	
D3	US5,908,826	Free-dried preparation containing monoclonal antibody	01-jun-1999
D4	US5,945,098	Stable intravenously-administrable immune globulin preparation	31-ago-1999
D5	WO0212501	Anti-dual integrin antibodies, compositions, methods and uses	14-feb-2002

12. Análisis de patentabilidad (según requisito) (artículo 14 D486)

12.1 Novedad (Artículo 16 D486 de la C.A.N.)

-La presente solicitud reclama las formulaciones farmacéuticas que comprenden una inmunoglobulina, un amortiguador de fosfato, un polisorbato y cloruro de sodio.

-Las anterioridades D1-D5 describen composiciones farmacéuticas que comprenden inmunoglobulinas, en los valores de pH de las soluciones a los que los anticuerpos se disuelven y que es compatible fisiológicamente pH de 4 a 8,1 más apropiado de 6,1 a 8,1 y aun más apropiado de 6,5, a 7,8, para mantener el pH de la composiciones estabiliza con buffer fosfato, se isotóniza la composición con cloruro de sodio y se utiliza tensioactivos los polisorbitos denominados tweenes entre ellos tween 80, igualmente se le agrega aminoácidos tal como histidina,

Vease D1 (EP0841067) por ejemplo pg 4 renglones 1-5, 12-46 y los ejemplos 7-10.

-D2 (WO9822136) véase especialmente reivindicación 6, que comprende todos los componentes de la solicitud en estudio, pg. 33 tabla del ejemplo 10; pg. 9.

-D3 (US5,908,826) véase especialmente col. 1 renglones 50-68; col.2 renglones 44-66; col.3 renglones 45- 58; col 4. Renglones 10-20, 35-44, 52-56; col. 5 renglones 14-17, 28-31, 40-50; ejem.1.

-D4 (US5,945,098) véase especialmente col.5 rengl. 1-30, 41-42, 54-63; ejem.II, III, V, reiv. 1-14.

-D5 (WO0212501) véase especialmente pg.33 tabla del ejemplo 10; pg.9 y reiv.6.

Tal como se observa la presente solicitud carece de novedad, ya que las anterioridades citadas se anticipan a describir las formulaciones que en la presente solicitud se reclaman como nuevas e inventivas.

-En cuanto al nivel inventivo es obvio que cualquier solicitud que tenga afectada su novedad carece de nivel inventivo.

13. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

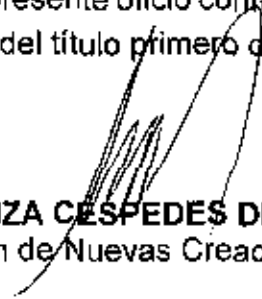
Nº	Documento Nº	Reivindicaciones afectadas	Requisito que afecta
D1	EP0841067	1-44	Novedad Nivel inventivo
D2	WO9822136	1-44	Novedad

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152

			Nivel inventivo
D3	US5,908,826	1-44	Novedad Nivel inventivo
D4	US5,945,098	1-44	Novedad Nivel inventivo
D5	WO0212501	1-44	Novedad Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha
19 Dic. 2008

CONCEPTO TÉCNICO No.	EXAMINADOR TÉCNICO Código No.202012
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United States Patent [19]
Sarno et al.

[11] Patent Number: 5,945,098
[45] Date of Patent: Aug. 31, 1999

[54] STABLE INTRAVENOUSLY-ADMINISTRABLE IMMUNE GLOBULIN PREPARATION

[75] Inventors: Maria Erlinda C. Sarno, Caracas, Venezuela; Anthony Vasquez, Norwalk, Conn.; San-Gee Yung, Rialto, Calif.; Clifford R. Graft, Lakeview Terrace, all of Calif.

[73] Assignee: Baxter International Inc., Deerfield, Ill.

[*] Notice: This patent issued on a continued prosecution application filed under 37 C.F.R. 1.53(d), and is subject to the twenty year patent term provisions of 35 U.S.C. 154(a)(2).

[21] Appl. No.: 08/935,294
[22] Filed: Sep. 22, 1997

Related U.S. Application Data

[63] Continuation of application No. 08/904,854, filed Jul. 30, 1996, abandoned, which is a continuation of application No. 08/317,214, filed Oct. 3, 1994, abandoned, which is a continuation of application No. 08/178,432, filed Jan. 6, 1994, abandoned, which is a continuation of application No. 07,866,089, filed Apr. 6, 1992, abandoned, which is a continuation of application No. 07,473,354, filed Feb. 1, 1990, abandoned.

[51] Int. Cl.⁶ A61K 38/21
[52] U.S. Cl. 424/85.5; 514/2; 514/12; 514/21; 530/387.1; 530/389.1; 530/380; 520/390.5
[58] Field of Search 424/85.5, 514/2, 514/12, 21; 530/387.1, 389.1, 380, 390.5

[56] References Cited

U.S. PATENT DOCUMENTS

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4,362,661	12/1980	Oto et al.	424/85.5
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4,374,763	2/1981	Takagi	424/85.8
4,364,994	5/1981	Sato et al.	424/85.1
4,396,608	6/1981	Urold	424/85.8
4,439,421	3/1984	Hooper et al.	424/85
4,477,432	10/1984	Hendic	424/85.1
4,482,480	11/1984	Curry et al.	530/387
4,540,573	9/1985	Neumath et al.	424/85
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4,789,545	12/1988	Roads et al.	424/101
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5,177,294	1/1993	Saras et al.	530/412

FOREIGN PATENT DOCUMENTS

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53 47215	4/1978	Japan	
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Fernandes et al., "Preparation of a Stable Intravenous Gamma-Globulin: Process Design and Scale-Up," *Vox Sang*, 39: 101-112 (1980)
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Primary Examiner—Chaya U. Nayala
Attorney, Agent, or Firm—Rothwell, Figg, Ernst & Kutz, P.C.

[57] ABSTRACT

Stable intravenously-administrable immune globulin preparations are stabilized against aggregation and polymerization and rendered isotonic with amino acid(s) and non-ionic detergents, polysorbate and polyethylene glycol. The immune globulins are derived from human or animal sources, or from hybridomas. Optional, additional stabilizers include various physiologically-acceptable carbohydrates and salts. Polyvinylpyrrolidone can be used in addition to the amino acid(s). Apart from the immunoglobulin itself, the preparations are otherwise essentially protein free. The preparations are useful in immunotherapy and as diagnostic reagents.

15 Claims, No Drawings

D4

even further. At these higher protein concentrations the immunoglobulin molecules appear to stabilize themselves. The optimal detergent content is obtained by formulation experiments well known to those skilled in the art. In any case, it appears that a desirable detergent concentration provides a balance of hydrophilic and hydrophobic properties in the protein solution so that the immunoglobulin is less prone to denature. The optimal detergent concentration will be the minimal amount that will prevent particles from coming out of solution, will not cause toxic effect and will not adversely alter the efficacy of the final product under the selected storage conditions.

An amount of one or more amino acids effective to impart a physiologically-acceptable tonicity (i.e. about 300 mOsm/l) to the preparation and to maintain said immune serum globulin in monomeric form also is added to the solution. Preferred amino acids include glycine, lysine, arginine and phenylalanine, with glycine being particularly preferred. A glycine concentration of about 0.2 M generally is preferred.

The IgG solution can further comprise polyvinylpyrrolidone ("PVP") at certain pH ranges, typically pH 4 to pH 7, in addition to or in place of the amino acid(s) as an agent for preventing polymerization and/or precipitation of the gamma globulin. PVP alone typically does not provide long term stabilization of immunoglobulins, but PVP does contribute an effective long term stabilizer in the preparations of this invention. The amount of PVP useful in the preparations is limited by its toxicity and insolubility at high concentrations. From 0.1 to 1 percent (w/v) of PVP K-15 (or other water soluble grade) is useful to prevent precipitation. Amounts within this range are soluble and well below toxic levels.

The addition of PVP does not provide an isotonic solution (except when the PVP is added at an acceptably high level). Therefore, PVP generally is added to the IgG solutions in combination with other additives such as amino acids, physiologically-acceptable salts or carbohydrates which can be used to adjust for tonicity. Useful carbohydrates include sugars such as glucose, mannose, fructose, galactose, dextran, fructose, lactose, sucrose, maltose, maltotriose and sorbitol. Non-reducing carbohydrates are preferred. Almost any physiologically acceptable salt, such as sodium chloride, sodium acetate, sodium citrate, etc., can be used to adjust the tonicity of the preparations. Examples include the alkali metal, alkaline earth metal, ammonium and phosphate salts as well as various organic acids (including but not limited to oxalate, valerate, oleate, lactate, borate, benzoate, lactate, tosylate, citrate, maleate, fumarate, succinate and tartrate). Sodium chloride and sodium citrate are preferred salts. The specific amount of any particular tonicity-adjusting agent(s) needed to provide an isotonic solution will vary from preparation to preparation. The artisan will readily determine appropriate amounts, however.

It has been found that the pH of the immune globulin preparation is an important factor relative to the IgG monomer content of the final product. In the preparations of the present invention the pH can range from 4.0 to 7.4. Within this range, a lower pH is desired, however, to ensure maximum monomer content. A pH of 4.2±0.5 is preferred for a 5 percent immune serum globulin preparation. Ten percent preparations, however, are most stable at a pH of 5.3±0.2. Optimal pH is obtained by formulation techniques well known to those skilled in the art. For example, optimal pH can be determined from size exclusion chromatography determinations as well as heat stability data and amino acid analysis of the various preparations under differing pH conditions.

5,945,098

Immune globulin solutions, including immune globulin solutions useful as starting materials for the present preparations, often include minor amounts of poly(ethylene glycol) ("PEG") as a stabilizer. While PEG alone cannot provide a preparation as stable as those described herein, its presence is believed to be important to the overall stability of any immune globulin solution, including those of the present invention. Thus, if PEG is not already present in the starting source of immune globulin, a small amount (typically less than 0.2 gram %) should be included in the preparations of the invention.

It will be apparent that the appropriate concentrations of the various stabilizers in the present immune globulin compositions will vary with protein concentration. Normal variations among pooled sera also will cause adjustments in stabilizer requirements. Those skilled in the art will readily determine acceptable stabilizer levels based on the teachings herein.

The invention is illustrated by the following examples. All percentages are by weight unless otherwise noted.

EXAMPLE I

Glycine at concentrations of 0.2±0.1M is added to aqueous IgG solutions purified according to the method taught in U.S. Pat. No. 5,177,194 from Cohn Fr. II, I, III, or other more crude IgG sources which contain residual amounts of polyethylene glycol, usually less than 0.2 g %. The protein concentration is then adjusted to approximately 5% either by desalting and dilution of a purified IgG precipitate or concentration through the use of tangential flow ultrafiltration and other suitable means of concentrating proteins. The pH is adjusted to 5.0±0.0 and Polysorbate 80 (Tween[®] 80) is then added in a 0.003±0.001% level. The IgG solution is sterile filtered and filled into final containers for use.

EXAMPLE II

Glycine at concentrations of 0.2±0.1M is added to aqueous IgG solutions purified from Cohn Fr. II, Cohn Fr. III filtrate, or other more crude IgG sources. The protein concentration is then adjusted to approximately 10% and the pH is adjusted to 6.0±0.0. Polysorbate 80 (Tween[®] 80) is then added in a 0.007±0.003% level. The IgG solution is sterile filtered and filled into final containers for use.

EXAMPLE III

Glycine at concentrations of 0.2±0.1M, PEG at concentrations of 0.01% and polysorbate at concentrations of approximately 0.001% are added to an aqueous solution of purified monoclone antibody derived from hybridomas. The concentration of the antibody is approximately 2 mg/ml and the solution is kept at pH 5.2 using 10 mM sodium citrate or sodium phosphate as buffer. This preparation can be used as a diagnostic reagent or can be sterile filtered and filled into final containers for intravenous or intramuscular administration.

EXAMPLE IV

Glycine at concentrations of 0.2±0.1M, and polyvinylpyrrolidone at concentrations ranging from 0.1 to 1% is added to aqueous IgG solutions purified from Cohn Fr. II, Cohn Fr. III filtrate, or other more crude IgG sources which contain residual amount of PEG (typically less than 0.2%). The protein concentration is then adjusted to the desired value, usually 5 or 10%, by known dilution or concentration techniques such as ultrafiltration. The pH is adjusted to

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5. (b) (i) Polysorbate 80 (Tween[®] 80) is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

EXAMPLE 1

1. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

2. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

3. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

4. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

5. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

1. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

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5. A mixture of polysorbate 80 (Tween[®] 80) and catholyte is added to a solution, typically, 0.001 to 0.01 percent, and the solution is stirred for 10 to 30 minutes. The pH is adjusted to 7.0 to 7.5. The solution is then filtered and the filtrate is used for the assay.

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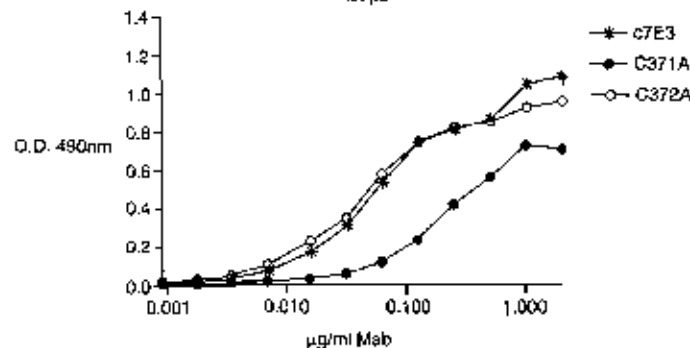
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(54) Title: ANTI-DUAL INTEGRIN ANTIBODIES, COMPOSITIONS, METHODS AND USES

Binding of GenPharm Mabs to
 $\alpha V\beta 3$ 

(57) Abstract: The present invention relates to at least one novel anti-dual integrin antibodies, including isolated nucleic acids that encode at least one anti-dual integrin antibody, dual integrin vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.

WO 02/12501

PCT/US01/24784

Anti-dual integrin antibody compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Genaro, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the anti-dual integrin antibody, fragment or variant composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, asparagine, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melzitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, malitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

Anti-dual integrin antibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, anti-dual integrin antibody compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar),

dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

5 These and additional known pharmaceutical excipients and/or additives suitable for use in the anti-dual integrin antibody, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

Formulations

As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one anti-dual integrin antibody in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one anti-dual integrin antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held

over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater.

The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one anti-dual integrin antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one anti-dual integrin antibody in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one anti-dual integrin antibody used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

The range of at least one anti-dual integrin antibody in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 μ g/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transnasal, or osmotic or micro pump methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monooleate), Tween 80 (polyoxyethylene (20) sorbitan monolaurate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or

26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

5 Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

10 For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical
15 stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Alternative Administration

20 Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one anti-dual integrin antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

25 dual integrin antibodies of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

Parenteral Formulations and Administration

30 Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting
35 agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an



United States Patent [19]

5,908,826

Patent Number: 5,908,826

Date of Patent: Jun. 1, 1999

Freeze-dried preparation

Containing monoclonal antibody

Inventors: Takanori Fukuda, Yukio Shimazaki, Yoshiyuki Kawamura, Shiro Takagi, all of Miyagi, Japan

Assignee: Miltenyi Biotec Chemicals Inc., Japan

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preventing denaturation of antibody during freeze-drying treatment thereof in the pH range where the carboxylic acid or its salt does not exert a buffer action. Regarding a pH range of an antibody solution, if an antibody solution having a low pH value is intravenously injected into a patient, an effect occurs. Where an antibody solution is used as an injection, it is desirable to have a pH value in an approximately neutral pH range. However, utilization of such an antibody solution in a neutral pH range is not suggested in WO 89/11297.

For the purpose of inactivating viruses which could contaminate immunoglobulins in preparing them from sera or plasma, immunoglobulins are often heated to the point of their solutions. For instance, JP-A 63-310731, 64-194035, 61-191612 and 57-140724 disclose the addition of carboxylic acids to said globulin solutions for this purpose. JP-A 64-78730 and 64-78733 disclose the addition of sodium acetate to immunoglobulin preparations and heating them in a dry state. However, all of them merely mention the addition of carboxylic acids for the purpose of stabilizing immunoglobulin preparations in the heat-treatment. It has not been known whether or not carboxylic acids and their salts would be useful for preventing denaturation of antibodies during freeze-drying and also for preventing a decrease of their antigen-binding activity owing to said denaturation.

PROBLEM TO BE SOLVED BY THE INVENTION

The object of the present invention is to provide stable freeze-dried preparations of monoclonal antibodies, which are free from denaturation of the monoclonal antibodies during freeze-drying and from a decrease of their antigen-binding activity owing to said denaturation.

MEANS FOR SOLVING THE PROBLEM

The present inventor have found that gelatin, carboxylic acids or their salts are effective for stabilizing a monoclonal antibody in freeze-drying it. Namely, they have found that, by addition of gelatin to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying as well as decrease of its antigen-binding activity may be prevented and that, by the addition of carboxylic acid or its salt to a solution containing a monoclonal antibody to be freeze-dried, denaturation of the monoclonal antibody during freeze-drying as well as decrease of its antigen-binding activity owing to said denaturation may be prevented over a broad pH range and even at a pH value being outside the range where a buffer action is exhibited. On the basis of these observations, applicants have found that it is possible to prepare a stable and highly safe preparation of a monoclonal antibody.

Specifically, the present invention provides a freeze-dried preparation containing a monoclonal antibody and gelatin as well as a preparation prepared by freeze-drying a solution containing a monoclonal antibody and carboxylic acid or its salt and having pH between 6.1 and 8.1.

The monoclonal antibody to be used in the present invention may be any monoclonal antibody that is generally obtained from human beings, mice, rats and others, and the origins and the producing means are not specifically defined. For instance, the monoclonal antibody for use in the present invention may be obtained from a culture supernatant as obtained by cultivating antibody-producing cells as prepared by known methods such as a cell fusion method or a transformation method, or by cultivating cells into which a

cloned antibody gene has been incorporated, or from ascites, etc., of a mouse into which such antibody-producing cells have been transplanted. For purifying the monoclonal antibody obtained from such a cell culture supernatant or mouse ascites or the like, usable are various purification methods such as ammonium sulfate salting-out, ion exchange chromatography, gel filtration, affinity chromatography, ultrafiltration, adsorption chromatography and hydrophobic chromatography. The immunoglobulin classes of the monoclonal antibody to be used in the present invention are not specifically defined, but they are not specifically defined. A monoclonal antibody of any immunoglobulin class can be used in the present invention. Above all, IgM is less stable than those of other immunoglobulin classes. Therefore, the stabilizing method is effective for the IgM class monoclonal antibody may easily be applied to monoclonal antibodies of other immunoglobulin classes. In the present invention, a single monoclonal antibody may be used or several monoclonal antibodies may be also used as a mixture of them with no problem.

Gelatin may be grouped into two types (neutral type and acidic type) according to the methods of preparing it, of which the isoelectric points are different from each other. Both of them may be used in the present invention. In addition, chemically modified gelatins such as carboxymethylated or modified liquid gelatins may be also used.

As carboxylic acid, usable are, for example, citric acid, acetic acid, oxalic acid, succinic acid and fumaric acid. Citric acid is preferable. As salt of carboxylic acid, usable are, for example, sodium citrate, potassium citrate, sodium acetate, potassium acetate, sodium malate, potassium oxalate, sodium succinate, potassium succinate, sodium fumarate and potassium fumarate. Sodium citrate is preferable.

For the purpose of stabilizing the monoclonal antibody or for the purpose of pH adjusting, immunizing and buffering the monoclonal antibody-containing solution to be freeze-dried, inorganic salt, monosaccharide, disaccharide, sugar alcohol or amino acid may be further added, in addition to gelatin or carboxylic acid or its salt.

As inorganic salt, usable are, for example, sodium chloride, potassium chloride and magnesium chloride. Sodium chloride is preferable.

As monosaccharide, usable are, for example, glucose, fructose, galactose and fructose. Glucose or fructose is preferable.

As disaccharide, usable are, for example, maltose, sucrose and lactose. Maltose or sucrose is preferable.

As sugar alcohol, usable are, for example, sorbitol and mannitol. Mannitol is preferable.

As amino acid, usable are, for example, glycine, alanine, valine, leucine, isoleucine, tyrosine, phenylalanine, serine, threonine, glutamic acid, glutamine, asparagine, aspartic acid, arginine, lysine, histidine, proline, tryptophan, methionine and cysteine. Glycine or arginine is preferable.

For producing the freeze-dried preparation of the present invention, a monoclonal antibody solution containing gelatin or carboxylic acid or its salt may be freeze-dried. Preferably, the monoclonal antibody solution is added to a buffer containing gelatin or carboxylic acid or its salt having an adjusted pH value; or gelatin or carboxylic acid or its salt is added to a monoclonal antibody-containing solution. The concentration of the monoclonal antibody in the solution to be used in the present invention is from 0.01 mg/ml to 50 mg/ml, preferably from 0.1 mg/ml to 10 mg/ml. The amount of gelatin is from 1:100 to 100 parts by weight to one part

by weight of the monoclonal antibody. Preferably, it is from 1:10 to 10 parts by weight to one part by weight of the same. The concentration of carboxylic acid or its salt to be added is from 2 mmol/L to 500 mM, preferably from 10 mM to 200 mM.

The pH value of the solution for dissolving the monoclonal antibody to be freeze-dried is from 4.1 to 8.1 when gelatin is added thereto; or it is from 6.1 to 8.1, preferably from 6.5 to 7.5, when carboxylic acid is added or both gelatin and carboxylic acid are added. Adjustment of the pH value of the solution may be done using organic acids, inorganic acids, inorganic salts or other compounds which are generally used for pH adjustment, singly or in combination of two or more of them. As a compound usable for such pH adjustment, there are mentioned, for example, citric acid, sodium citrate, potassium citrate, phosphoric acid, sodium phosphate, potassium phosphate, hydrochloric acid, tris(hydroxymethyl)aminomethane, acetic acid, sodium acetate, potassium acetate, sodium hydroxide, boric acid, sodium borate, and potassium borate. The concentration of the buffer solution for dissolving the monoclonal antibody is from 5 mM to 500 mM, preferably from 10 mM to 500 mM. As mentioned above, carboxylic acid or its salt may be also used for pH adjustment of a monoclonal antibody-containing solution, and the above-mentioned amount of the acid or its salt indicates the total amount thereof in the solution including the amount for pH adjustment.

The thus prepared monoclonal antibody solution may be stable when freeze-dried directly as it is. It is also possible to add therein a surfactant such as TWEEN 20 or TWEEN 80, a human or bovine albumin, or a chelating agent such as EDTA, for the purpose of stabilizing the solution or of preventing adhesion of the monoclonal antibody to the container containing the solution.

Freeze-drying of the monoclonal antibody solution may be carried out by any ordinary known method, and the drying temperature and the vacuum degree in the method may be selected suitably.

EXAMPLES

Next, the present invention will be explained by way of the following examples, which are, however, not limitative. IgM is exemplified herein as a monoclonal antibody in the present invention. This is because, as mentioned above, IgM is less stable than antibodies of other immunoglobulin classes (e.g., IgG, IgA and IgE), and therefore the stabilizing effect to be verified in IgM may be easily applied to antibodies of other immunoglobulin classes.

Example 1

Cells of Epstein-Barr virus (EB virus) transformed cell line MP-503 (FERM BP-1586) producing an IgM antibody reactive to Group B serotype *Pseudomonas aeruginosa* were cultured, and a human monoclonal antibody was purified from the culture supernatant by ammonium sulfate salting-out, gel filtration with SEPHACRYL S-300 (Pharmacia Co.) and column chromatography with a hydroxyapatite HPLC column (Mitsubishi Chemicals, Inc.) and BLUE-SEPHAROSE (Pharmacia Co.). The monoclonal antibody is obtained by these methods had a purity of 99% or higher, as analyzed by SDS-polyacrylamide gel electrophoresis and HPLC with a gel filtration column. The monoclonal antibody was dissolved in a phosphate-buffered physiological saline having an adjusted pH value of 7.4 (hereinafter referred to as PBS), to have a final concentration of 0.1 mg/ml. On the other hand, gelatin (high-grade gelatin; Nippi

Co., type A (neutral gelatin) and type B (acidic gelatin)) was added thereto to have a final concentration of 0.001 to 1%. The resulting solution was then put in 2 ml-volume polypropylene cryotubes (Coring Co.) under a sterilized condition, each in an amount of 0.5 ml, and frozen therein at -80°C . These were freeze-dried in vacuum. After drying, the same amount, as that before freeze-drying, of a distilled water for injection was added to the freeze-dried product so that the product was dissolved. The antigen-binding activity of the monoclonal antibody in the resulting solution was measured.

Measurement of the antigen-binding activity of the anti-*Pseudomonas aeruginosa* antibody was carried in the manner mentioned below. A lipopolysaccharide (LPS), as prepared from formalin-killed cells of Group B serotype *Pseudomonas aeruginosa* ATCC 27551 by Tanshe et al.'s method (Metsikjalkonsalo et al., (1976) 1793-1801), was dissolved in PBS to have a concentration of 1 mg/ml, and this was diluted by 500-fold with 0.1 M phosphate buffer (pH 7.0). The thus diluted solution was then put in wells of a 96-well EIA plate (Immunolog-600; Greiner Co.) in an amount of 50 μl /well. The plate was allowed to stand at 4°C . overnight for coating, and it was then washed with PBS containing 0.05% TWEEN 20 (hereinafter referred to as a "washing solution"). A PBS containing 0.5% bovine serum albumin (hereinafter referred to as a "blocking solution") was added to each well in an amount of 200 μl /well, and the plate was then shaken at room temperature for one hour so that the non-specific protein-binding sites were saturated. After the blocking solution was removed, solutions of a sample to be tested, each having a multi-fold diluted concentration in order from a determined concentration, were put in the wells each in an amount of 100 μl /well, and the plate was then shaken for 2 hours at room temperature. After washing with the washing solution (four times), a peroxidase-labeled goat anti-human IgM antibody (Tago Co.) was diluted by 1000-fold with the blocking solution, and was put in each well in an amount of 100 μl /well, and the plate was shaken at room temperature for 2 hours. After washing with the washing solution four times and then with 0.1 M citric acid buffer (pH 4.0) one time, a substrate solution containing 1 mg/ml of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 0.005% hydrogen peroxide in the same buffer was put into each well in an amount of 50 μl /well, and the plate was then shaken at room temperature. After 30 minutes, 2% succinic acid was added to each well in an amount of 50 μl /well so that the enzymatic reaction therein was stopped. The absorbance at 414 nm was measured with a 96-well plate reader (Nippon InterMed Co.). Double logarithmic plotting was done between the reciprocal of the diluting magnification and the absorbance, and the diluting magnification to show the absorbance of being 0.1 was obtained and indicated as the antigen-binding activity of the sample tested.

The results are shown in Table 1, as a relative activity on the basis of the antigen-binding activity of the sample before being frozen. It was shown that the monoclonal antibody was freeze-dried without addition of gelatin thereto, the antigen-binding activity noticeably decreased. As opposed to the case, when gelatin was added, the antigen-binding activity was well recovered even in the freeze-dried product, and the effect depended upon the concentration of the gelatin added.

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(54) Titel: STABLE LYOPHILIZED PHARMACEUTICAL SUBSTANCES FROM MONOCLONAL OR POLYCLONAL ANTIBODIES		
(54) Bezeichnung: STABILE LYOPHILISIERTE PHARMAZEUTISCHE ZUBEREITUNGEN VON MONO- ODER POLYKLONALEN ANTIKÖRPERN		
(57) Abstract: Disclosed are lyophilized pharmaceutical substances from monoclonal or polyclonal antibodies, containing a sugar or amino sugar as well as a surfactant and a stabilizing agent. Also disclosed are the methods of preparing said stable lyophilizates as well as the use of a sugar or amino sugar, an amino acid and a surfactant as stabilizers for therapeutic and diagnostic agents containing antibodies. (57) Zusammenfassung: Die Erfindung betrifft lyophilisierte pharmazeutische Zubereitungen von mono- oder polyclonalen Antikörpern, die einen Zucker oder Aminosucker, eine Aminosäure und ein Tensid als Stabilisierungsmittel enthalten. Außerdem betrifft die Erfindung ein Verfahren zur Herstellung dieser stabilen Lyophilisate sowie die Verwendung eines Zuckers oder Aminosuckers, einer Aminosäure und eines Tensids als Stabilisatoren von antikörperhaltigen therapeutischen oder diagnostischen Mitteln.		

DZ

untere Grenze für den Zuckergehalt kommen beispielsweise Mengen ab 10, 50 bzw. 100 mg in Frage. Bevorzugte Bereiche sind 200 - 1000 mg, insbesondere 400 - 800 mg. Die hier genannten Mengenangaben pro Einzeldarreichungsform beziehen sich Einzel-darreichungsformen, die als Lyophilisate in den Handel kommen. Derartige Lyophilisate sind vorzugsweise in Injektionsflaschen mit einem Volumen von 10 ml abgefüllt. Nach Auflösung der Lyophilisate mit einer Rekonstitutionslösung von 10 ml werden flüssige Darreichungsformen erhalten, die direkt verabreicht werden können. Die Zucker-konzentration in diesen Injektionslösungen beträgt bis zu 200 mg/ml, vorzugsweise bis zu 100 mg/ml, basierend auf den eingangs genannten Mengenangaben der verwendeten Zucker.

Als erfindungsgemäß verwendete Aminosäuren kommen basische Aminosäuren in Frage, wie beispielsweise Arginin, Lysin, Histidin, Ornithin u.a., wobei die Aminosäuren vorzugsweise in Form ihrer anorganischen Salze (vorteilhaft in Form der Phosphor-säuresalze, d.h. als Aminosäurephosphate) eingesetzt werden. Für den Fall, daß die freien Aminosäuren eingesetzt werden, erfolgt die Einstellung des gewünschten pH-Wertes durch Zugabe einer geeigneten physiologisch verträglichen Puffersubstanz, wie z.B. einer anorganischen Säure, insbesondere von Phosphorsäure, Schwefelsäure, Essigsäure, Ameisensäure oder deren Salze. Der Einsatz von Phosphaten hat hierbei insbesondere den Vorteil, daß besonders stabile Lyophilisate erhalten werden. Es hat sich gezeigt, daß es vorteilhaft ist, wenn die Zubereitungen im wesentlichen frei sind von organischen Säuren, wie z.B. Äpfelsäure, Weinsäure, Zitronensäure, Bernsteinsäure, Fumarsäure, etc., bzw. die entsprechenden Anionen (Malate, Oxalate, Citrate, Succinate, Fumarate, etc. nicht enthalten sind.

Als Aminosäuren kommen bevorzugt Arginin, Lysin oder Ornithin in Frage. Daneben können auch saure Aminosäuren, wie z.B. Glutaminsäure und Asparaginsäure, oder neutrale Aminosäuren, wie z.B. Isoleucin, Leucin und Alanin, bzw. aromatische Aminosäuren, wie z.B. Phenylalanin, Tyrosin oder Tryptophan, Verwendung finden. Der Aminosäuregehalt in den erfindungsgemäßen wäßrigen Zubereitungen beträgt bis zu 100 mg/ml, bevorzugt bis zu 50 mg/ml oder bis zu 30 mg/ml. Als untere Grenze kommen

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Beispiel 10

Aus der folgenden Zusammenstellung sind die Bestandteile der Rezeptur 15 zu entnehmen. Bei dem verwendeten Antikörper handelt es sich um Anti-L-Selektin. Aus den in Tabelle 13 a angegebenen Daten zur Untersuchung der Stabilität geht hervor, daß die verwendete Rezeptur eine hinreichend gute Stabilisierung ermöglicht.

Zusammensetzungen der Rezeptur 15:

	Rezeptur 15
Anti-L-Selektin	7,0 mg
Phosphatpuffer	15 mM
Natriumchlorid	30 mM
Saccharose	68,0 mg
Arginin	10,0 mg
Phosphorsäure	ad pH 6,5
Tween 20	0,1 mg
Wasser für Injektionszwecke	ad 1,0 ml

MAK-HBV und Anti-L-Selektin Lyophilisate.

Zur Lösung des Anti-L-NGFR in einem Phosphatpuffer wird eine wässrige Lösung mit den Zusatzstoffen Zucker, Aminosäure und Tensid mit pH 5 bis 8 beigemischt. Der Zusatz von Phosphatsalzen erfolgt in solchen Mengen, so daß die zuvor definierten Konzentrationen erhalten werden. Anschließend erfolgt die Sterilfiltration und Lyophilisation der so hergestellten Lösung. Nach Lyophilisation erhält man einen optisch einwandfreien Lyophilisationskuchen. Der Antikörper Anti-L-NGFR bleibt stabil. Nach Rekonstitution des Lyophilisates mit Wasser für Injektionszwecke erhält man eine klare Lösung.

Tabelle 13 a: Stabilitätsdaten Rezeptur 15 bei 25°C

	Lagerung 4 Wochen bei 25°C			Lagerung 13 Wochen bei 25°C		
	I	II	III	I	II	III
Rez. 15: 7 mg/1 ml	>99	n.n.	2,5	>99	n.n.	2,9

Patentansprüche

1. Stabile lyophilisierte pharmazeutische Zubereitung von mono- oder polyklonalen Antikörpern, enthaltend einen Zucker oder einen Aminozucker, eine Aminosäure und ein Tensid.
2. Zubereitung nach Anspruch 1, dadurch gekennzeichnet, daß die Zubereitung im wesentlichen frei von Polyethylenglykolen und/oder frei von proteinartigen pharmazeutisch üblichen Hilfsstoffen ist.
3. Zubereitung nach Anspruch 1 oder 2 bestehend im wesentlichen aus
 - a) einem mono- oder polyklonalen Antikörper,
 - b) einem Zucker oder Aminozucker,
 - c) einer Aminosäure,
 - d) einer als Puffersubstanz wirkenden anorganischen Säure und
 - e) einem Tensid
4. Zubereitung nach einem der Ansprüche 1 - 3, dadurch gekennzeichnet, daß der Zucker ein Mono-, Di- oder Trisaccharid ist, vorzugsweise Saccharose, Maltose, Trehalose oder Raffinose.
5. Zubereitung nach einem der Ansprüche 1 - 4, dadurch gekennzeichnet, daß der Aminozucker Glucosamin, N-Methylglucosamin, Galactosamin oder Neuraminsäure ist.
6. Zubereitung nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, daß die Aminosäure eine basische, saure oder neutrale Aminosäure ist, vorzugsweise Arginin, Lysin, Histidin, Ornithin, Isoleucin, Leucin, Alanin, Glutaminsäure oder Asparaginsäure.

7. Zubereitung nach einem der Ansprüche 1 bis 6, dadurch gekennzeichnet, daß das Tensid ein Polysorbat oder ein Polyoxyethylen-polyoxypropylen-Polymer ist.
8. Zubereitung nach einem der Ansprüche 1 bis 7, dadurch gekennzeichnet, daß sie physiologisch verträgliche Hilfsstoffe aus der Gruppe der Säuren, Basen, Puffer und/oder Isotonisierungsmittel enthält.
9. Wäßrige pharmazeutische Zubereitung von mono- oder polyklonalen Antikörpern erhältlich durch Redissolution des Lyophilisats nach einem der Ansprüche 1 bis 8.
10. Wäßrige pharmazeutische Zubereitung nach Anspruch 9, dadurch gekennzeichnet, daß die Lösung einen pH-Wert von 5 - 8, vorzugsweise von 6 - 7,4 aufweist.
11. Verfahren zur Herstellung einer lyophilisierten pharmazeutischen Zubereitung gemäß einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, daß man eine wäßrige Zubereitung, enthaltend einen monoklonalen oder polyklonalen Antikörper als Wirkstoff und einen Zucker oder Aminozucker, eine Aminosäure und ein Tensid als Zusatzstoffe sowie gegebenenfalls weitere pharmazeutische Hilfsstoffe, herstellt und die Lösung anschließend lyophilisiert.
12. Verwendung der Kombination von Hilfsstoffen bestehend aus a) einem Zucker oder Aminozuckers, b) einer Aminosäure und c) eines Tensids zur Herstellung stabiler antikörperhaltiger therapeutischer oder diagnostischer Mittel

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(54) **Freeze-dried preparation containing monoclonal antibody**

(57) The present invention relates to a freeze-dried monoclonal antibody preparation containing a carboxylic acid or its salt.

According to the present invention, by adding a carboxylic acid or its salt, it has become possible to prevent denaturation of monoclonal antibodies during freeze-drying. The present invention provides, therefore, a freeze-dried monoclonal antibody preparation having a stabilized antigen-binding activity.

The freeze-dried monoclonal antibody preparation of the present invention may be used, like other immunoglobulin preparations, as an adjuvant for immunotherapy for prophylaxis and treatment of bacterial infectious diseases and viral infectious diseases.

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body of any globulin class can be used in the present invention. Above all, IgM is less stable than those of other globulin classes. Therefore, the stabilizing method effective to IgM class monoclonal antibody may easily be applied to monoclonal antibodies of other globulin classes. In the present invention, a single monoclonal antibody may be used or plural monoclonal antibodies may be also used as a mixture of them with no problem.

Gelatin may be grouped into two types (neutral type and acidic type) according to the methods of preparing it, of which the isoelectric points are different from each other. Both of them may be used in the present invention. In addition, chemically modified gelatins such as copolygelatin or modified liquid gelatins may be also used.

As carboxylic acid, usable are, for example, citric acid, acetic acid, malic acid, succinic acid and fumaric acid. Citric acid is preferable. As salt of carboxylic acid, usable are, for example, sodium citrate, potassium citrate, sodium acetate, potassium acetate, sodium oxalate, potassium oxalate, sodium succinate, potassium succinate, sodium fumarate and potassium fumarate. Sodium citrate is preferable.

For the purpose of stabilizing the monoclonal antibody or for the purpose of pH adjusting, isotonicating and buffering the monoclonal antibody-containing solution to be freeze-dried, inorganic salt, monosaccharide, disaccharide, sugar alcohol or amino acid may be further added, in addition to gelatin or carboxylic acid or its salt.

As inorganic salt, usable are, for example, sodium chloride, potassium chloride and magnesium chloride. Sodium chloride is preferable.

As monosaccharide, usable are, for example, glucose, mannose, galactose and fructose. Glucose or mannose is preferable.

As disaccharide, usable are, for example, maltose, sucrose and lactose. Maltose or sucrose is preferable.

As sugar alcohol, usable are, for example, sorbitol and mannitol. Mannitol is preferable.

As amino acid, usable are, for example, glycine, alanine, valine, leucine, isoleucine, tyrosine, phenylalanine, serine, threonine, glutamine, glutamic acid, asparagine, aspartic acid, arginine, lysine, histidine, proline, tryptophan, methionine and cysteine. Glycine or arginine is preferable.

For producing the freeze-dried preparation of the present invention, a monoclonal antibody solution containing gelatin or carboxylic acid or its salt may be freeze-dried. Preferably, a monoclonal antibody solution is added to a buffer containing gelatin or carboxylic acid or its salt and having an adjusted pH value, or gelatin or carboxylic acid or its salt is added to a monoclonal antibody-containing solution. The concentration of the monoclonal antibody in the solution to be used in the present invention is from 0.01 mg/ml to 50 mg/ml, preferably from 0.1 mg/ml to 10 mg/ml. The addition amount of gelatin is from 1/100 to 100 parts by weight to one part by weight of the monoclonal antibody. Preferably, it is from 1/10 to 10 parts by weight to one part by weight of the same. The concentration of carboxylic acid or its salt to be added is from 2 mM to 500 mM, preferably from 10 mM to 200 mM.

The pH value of the solution of dissolving the monoclonal antibody to be freeze-dried is from 4.0 to 8.1 when gelatin is added thereto; or it is from 6.1 to 8.1, preferably from 6.5 to 7.8, when carboxylic acid is added or both gelatin and carboxylic acid are added. Adjustment of the pH value of the solution may be done by use of organic acids, inorganic acids, inorganic salts or other compounds which are generally used for pH adjustment, singly or in combination of two or more of them. As compound usable for such pH adjustment, there are mentioned, for example, citric acid, sodium citrate, potassium citrate, phosphoric acid, sodium phosphate, potassium phosphate, hydrochloric acid, bis(hydroxymethyl)aminomethane, acetic acid, sodium acetate, potassium acetate, sodium hydroxide, boric acid, sodium borate, and potassium borate. The concentration of the buffer of dissolving the monoclonal antibody is from 5 mM to 500 mM, preferably from 10 mM to 500 mM. As mentioned above, carboxylic acid or its salt may be also used for pH adjustment of a monoclonal antibody containing solution, and the above-mentioned amount of the acid or its salt indicates all the amount thereof in the solution including one for pH adjustment.

The thus prepared monoclonal antibody solution may be well stable when freeze-dried directly as it is. It is also possible to add thereto a surfactant such as Tween 20 or Tween 80, a human or bovine albumin, or a chelating agent such as EDTA, for the purpose of isotonicating the solution or of preventing adhesion of the monoclonal antibody to the container containing the solution.

Freeze-drying of the monoclonal antibody solution may be carried out by any ordinary known method, and the drying temperature and the vacuum degree in the method may be selected suitably.

EXAMPLES

Next, the present invention will be explained by way of the following examples, which are, however, not limitative. IgM is exemplified herein as a monoclonal antibody in the present invention. This is because, as mentioned above, IgM is less stable than antibodies of other globulin classes (e.g., IgG, IgA and IgE), and therefore the stabilizing effect to be verified in IgM may be easily applied to antibodies of other globulin classes.

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