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DIRECCIÓN DE NUEVAS CREACIONES
PRESENTACIÓN DE OPOSICIÓN

1. Oposición a solicitud de:

- ☒ Patente de invención
☐ Registro de Diseño Industrial
☐ Patente de Modelo de Utilidad
☐ Esquema de Trazado de Circuitos Integrados

Número de expediente: 10 091445

Solicitante: CHUGAI SEIYAKU KABUSHI KAISHA- F. HOFFMANN- LA ROCHE AG.

Apoderado: LUZ CLEMENCIA DE PAEZ

Título de la Nueva Creación: PREPARACION DE SOLUCION QUE CONTIENE ANTICUERPOS EN UNA ALTA CONCENTRACION.

Gaceta: 645 de 31 de mayo de 2012, No. de publicación 630

2. Datos del Opositor

☐ Persona Natural

☒ Persona Jurídica

TECNOQUIMICAS S.A.

Nombre o denominación /Razón social completa

Documento de identificación: C.C. ☐ C.E. ☐ NIT ☒ Otro ☐ Cuál

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3. Datos del representante o apoderado:

REYES VILLAMIZAR JOSE LUIS

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Apellidos y Nombre

Documento de identificación

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En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación

6. Fundamentos de la oposición

Causal: Incumplimiento de requisitos de patentabilidad (Título II, Capítulo I, Decisión 486 de 2000)

Justificación: VER ANEXO

Prórroga: SI ☒ NO ☐

7. Anexos

☒ Comprobante de pago de la tasa para la presentación de la solicitud No. 12 0089494

☒ Poder, si fuera el caso con el que se acredita la representación.

☒ Pruebas de los fundamentos de la oposición

☒ Copias para traslado

Fecha: 12 0089494

8. Firma

JOSÉ LUIS REYES VILLAMIZAR

Nombre y apellidos

Firma

79.152.473

C.C.



44655

T.P.

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente 10-091-445

TÍTULO SOLICITADO: "PREPARACIÓN DE SOLUCIÓN QUE CONTIENE ANTICUERPOS EN UNA ALTA CONCENTRACIÓN"

PUBLICACIÓN: Gaceta 645 de 31 de Mayo de 2012, N° de publicación 630.

PRIORIDAD: JP 2007-0336310 de 27/12/2007

SOLICITANTE: CHUGAI SEIYAKU KABUSHIKI KAISHA Y
F. HOFFMANN - LA ROCHE AG

APODERADO: LUZ CLEMENCIA DE PAEZ

TRÁMITE: PRESENTACIÓN DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUÍMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42 de la Decisión 486 de 2000, muy respetuosamente me permito formular la siguiente

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente **10-091-445**, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **TECNOQUÍMICAS S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias

farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud en curso, que se refiere a preparaciones estables en solución de anticuerpos anti-IL6 que comprenden los aminoácidos arginina y metionina, adecuadas para administración subcutánea y que previenen la formación de dímeros y la desaminación durante el almacenamiento prolongado, carece de los requisitos fundamentales para otorgársele el privilegio de patente de invención, particularmente en materia de Novedad y Nivel Inventivo si se tiene en cuenta la materia revelada entre otros, en documentos como:

- La publicación internacional WO 03/072060 de 04/09/2003 titulada "Polypeptide Formulation".
- La publicación internacional WO 2005/049078 de 02/06/2005 titulada "Immunoglobulin preparations having increased stability".
- El documento de patente US 2003/0190316 de 09/10/2003 titulada "Stabilized antibody-containing preparations".

La solicitud presenta un pliego de (20) reivindicaciones que obedecen a una formulación líquida estable que contiene anticuerpos que comprenden arginina y metionina; la reivindicación 19 por su parte, menciona un método para inhibir la desamidación de moléculas de un anticuerpo en una formulación líquida que contiene el anticuerpo, que comprende agregar arginina a la formulación líquida y la reivindicación 20 la invención emplea arginina y metionina.

Según la solicitud en trámite, los aminoácidos arginina y/o metionina pueden emplearse indistintamente en la formulación, lo que no permite establecer las características esenciales de la formulación dado que son incorporados opcionalmente durante las reclamaciones (ver por ejemplo las reivindicaciones independientes 1, 8 y 19); en consecuencia, la solicitud no cumpliría con requisitos de unidad de invención ya que claramente hay una invención en la que la formulación comprende arginina, otra en la que comprende metionina, y una adicional que comprende los dos aminoácidos.

Adicionalmente, la formulación inicialmente es de carácter general (primeras reclamaciones), haciendo pensar que la invención esta enfocada hacia la formulación únicamente pero, posteriormente (reivindicación 7) la invención

cambia a ser una formulación de anticuerpo anti-IL6, lo que no obedece a una extrapolación razonable de lo ejemplificado, ya que esta formulación no puede presentar las mismas propiedades de estabilidad o inhibición de desamidación sobre dos anticuerpos diferentes, así como tampoco se puede demostrar su efectividad sobre los anticuerpos que no han sido evaluados. Lo que significa sin más, que la solicitud colombiana en trámite no presenta pleno sustento en la descripción.

Por otra parte, existen modalidades particulares de la solicitud de invención como se reclama en las reivindicaciones 19 – 20 que indican métodos para desamidar anticuerpos, lo que no puede ser considerado ya que la reclamación no se presenta sobre un producto o procedimiento establecido, sino sobre la función de la formulación de una “eventual” desamidación del anticuerpo en cuestión

Objeciones a la patentabilidad basados en la publicación internacional WO 03/072060 de 04/09/2003.

El documento WO 03/072060 “Polypeptide Formulation”, se refiere a composiciones farmacéuticas acuosas de polipéptidos que contienen dominios Fc, como inmunoglobulinas (ver abstract); adicionalmente, en dicho documento se indica que la arginina es un inhibidor de la agregación de polipéptidos que contienen el dominio Fc (ver reivindicación 1), que la histidina puede estar presente como un agente buffer (reivindicación 3), que se puede emplear un surfactante tipo Tween, SDS, o polisorbato, (reivindicación 11) y que el pH debe estar entre 5.5 a 7.8 (reivindicación 18).

Si tenemos en cuenta lo anterior y lo comparamos con lo revelado en el capítulo reivindicatorio de la solicitud colombiana en trámite tenemos: en la parte descriptiva se menciona que la adición de arginina en la solución, particularmente suprime la generación de dímeros; en la reivindicación 2 se describe que la histidina se incluye como buffer; en la reivindicación 3 la formulación comprende un surfactante, que puede ser escogido de Polioxietilen sorbitanes como son los Polisorbatos (Tween 20, Tween 80), entre otros, tal como se evidencia en la parte descriptiva; en la reivindicación 11 se señala que el pH de la formulación en solución es 4-8; Como vemos, se mencionan los mismos componentes específicos de la formulación e incluso se reclaman como fundamentales al presentarse en las reivindicaciones tal como se observa en los párrafos anteriores.

Continuando con el análisis, vemos que la descripción de la citada anterioridad (pág 3) tiene el mismo objeto de la solicitud en trámite y se enfocan en el mismo problema técnico:

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DETAILED DESCRIPTION

In long-term storage of pharmaceutical compositions containing polypeptides, including aqueous and lyophilized formulations, active polypeptides can be lost due to aggregation and/or degradation. Thus, the present invention is directed to an aqueous formulation that surprisingly allows for stable long-term storage of a pharmaceutical composition wherein the active ingredient in the composition is a polypeptide having an Fc domain of an antibody. This formulation is useful, in part, because it is more convenient to use for the patient, as this formulation does not require any extra steps such as rehydrating.

Luego, en las paginas siguientes se menciona que el inhibidor de agregación que consiste en L- arginina debe estar presente en la formulación de la inmunoglobulina determinada, veamos:

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In one embodiment, the formulation is comprised of an Fc domain containing polypeptide, an aggregation inhibitor selected from group consisting of L-arginine and L-cysteine, and, optionally, a buffer, a tonicity modifier and additional excipients as necessary. L-arginine has been used to assist refolding of insoluble polypeptides, particularly those expressed to high levels in inclusion bodies in bacteria. However, L-arginine has not been utilized successfully to enhance stability of Fc domain containing polypeptides in pharmaceutical compositions (Soejima et al., 2001, J. Biochem., 130:369-277).

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Adicionalmente, la anterioridad citada menciona específicamente los anticuerpos adecuados para las formulaciones empleadas, incluso se observa claramente la mención textual del anticuerpo anti IL-6 receptor (página 7, línea 5) ejemplificado a su vez en la solicitud en trámite, demostrando que la materia en estudio no presenta novedad alguna.

- Examples of antibodies or antibody/cytotoxin or antibody/luminophore conjugates contemplated for use in the invention include those that recognize one or more of the following antigens: CD2, CD3, CD4, CD8, CD11a, CD14, CD18, CD20, CD22, CD23, CD25, CD33, CD40, CD44, CD52, CD80 (B7.1), CD86 (B7.2), CD147, IL-4, IL-5, IL-8, IL-10, IL-2 receptor, IL-4 receptor, IL-6 receptor, IL-13 receptor, PDGF- β , VEGF, TGF, TGF- β 2, TGF- β 1, EGF receptor, VEGF receptor, C5 complement, IgE, tumor antigen CA125, tumor antigen MUC1, PEM antigen, LCG (which is a gene product that is expressed in association with lung cancer), HER-2, a tumor-associated glycoprotein TAG-72, the SK-1 antigen, tumor-associated epitopes that are present in elevated levels in the sera of patients with colon and/or pancreatic cancer, cancer-associated epitopes or polypeptides expressed on breast, colon, squamous cell, prostate, pancreatic, lung, and/or kidney cancer cells and/or on melanoma, glioma, or neuroblastoma cells, TRAIL receptors 1, 2, 3 and 4, the necrotic core of a tumor, integrin alpha 4 beta 7, the integrin VLA-4, B2 integrins, TNF- α , the adhesion molecule VAP-1, epithelial cell adhesion molecule (EpCAM), intercellular adhesion molecule-3 (ICAM-3), leukointegrin adhesin, the platelet glycoprotein gp IIb/IIIa, cardiac myosin heavy chain, parathyroid hormone, rNAPc2 (which is an inhibitor of factor VIIa-tissue factor), MHC I, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), tumor necrosis factor (TNF), CTLA-4 (which is a cytotoxic T lymphocyte-associated antigen), Fc- γ -1 receptor, HLA-DR 10 beta, HLA-DR antigen, L-selectin, IFN- γ , Respiratory Syncytial Virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV), Streptococcus mutans, and Staphylococcus aureus.

Por último y remitiéndonos exclusivamente a lo revelado en la reivindicación 19 que menciona: “un método para inhibir la desamidación de moléculas de un anticuerpo en una formulación líquida que contiene el anticuerpo, que comprende agregar arginina a la formulación líquida”, se observa que el documento citado incluye todas las características técnicas mencionadas por lo que destruye la novedad y el nivel inventivo de la solicitud.

Y aunque en la anterioridad tantas veces citada no se mencione la intención de la formulación de inhibir la desamidación de las moléculas de anticuerpo, este resulta ser únicamente un efecto técnico a alcanzar dado por la estructura de la formulación que en esencia es la misma.

Objeciones a la patentabilidad basados en la publicación Internacional WO 2005/049078 de 02/06/2005.

El documento WO 2005/049078 “Immunoglobulin preparations having increased stability”, describe una preparación de proteína estable que contiene un aminoácido estabilizante (ver abstract) seleccionado de arginina, metionina y similares (ver reivindicaciones 1, 2 y 19), e indica que la proteína obedece a una preparación de inmunoglobulina (reivindicaciones 14 y 15).

Protein preparations, in particular immunoglobulin preparations for intravenous injection, have been in use for quite some time. Proteins, and immunoglobulin in particular, tend to form aggregates and/or dimers and to fragment or denature. If such solutions are injected intravenously, aggregates can give rise to severe side reactions including anaphylactic shock. In order to avoid aggregation, fragmentation, etc in such protein solutions and to improve their stability, a number of treatments have been tried in the state of the art. For instance, intravenous IgG for clinical use are often lyophilised (freeze-dried) for improved stability on storage, but such preparations must be reconstituted with a diluent before use. The reconstitution step is inconvenient and time consuming and increases the likelihood of contamination of the product. Another way of improving immunoglobulin stability and storage that is well known in the art is the addition of protein-stabilising excipients to the IgG preparation. Known excipients include sugars, polyols, amino acids, amines, salts, polymers and

Página 1

Como se observa, en este documento se reclaman las formulaciones con los dos aminoácidos mencionados por la solicitud en estudio, y no solo se indican como posibles componentes, sino que se reclaman como ingredientes específicos para mejorar propiedades de la formulación.

- 4 -

Thus, the present invention provides a protein preparation having improved stability wherein the preparation comprises one or more stabilisers selected from the group consisting of non-polar and basic amino acids. Exemplary non-polar and basic amino acids, useful for the purposes of the present invention are histidine, arginine, lysine, ornithine (basic amino acids) and, isoleucine, valine, methionine, glycine and proline (non-polar amino acids). Particularly useful is proline. The stabiliser may be an amino acid of the group of non-polar or basic amino acids on its own, or it may be a combination of 2 or more such amino acids. The amino acids are preferably not used in combination with nicotinamide. The amino acid stabilisers may be natural amino acids, amino acid analogues, modified amino acids or amino acid equivalents. L-amino acids are preferred. When proline is used as the stabiliser, it is preferably L-proline. It is also possible to use proline equivalents, e.g. proline analogues.

Por otra parte, este documento también es enfático en reclamar las formulaciones dirigidas hacia inmunoglobulinas (ver página 5 de la solicitud a continuación), lo que corresponde, al igual manera que la solicitud en trámite, a anticuerpos y su formulación para mejorar la estabilidad.

15 Preferably, the protein preparation is an immunoglobulin preparation, in particular an antibody preparation wherein the antibodies may be of any idiotype but preferably IgG, IgA or IgM. IgG preparations are particularly preferred. The immunoglobulins can be polyclonal or monoclonal and can be isolated from human or animal blood or produced by other means, for instance by recombinant DNA technology or hybridoma technology. In
20 general, immunoglobulins are obtained from blood plasma by alcohol fractionation, which may be combined with other purification techniques like chromatography, adsorption or precipitation. The immunoglobulins may be treated with trace amounts of enzymes (e.g. pepsin) in order to reduce anti-complementary activity or they may be used whole.

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Como se observa, este documento menciona lo que es de conocimiento general en el arte en cuanto a la adición de arginina y/o metionina a formulaciones con anticuerpos con el fin de mejorar las propiedades de estabilidad, por lo que la materia objeto de la presente solicitud ya había sido divulgada y por lo tanto no cumple con el requisito de novedad.

Objeciones a la patentabilidad basados en el documento de patente US 2003/0190316 de 09/10/2003.

El documento US 2003/0190316 "Stabilized antibody-containing preparations", describe una preparación estabilizada de un anticuerpo en un buffer de histidina (reivindicación 1), e indica que el anticuerpo es un anti-IL6 receptor (reivindicación 3), que el aminoácido básico tal como arginina puede ser admitido (reivindicación 15) y que la preparación puede contener surfactante (página 14, último párrafo).

En este caso, y para ir más profundamente en la invención, el documento menciona anticuerpos anti-IL6 tal como los reclamados y ejemplificados en la solicitud en una formulación de histidina y arginina.

Esto se observa además en lo reclamado por el solicitante en la reivindicación 8 y dependientes donde se enuncia: "una formulación líquida estable que contiene un anticuerpo anti-IL-6 de receptor que comprende ya sea arginina o metionina", - entendiendo que la adición de metionina es una característica meramente opcional-, se concluye que todas las características esenciales de la solicitud en estudio se encuentra anticipada por éste documento, por lo tanto no puede admitirse la novedad de la solicitud.

En consecuencia, los tres documentos citados anticipan la materia reclamada por la solicitud, y no solo eso, sino que se enfocan en el mismo problema técnico y aportan la misma solución.

Por otra parte, y sobre la base del conocimiento de los documentos del estado de la técnica, una persona versada en la materia podría fácilmente concebir la mezcla de arginina y metionina como inhibidores de agregación o estabilizantes en un anticuerpo o en otra preparación de soluciones que contienen proteínas tal como se observó en el documento WO 2005/049078 y seleccionar como anticuerpo algún anti-IL6 de receptor como en US 2003/0190316, mezclando además surfactante y triptofano con el fin de mejorar la estabilización. De esta manera, todas las modalidades particulares de la invención son anticipadas por cada documento, o por la combinación de dos de los mismos.

Aunque como se ha visto, no se evidencian diferencias en la formulación del anticuerpo de la presente invención con los documentos citados, los efectos técnicos alcanzados por composiciones de arginina y/o histidina tampoco sorprenden al técnico medio versado en la materia en la medida en que los resultados de estabilidad de proteínas y en este caso anticuerpos anti-IL-6 de receptor son posibles gracias a la presencia de los mismos, como es de esperarse.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

Ruego que se tengan como tales, sin perjuicio de que el Despacho considere oficiosamente, en particular de las allegadas por el suscrito a los expedientes mencionados en este escrito, las siguientes:

- ❖ La publicación de patente WO 03/072060 "Polypeptide Formulation" de 04/09/2003; portada, página 3, 4, 7 y reivindicaciones.
- ❖ La publicación de patente WO 2005/049078 "Immunoglobulin preparations having increased stability" de 02/06/2005; portada página 1, 4, 5 y reivindicaciones.

- ❖ El documento de patente US 2003/0190316 de 09/10/2003 titulada "Stabilized antibody-containing preparations"; portada y reivindicaciones.

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos:

1. Certificado de existencia y representación legal de TECNOQUÍMICAS S.A.
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. Constancia de pago para sustentar la solicitud de prórroga a que se refiere el artículo 42, inciso 2º de la Decisión 486.
5. Copia para correr traslado al solicitante.
6. El mencionado en el acápite de pruebas.

VII. NOTIFICACIONES

El suscrito recibirá notificaciones en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 205, Fax (1) 621.25.42 de esta ciudad TECNOQUÍMICAS S.A., las recibirá en sus oficinas localizadas en la Carrera 1 N° 46-84 de Cali.

Señor Superintendente,



JOSÉ LUIS REYES VILLAMIZAR

C.C. 79'152.473 de Usaquén

T.P.A. 44.655 del C.S.J.

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- (71) Applicant (*for all designated States except US*): **IMMUNEX CORPORATION** [US/US]; Law Department, 51 University Street, Seattle, WA 98101 (US).
- (72) Inventors; and
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- (74) Agents: **ODRE, Steven, M.** et al.; Immunex Corporation, Law Department, 51 University Street, Seattle, WA 98101 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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WO 03/072060 A2

(54) Title: POLYPEPTIDE FORMULATION

(57) Abstract: The present invention relates to an aqueous pharmaceutical composition suitable for long-term storage of polypeptides containing an Fc domain of an immunoglobulin, methods of manufacture, methods of administration and kits containing same.

Figure 6: HIC Peak 1 and Pre-peak 1 data for lots A, B, C and D, and lot 1 stored for up to 1 year at 37°C.

Figure 7: HIC Peak 2 data for lots A, B, C and D, and lot 1 stored for up to 1 year at 2-8°C.

5 Figure 8: HIC Peak 2 data for lots A, B, C and D, and lot 1 stored for up to 1 year at 37°C.

Figure 9: HIC Peak 3 data for lots A, B, C and D, and lot 1 stored for up to 1 year at 2-8°C.

10 Figure 10: HIC Peak 3 data for lots A, B, C and D, and lot 1 stored for up to 1 year at 37°C.

Figure 11: Binding activity of lots A, B, C and D, and lot 1 stored for 12 months at -70, 2-8, 30, and 37°C.

Figure 12: Bioactivity of lots A, B, C and D, and lot 1 stored for 12 months at -70, 2-8, 30, and 37°C.

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DETAILED DESCRIPTION

In long-term storage of pharmaceutical compositions containing polypeptides, including aqueous and lyophilized formulations, active polypeptides can be lost due to aggregation and/or degradation. Thus, the present invention is directed to an aqueous formulation that surprisingly allows for stable long-term storage of a pharmaceutical composition wherein the active ingredient in the composition is a polypeptide having an Fc domain of an antibody. This formulation is useful, in part, because it is more convenient to use for the patient, as this formulation does not require any extra steps such as rehydrating.

25 As used herein, the phrase "pharmaceutical composition" is understood to refer to a formulation comprised of a polypeptide prepared such that it is suitable for injection and/or administration into a patient in need thereof. More particularly, a pharmaceutical composition is substantially sterile and does not contain any agents that are unduly toxic or infectious to the recipient. Further, it is to be understood that, as used herein, a solution or liquid formulation is meant to mean a liquid preparation that contains one or 30 more chemical substances dissolved in a suitable solvent or mixture of mutually miscible solvents.

In addition, as used herein, the term "about" is understood to mean that there can be variation in the concentration of a component of the described formulation that can be

to 5%, 10%, 15% or up to and including 20% of the given value. For example, if a formulation has about 10 mg/ml of an Fc domain containing polypeptide, this is understood to mean that a formulation can have between 8 to 12 mg/ml of the stated polypeptide.

5 In one embodiment, the formulation is comprised of an Fc domain containing polypeptide, an aggregation inhibitor selected from group consisting of L-arginine and L-cysteine, and, optionally, a buffer, a tonicity modifier and additional excipients as necessary. L-arginine has been used to assist refolding of insoluble polypeptides, particularly those expressed to high levels in inclusion bodies in bacteria. However, L-
10 arginine has not been utilized successfully to enhance stability of Fc domain containing polypeptides in pharmaceutical compositions (Soejima et al., 2001, J. Biochem., 130:369-277).

It is contemplated that the preparation of the composition should be done in consideration of limiting injection site discomfort. It is further contemplated that
15 additional active ingredients can also be included in the presently described composition, for example, to reduce injection site discomfort. Such active ingredients include, but are not limited to non-steroidal anti-inflammatory drugs such as, for example, tromethamine, in an appropriate dosage.

Polypeptides

20 In a particular embodiment the Fc domain containing polypeptide is a soluble form of the TNF receptor fused to an Fc domain (TNFR:Fc), however, it is to be understood that any polypeptide containing an Fc domain is suitable for use in the instant formulation. A commercially available TNFR:Fc is known as etanercept (Enbrel®, Immunex Corporation), which is a dimeric fusion polypeptide consisting of the
25 extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. The Fc component of etanercept contains the constant heavy 2 (CH2) domain, the constant heavy 3 (CH3) domain and hinge region, but not the constant heavy 1 (CH1) domain of human IgG1. It is to be understood that an Fc domain can contain one or all of the domains described
30 above. Etanercept is produced by recombinant DNA technology in a Chinese hamster ovary (CHO) mammalian cell expression system. It consists of 934 amino acids and has an apparent molecular weight of approximately 150 kilodaltons (Physicians Desk Reference, 2002, Medical Economics Company Inc.).

Examples of antibodies or antibody/cytotoxin or antibody/luminophore conjugates contemplated for use in the invention include those that recognize one or more of the following antigens: CD2, CD3, CD4, CD8, CD11a, CD14, CD18, CD20, CD22, CD23, CD25, CD33, CD40, CD44, CD52, CD80 (B7.1), CD86 (B7.2), CD147, IL-4, IL-5, IL-8, IL-10, IL-2 receptor, IL-4 receptor, IL-6 receptor, IL-13 receptor, PDGF- β , VEGF, TGF, TGF- β 2, TGF- β 1, EGF receptor, VEGF receptor, C5 complement, IgE, tumor antigen CA125, tumor antigen MUC1, PEM antigen, LCG (which is a gene product that is expressed in association with lung cancer), HER-2, a tumor-associated glycoprotein TAG-72, the SK-1 antigen, tumor-associated epitopes that are present in elevated levels in the sera of patients with colon and/or pancreatic cancer, cancer-associated epitopes or polypeptides expressed on breast, colon, squamous cell, prostate, pancreatic, lung, and/or kidney cancer cells and/or on melanoma, glioma, or neuroblastoma cells, TRAIL receptors 1, 2, 3 and 4, the necrotic core of a tumor, integrin alpha 4 beta 7, the integrin VLA-4, B2 integrins, TNF- α , the adhesion molecule VAP-1, epithelial cell adhesion molecule (EpCAM), intercellular adhesion molecule-3 (ICAM-3), leukointegrin adhesin, the platelet glycoprotein gp IIb/IIIa, cardiac myosin heavy chain, parathyroid hormone, rNAPc2 (which is an inhibitor of factor VIIa-tissue factor), MHC I, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), tumor necrosis factor (TNF), CTLA-4 (which is a cytotoxic T lymphocyte-associated antigen), Fc- γ -1 receptor, HLA-DR 10 beta, HLA-DR antigen, L-selectin, IFN- γ , Respiratory Syncytial Virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV), Streptococcus mutans, and Staphylococcus aureus.

The formulations of the invention can also be used for anti-idiotypic antibodies, or substantially similar polypeptides, including but not limited to anti-idiotypic antibodies against: an antibody targeted to the tumor antigen gp72; an antibody against the ganglioside GD3; or an antibody against the ganglioside GD2.

The Fc domain containing polypeptide suitable for storage in the present pharmaceutical composition can be produced by living host cells that express the polypeptide, such as hybridomas in the case of antibodies, or host cells that have been genetically engineered to produce the polypeptide in the case of fusion polypeptides or antibodies. Methods of genetically engineering cells to produce polypeptides are well known in the art. See, e.g., Ausubel et al., eds. (1990), Current Protocols in Molecular Biology (Wiley, New York). Such methods include introducing nucleic acids that encode and allow expression of the polypeptide into living host cells. These host cells can be

CLAIMS

What is claimed is:

1. A pharmaceutical composition that is a stable aqueous formulation comprising an Fc domain containing polypeptide and an aggregation inhibitor, wherein the aggregation inhibitor is L-arginine.
2. The composition of claim 1, further comprising a buffer.
3. The composition of claim 2, wherein the buffer is selected from the group consisting of sodium phosphate, histidine, potassium phosphate, sodium or potassium citrate, maleic acid, ammonium acetate, tris-(hydroxymethyl)-aminomethane (tris), acetate and diethanolamine.
4. The composition of claim 3, wherein the L-arginine is at a concentration of from about 10 mM to about 100mM.
5. The composition of any one of claims 1, 2, 3 or 4, further comprising a tonicity modifier.
6. The composition of claim 5, wherein the tonicity modifier is selected from the group consisting of arginine, cysteine, histidine, glycine, sodium chloride, potassium chloride, sodium citrate, sucrose, glucose and Mannitol.
7. The composition of claim 6, wherein the tonicity modifier is sodium chloride.
8. The composition of any one of claims 1, 2, 3, or 4, further comprising an excipient.
9. The composition of claim 6, further comprising an excipient.
10. The composition of claim 7, further comprising an excipient.

11. A composition of claim 8, wherein the excipient is selected from the group consisting of sucrose, lactose, glycerol, xylitol, sorbitol, Mannitol, maltose, inositol, trehalose, glucose, bovine serum albumin (BSA), human SA or recombinant HA, dextran, PVA, hydroxypropyl methylcellulose (HPMC), polyethyleneimine, gelatin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC), polyethylene glycol, ethylene glycol, glycerol, dimethylsulfoxide (DMSO), dimethylformamide (DMF), proline, L-serine, sodium glutamic acid, alanine, glycine, lysine hydrochloride, sarcosine, gamma-aminobutyric acid, Tween-20, Tween-80, SDS, polysorbate, polyoxyethylene copolymer, potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate, trimethylamine N-oxide, betaine, zinc ions, copper ions, calcium ions, manganese ions, magnesium ions, CHAPS, sucrose monolaurate, and 2-O-beta-mannoglycerate.
12. The composition of claim 11, wherein the excipient is sucrose.
13. A stable pharmaceutical composition comprising about 10 mg/ml to about 100 mg/ml TNFR:Fc, L-arginine, sodium phosphate, sodium chloride and sucrose.
14. The composition of claim 13, wherein L-arginine is about 10 mM to about 75 mM.
15. The composition of claim 13, wherein sodium phosphate is about 5 mM to about 100 mM.
16. The composition of claim 13, wherein sodium chloride is about 5 mM to about 200 mM.
17. The composition of claim 13, wherein sucrose is about 0.5% to about 1.5%.
18. The composition of claim 13, wherein pH is about 5.5 to about 7.8.
19. The composition of claim 13, having 25 mg/ml TNFR:Fc, 25 mM L-arginine, 25 mM sodium phosphate, 98 mM sodium chloride, 1% sucrose at pH 6.2.

20. The composition of any one of claims 1, 13 or 19, wherein the composition is liquid.
21. The composition of claim 20, wherein the composition is frozen.
22. A method of formulating a composition comprising combining isolated TNFR:Fc with L-arginine.
23. The method of claim 22, further comprising the steps of combining a buffer, a tonicity modifier, and an excipient with the composition.
24. The method of claim 22 or 23, wherein the composition is liquid.
25. A kit comprising a composition comprising an Fc domain containing polypeptide and L-arginine, and instructions for use of said composition.
26. The kit of claim 25, wherein the composition is liquid.
27. The kit of claim 25 or 26, wherein the composition is stored in a pre-filled sterile syringe.
28. The kit of claim 27, wherein the syringe is stored at about -20°C to about -70°C.
29. A method of treating a mammal in need thereof comprising administering a therapeutically effective amount of the pharmaceutical composition of any one of claims 4 or 13.
30. A method of accelerated stability testing of an Fc domain containing polypeptide in a pharmaceutical composition, wherein the composition comprises L-arginine, the steps of the method comprising storing the composition at 37°C and measuring the stability of the polypeptide after at least one month at 37°C.

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- (54) Title: IMMUNOGLOBULIN PREPARATIONS HAVING INCREASED STABILITY
- (57) Abstract: The present invention relates to a protein preparation having increased stability, comprising a stabiliser selected from the group consisting of non-polar and basic amino acids and having a pH of 4.0 to 5.2. The invention further relates to a pharmaceutical composition and a method of stabilising protein preparations.

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Immunoglobulin Preparations having increased stability**Description**

The present invention relates to a protein preparation having increased stability, comprising a stabiliser selected from the group consisting of non-
5 polar and basic amino acids and having a pH of 4.2 to 5.4. The invention further relates to a pharmaceutical composition and a method of stabilising protein preparations.

Protein preparations, in particular immunoglobulin preparations for
10 intravenous injection, have been in use for quite some time. Proteins, and immunoglobulin in particular, tend to form aggregates and/or dimers and to fragment or denature. If such solutions are injected intravenously, aggregates can give rise to severe side reactions including anaphylactic shock. In order to avoid aggregation, fragmentation, etc in such protein
15 solutions and to improve their stability, a number of treatments have been tried in the state of the art. For instance, intravenous IgG for clinical use are often lyophilised (freeze-dried) for improved stability on storage, but such preparations must be reconstituted with a diluent before use. The reconstitution step is inconvenient and time consuming and increases the
20 likelihood of contamination of the product. Another way of improving immunoglobulin stability and storage that is well known in the art is the addition of protein-stabilising excipients to the IgG preparation. Known excipients include sugars, polyols, amino acids, amines, salts, polymers and

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surfactants. Such stabilisation strategies in protein pharmaceuticals are abundant in the art. For example, US Patent 4,499,073 (Tenold) improves the stabilisation through the selection of pH and ionic strength. JP 54020124 discloses the addition of an amino acid to an intramuscular preparation to render it storage stable and safe. JP 57031623 and JP 57128635 disclose the use of arginine and/or lysine with NaCl in 5 to 15% IgG preparations to achieve long-term stability in an intramuscular preparation. JP 56127321 discloses the addition of a sugar alcohol to IgG which works better than the previously used glucose in suppressing aggregation. JP 4346934 discloses the use of low conductivity (less than 1 mmho), pH 5.3 to 5.7 and optionally one or more stabilisers including PEG, human serum albumin and mannitol. US 4,439,421 (Hooper) teaches the addition of a hydrophilic macromolecule, a polyol and another protein to stabilise against ACA (anti-complement activity) generation. US 5,945,098 (Sarno) discloses the stabilisation of isotonic solutions by the addition of amino acids (0.1 to 0.3 M glycine), and non-ionic detergents (polysorbate) and PEG. US 4,186,192 (Lundblad) discloses various additives including amino acids, however, without specifying the use of single specific amino acids. This disclosure includes the stabilisation of IgG with maltose and additionally glycine to 0.1 M. US 4,362,661 (Ono) discloses the use of neutral and basic amino acids to impart stability on a 5% IgG product. All the above mentioned documents disclose IgG preparations of an acidic but still relatively high pH of above 5.2.

In addition to preventing the formation of immunoglobulin aggregates, it has also been recognised that dimer formation, in particular of IgG, can be detrimental to IgG preparations for intravenous use. Although IgG dimers are not known to cause anaphylactic shock, it has nevertheless been found that IgG preparations with a high dimer content are less well tolerated on intravenous injection and can give rise to undesirable side effects including fever, nausea and sometimes lowered blood pressure. Hypotensive side effects have been detected in a rat model by Bleaker et al. (Vox Sanguinis

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52, 281-290, 1987), and this also shows an apparent correlation with the dimer content. Dimer formation is less of a problem when an IgG preparation is lyophilised shortly after it is produced. However, if the preparation is intended for storage in non-lyophilised liquid form, dimer concentration
5 increases with storage time.

US patent 5,871,736 (Bruegger et al.) discloses immunoglobulin preparations, particularly liquid preparations of IgG for intravenous infusion which comprise one or more amphiphilic stabilisers in order to stabilise
10 against dimer formation. The amphiphilic stabilisers include nicotinic acid and its derivatives, in particular nicotinamide, and, mainly in conjunction with the above, amino acids having uncharged lipophilic side chains, e.g. phenylalanine, methionine, leucine, isoleucine, proline and valine. The experimental disclosure of this prior art document discloses amino acids
15 always in conjunction with nicotinamide, and the concentrations disclosed for the amino acids are 200 mmol/litre for proline, 80 mmol/litre for glycine and 120mmol/litre for isoleucine.

The pH range for the preparations disclosed in US 5,871,736 is broadly
20 given as being between 4 and 8, but the actual disclosure of the Examples teaches a pH of 5.3.

Although the above US patent discloses IgG preparations in which dimer formation has been suppressed to a certain degree, it is still desirable to
25 provide protein preparations, in particular immunoglobulin preparations, which show improved stabilisation, in particular at ambient temperature.

The inventors have found that a surprisingly high degree of stabilisation of liquid protein preparations can be achieved by adjusting the pH of the final
30 preparation to between 4.2 and 5.4 and by adding as a stabiliser, a basic or non-polar amino acid.

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Thus, the present invention provides a protein preparation having improved stability wherein the preparation comprises one or more stabilisers selected from the group consisting of non-polar and basic amino acids. Exemplary non-polar and basic amino acids, useful for the purposes of the present invention are histidine, arginine, lysine, ornithine (basic amino acids) and, isoleucine, valine, methionine, glycine and proline (non-polar amino acids). Particularly useful is proline. The stabiliser may be an amino acid of the group of non-polar or basic amino acids on its own, or it may be a combination of 2 or more such amino acids. The amino acids are preferably not used in combination with nicotinamide. The amino acid stabilisers may be natural amino acids, amino acid analogues, modified amino acids or amino acid equivalents. L-amino acids are preferred. When proline is used as the stabiliser, it is preferably L-proline. It is also possible to use proline equivalents, e.g. proline analogues.

Surprisingly, it was found that the addition of amino acids on their own, without other stabilisers (such as nicotinamide), and the adjustment of the pH of the final preparation markedly increases the stability of those preparations, particularly at ambient temperature. The increased stability is demonstrated by better stability of the preparations at temperatures between about 2°C and about 40°C, particularly at ambient temperature which preferably ranges from about 10°C, more preferably from about 15°C, more preferably from about 20°C to about 30°C, most preferably to about 25°C. The increased stability of the preparations of the invention is also visible at higher temperatures of about 30°C to about 40°C, including body temperature of about 37°C. Preferably, the increased stability is alternatively or additionally further defined as improved storage time, decreased fragmentation, decreased aggregate formation, decreased dimer formation or/and decreased discolouring. The improved storage time means that the preparations of the invention are preferably stable for at least 30 days, preferably at least 60 days, more preferably at least 90 days, more preferably at least 120 days, more preferably even longer than that.

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Decreased aggregation preferably means that the preparations show a lower percentage of aggregates (in particular in case of Ig) than conventional preparations. Preferably, the dimer content of the preparations is below about 12%, preferably below about 10%, more preferably below about 8%.

5 Decreased colouring preferably means that the optical density of the formulations of the invention is between about 20 % and 60% lower than of conventional formulations.

10 In general, the protein preparations of the present invention are liquid formulations which are useful for intravenous injection. Such preparations can be stored and are stable in liquid form and thus do not require lyophilisation or other treatment and can be readily used.

15 Preferably, the protein preparation is an immunoglobulin preparation, in particular an antibody preparation wherein the antibodies may be of any idiotype but preferably IgG, IgA or IgM. IgG preparations are particularly preferred. The immunoglobulins can be polyclonal or monoclonal and can be isolated from human or animal blood or produced by other means, for instance by recombinant DNA technology or hybridoma technology. In
20 general, immunoglobulins are obtained from blood plasma by alcohol fractionation, which may be combined with other purification techniques like chromatography, adsorption or precipitation. The immunoglobulins may be treated with trace amounts of enzymes (e.g. pepsin) in order to reduce anti-complementary activity or they may be used whole.

25 The preparations can be obtained by methods known in the art, except that the pH of the final preparation is adjusted to a relatively high but acidic pH, namely in the range of about pH 4.2 to 5.4. It has been found that this pH range is particularly useful for improving the storage of characteristics of
30 immunoglobulin preparations. The pH range is preferably from 4.5 to about 5.2, a pH range of about 4.6 to 5.0 being particularly preferred, pH 4.8 being especially preferred.

Claims

5

1. A stable protein preparation, wherein the preparation comprises one or more stabilisers selected from the group consisting of non-polar and basic amino acids and wherein the preparation has a pH of 4.2 to 5.4.

10

2. The preparation of claim 1, wherein the one or more stabilisers are selected from the group consisting of histidine, arginine, lysine, ornithine, isoleucine, valine, methionine, glycine and proline.

15

3. The preparation of claims 1 or 2, wherein the stabiliser is proline.

4. The preparation of claim 3, wherein proline is L-proline.

20

5. The preparation of any one of the preceding claims, wherein it has a pH of 4.5 to 5.2.

6. The preparation of claim 5, wherein it has a pH of 4.6 to 5.0.

25

7. The preparation of any one of the preceding claims, wherein it comprises the stabiliser at a final concentration of at least 0.2 M.

8. The preparation of claim 7, wherein it comprises the stabiliser at a final concentration of 0.2 to 0.4 M.

30

9. The preparation of claim 8, wherein it comprises the stabiliser at a final

concentration of 0.25 M.

10. The preparation of any one of the preceding claims, wherein its protein concentration is from 5 to 25 % w/v.
- 5 11. The preparation of claim 10, wherein its protein concentration is from 15 to 20% w/v for subcutaneous administration.
- 10 12. The preparation of claim 10, wherein its protein concentration is from 6 to 15 % w/v, for intravenous administration.
- 15 13. The preparation of claim 12, wherein its protein concentration is from 8 to 12 % w/v.
14. The preparation of any one of the preceding claims, wherein it is an immunoglobulin preparation.
15. The preparation of any one of the preceding claims, wherein it is an IgG, IgA or IgM preparation.
- 20 16. A pharmaceutical composition comprising the protein preparation of one of the preceding claims and pharmaceutically acceptable additives.
17. The pharmaceutical composition of claim 16, wherein it comprises the immunoglobulin preparation of any one of claims 11 to 15 for a dosage of 0.2 to 2.0 g immunoglobulin per kg bodyweight per day.
- 25 18. A method of stabilising protein preparations, in particular immunoglobulin preparations, comprising providing an aqueous protein solution and adding one or more stabilisers selected from the group consisting of basic and non-polar amino acids, wherein the pH of the solution is adjusted to a pH of about 4.2 to 5.4.
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19. The method of claim 18, wherein the stabiliser is selected from the group consisting of histidine, arginine, lysine, ornithine, isoleucine, valine, methionine and proline.
 20. The method of claim 18 or 19, wherein the pH is adjusted to 4.8.
 21. The method of any one of claims 18 to 20, wherein the final stabiliser concentration is adjusted to 0.2 to 0.4 M.



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(54) **STABILIZED ANTIBODY-CONTAINING PREPARATIONS**

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(57) **ABSTRACT**

The present invention provides stabilized preparations containing an antibody in a glycine buffer and/or a histidine buffer and also provides processes for preparing a protein-containing stabilized preparation, comprising adjusting the pH with a basic amino acid or a basic amino acid derivative or a salt thereof.

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STABILIZED ANTIBODY-CONTAINING PREPARATIONS

FIELD OF THE INVENTION

[0001] The present invention relates to antibody-containing preparations, particularly stabilized antibody-containing preparations with low loss of active ingredients even after long-term storage. The present invention also relates to processes for preparing protein-containing stabilized preparations, comprising adjusting the pH with a basic amino acid or a basic amino acid derivative or a salt thereof.

BACKGROUND ART

[0002] A number of injectable protein-containing preparations are supplied to the market, in which various measures are taken to provide stabilized protein-containing preparations with low loss of active ingredients even after long-term storage. Protein-containing preparations are prepared by dissolving active ingredients and various additives such as diluents, solubilizers, excipients, soothing agents, buffering agents, sulfur-containing reducing agents, antioxidants, stabilizers, surfactants, etc. in a buffer.

[0003] A problem generally associated with the storage of proteins stored as concentrated solutions is their deterioration as exemplified by the formation of insoluble aggregates and that must be prevented.

[0004] For example, antibodies such as immunoglobulins, monoclonal antibodies and humanized antibodies are unstable proteins liable to physical or chemical changes such as association or aggregation under stresses of filtration, concentration and heating for removing viruses during the purification process.

[0005] A conventional method widely used for inhibiting deterioration of proteins to stably store them is stabilization by freeze-drying. However, it was necessary to add some agent for protecting against freezing to avoid denaturation or chemical changes that might be caused by mechanical stresses during freezing and freeze-drying.

[0006] A stabilization effect was found by adding as a stabilizer for inhibiting chemical or physical changes, polymers or polyols including proteins such as human serum albumin or purified gelatin or oligomers such as amino acids and surfactants. However, the addition of biopolymers such as proteins as stabilizers had problems such as the necessity of a very complex step for removing contaminants such as viruses derived from the stabilizers. Moreover, heat treatments for inactivating viruses sometimes caused problems such as association or aggregation due to heat stresses.

[0007] Interleukin-6 (IL-6) receptor is a ligand-binding protein having a molecular weight of about 80 KD to which IL-6 binds. Anti-IL-6 receptor antibodies were found to have a therapeutic effect on various IL-6-mediated diseases such as immune disorders, inflammatory diseases or lymphocyte tumors by blocking IL-6 signal transduction in immature myeloma cells to inhibit biological activities of IL-6 (Tsunenari, T. et al., Blood, 90:2437, 1997; Tsunenari T. et al., Anticancer Res. 16:2537, 1996). We also found that anti-IL-6 receptor antibodies have a therapeutic effect on immature myeloma cells (JP-A-8-099902).

[0008] We succeeded in the mass production of a reshaped humanized antibody hPM-1 as one of such anti-IL-6 recep-

tor antibodies, and have tried to formulate this purified anti-IL-6 receptor antibody into pharmaceutical preparations.

[0009] Similarly to other protein preparations, an important factor for formulation of anti-IL-6R antibodies is chemical and physical stability. Especially, humanized anti-IL-6 receptor antibodies are unstable proteins liable to physical or chemical changes such as association or aggregation under stresses of filtration, concentration and heating for virus elimination during the purification process.

[0010] Thus, there is a demand for the development and commercialization of preparations containing an antibody, especially a humanized anti-IL-6 receptor antibody, which are stable even after long-term storage.

[0011] As for various physiologically active proteins, it is also necessary to establish preparation conditions and storage conditions for maintaining their structures and activities in order to supply them as pharmaceuticals in constant amount and with high quality. Especially, it would be desirable to develop a method for inhibiting formation of insoluble aggregates in protein solutions.

DISCLOSURE OF THE INVENTION

[0012] As a result of careful studies to achieve the above objects, we accomplished the present invention on the basis of the finding that heat-induced aggregation is controlled by formulating a humanized anti-interleukin-6 receptor antibody in a glycine buffer and/or histidine buffer and that such formulations are further stabilized by adding glycine and/or sucrose.

[0013] We also accomplished the present invention on the basis of the finding that aggregation can be reduced and stabilization effect can be increased by adjusting the pH with a basic amino acid or a basic amino acid derivative or a salt thereof.

[0014] Accordingly, the present invention provides:

[0015] (1) a stabilized preparation containing an antibody in a glycine buffer and/or a histidine buffer;

[0016] (2) the stabilized preparation as defined in (1) above wherein the antibody is a chimeric antibody or a humanized antibody;

[0017] (3) the stabilized preparation as defined in (1) or (2) above wherein the antibody is an anti-interleukin-6 receptor antibody;

[0018] (4) the stabilized preparation as defined in (3) above wherein the anti-interleukin-6 receptor antibody is a humanized anti-interleukin-6 receptor antibody;

[0019] (5) the stabilized preparation as defined in (1) above wherein the concentration of the glycine buffer and/or histidine buffer is 5 mM-200 mM;

[0020] (6) the stabilized preparation as defined in any one of (1) to (5) above which contains glycine and/or sucrose as an isotonicizing agent;

[0021] (7) the stabilized preparation as defined in (6) above containing 0.05-1 M glycine and/or sucrose;

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[0144] FIG. 11 shows the results of comparison of the effect of histidine in two samples at pH 6.0, i.e. samples 6-2 and 6-5. In both samples, 5-10 mM histidine was the most effective.

[0145] FIG. 12 shows the results of comparison of sample 6-1 (5 mM phosphate/Na, pH 6.5), sample 6-2 (5 mM phosphate/His, pH 6.0) and sample 6-5 (5 mM phosphate/Na, pH 6.0). The aggregate content was the lowest in 6-2 followed by 6-5 and then 6-1. This confirmed that antibody hPM-1 is more stable at pH 6.0. Samples 6-2 and 6-5 differ in the means for adjusting pH to 6.0. That is, sample 6-5 was adjusted to pH 6.0 with NaOH, while sample 6-2 was adjusted with concentrated histidine used as a base. Thus, stabilization effect is greater when histidine is used than when conventional NaOH is used to adjust the pH to 6.0 similarly to pH 6.5.

[0146] FIG. 13 shows the effect of sucrose (50-200 mM) added to sample 6-2. Effective prevention of aggregation was observed as the sucrose concentration increased.

[0147] FIG. 13 also shows the effect of glycine (50-200 mM) added to sample 6-5. Remarkably effective prevention of aggregation was observed as the glycine concentration increased.

[0148] FIG. 14 shows a comparison of the effect of 200 mM glycine or sucrose in two samples at pH 6.0 (6-2 and 6-5). Aggregation was less in 6-2 than 6-5. It was further confirmed from these results that pH adjustment is more effective with histidine than NaOH.

1. A stabilized preparation containing an antibody in a glycine buffer and/or a histidine buffer.
2. The stabilized preparation of claim 1 wherein the antibody is a chimeric antibody or a humanized antibody.
3. The stabilized preparation of claim 1 or 2 wherein the antibody is an anti-interleukin-6 receptor antibody.
4. The stabilized preparation of claim 3 wherein the anti-interleukin-6 receptor antibody is a humanized anti-interleukin-6 receptor antibody.
5. The stabilized preparation of claim 1 wherein the concentration of the glycine buffer and/or histidine buffer is 5 mM-200 mM.
6. The stabilized preparation of any one of claims 1 to 5 which contains glycine and/or sucrose as an isotonicizing agent.
7. The stabilized preparation of claim 6 containing 0.05-1 M glycine and/or sucrose.
8. The stabilized preparation of any one of claims 1 to 7, which does not contain NaCl as an isotonicizing agent.
9. A stabilized preparation containing glycine and/or sucrose as an isotonicizing agent as well as a humanized anti-interleukin-6 receptor antibody in a glycine buffer and/or a histidine buffer.
10. The stabilized preparation of any one of claims 1 to 9 having a pH of 5-8.
11. A method for stabilizing an antibody preparation, comprising incorporating an antibody in a glycine buffer and/or a histidine buffer.

12. The method of claim 11, comprising incorporating glycine and/or sucrose as an isotonicizing agent.

13. A method for stabilizing an anti-interleukin-6 receptor antibody preparation, comprising incorporating glycine and/or sucrose as an isotonicizing agent as well as a humanized anti-interleukin-6 receptor antibody in a glycine buffer and/or a histidine buffer.

14. A process for preparing a stabilized preparation containing a physiologically active protein, comprising adjusting the pH with a basic amino acid or a basic amino acid derivative or a salt thereof.

15. The process of claim 14 wherein the basic amino acid is one or more members selected from histidine, arginine and lysine.

16. The process of claim 15 wherein the basic amino acid is histidine.

17. The process of any one of claims 14 to 16 wherein the physiologically active protein is a recombinant protein.

18. The process of any one of claims 14 to 17 wherein the physiologically active protein is an antibody.

19. The process of claim 18 wherein the antibody is a chimerized antibody or a humanized antibody.

20. The process of claim 18 or 19 wherein the antibody is an anti-interleukin-6 receptor antibody.

21. The process of claim 20 wherein the anti-interleukin-6 receptor antibody is a humanized anti-interleukin-6 receptor antibody.

22. A stabilized preparation containing an antibody in a histidine buffer and having a pH of 5-7.5.

23. The stabilized preparation of claim 22 wherein the antibody is an anti-interleukin-6 receptor antibody.

24. The stabilized preparation of claim 23 wherein the anti-interleukin-6 receptor antibody is a humanized anti-interleukin-6 receptor antibody.

25. The stabilized preparation of claim 24 having a pH of 5.5-6.2.

26. The stabilized preparation of claim 25 wherein the concentration of histidine is 1-50 mM.

27. The stabilized preparation of claim 26 wherein the concentration of histidine is 3-20 mM.

28. The stabilized preparation of claim 27 wherein the concentration of histidine is 5-10 mM.

29. The stabilized preparation of claim 24 having a pH of 6.2-7.5.

30. The stabilized preparation of claim 29 wherein the concentration of histidine is 5-200 mM.

31. The stabilized preparation of claim 30 wherein the concentration of histidine is 10-150 mM.

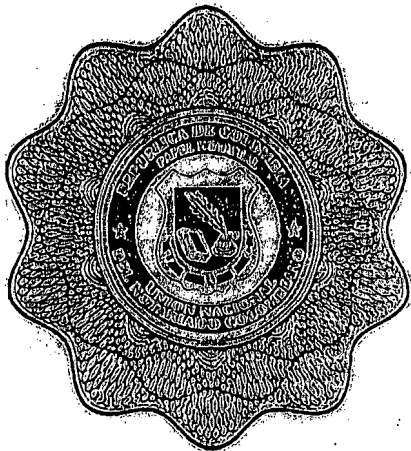
32. The stabilized preparation of claim 31 wherein the concentration of histidine is 25-100 mM.

33. The stabilized preparation of any one of claims 22 to 32 further containing glycine and/or sucrose.

34. The stabilized preparation of claim 33 containing 0.05-1 M glycine and/or sucrose.

35. The stabilized preparation of any one of claims 22 to 34, which has its pH adjusted with a basic amino acid or a basic amino acid derivative or a salt thereof.

* * * * *



ESCRITURA NUMERO CUATRO MIL QUINIENTOS - 31
TREINTA Y NUEVE (No.4.539) -----
En la ciudad de Cali, Capital del Depar-
tamento del Valle del Cauca, República de
Colombia, a los VEINTITRES (23) días -
del mes de OCTUBRE del año DOS MIL UNO --

(2.001), ante mi, JORGE ENRIQUE CAICEDO ZAMORANO, Notario
T E R C E R O (3o.) del Círculo de Cali, - - - - -

compareció JUAN MANUEL BARBERI OSPINA mayor de edad, vecino de Cali,
identificado con la cédula de ciudadanía número 14.949.561 de Cali, y dijo: ----

PRIMERO.- Que obra en su carácter de Representante Legal de en nombre y
representación de TECNOQUIMICAS S.A. sociedad comercial organizada y existente
conformidad a las leyes colombianas, con domicilio y sede principal en Cali constituida
por escritura pública número 2670 de la Notaría Segunda de Cali de fecha 12 de
junio de 1957, reformada en distintas oportunidades, según consta en el certificado
expedido por la Cámara de Comercio de Cali -----

SEGUNDO: Que en tal carácter, por este público instrumento, concede Poder Especial
pero amplio y suficiente a JOSE LUIS REYES VILLAMIZAR, mayor de edad, vecino de
Bogotá, identificado con C.C. # 79.152.473 de Isaquén, abogado en ejercicio, portador
de la TAP 44655 del C.S. de la J., domiciliado en Bogotá, D.C., República de Colombia
con dirección carrera 7 No. 88.23 oficina 205, de la misma ciudad, como su representante
con poder especial, amplio y suficiente, para obtener la protección de nuestra propiedad
industrial y contra la competencia desleal, a cuyo efecto lo autorizo, para que nos
represente ante cualesquiera entidad o persona, ejerzan o no jurisdicción, especialmente
ante las del orden administrativo, contencioso-administrativo y judicial, en todo lo
concerniente a los siguientes asuntos: a) Obtención, formulación de observaciones y
oposición a patentes de invención, modelos y dibujos industriales y el registro de
licencias de uso de esos derechos; b) Las acciones de oposición, cancelación, nulidad,
medidas cautelares, concesión de licencias y en general los procesos civiles, penales,
administrativos o contencioso-administrativos relacionados con estos derechos u otros de
diferente naturaleza; acciones y procesos que promovamos o se nos promuevan; y c) para

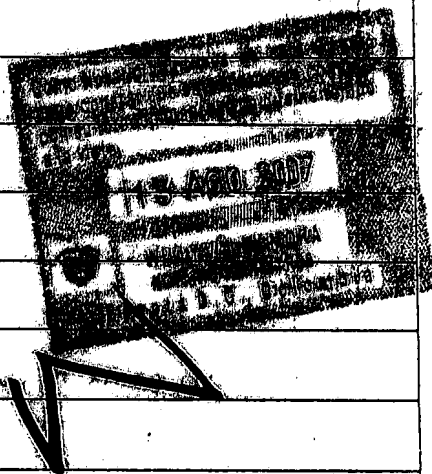
Como Notario Ventitas de
hago constar que esta fotocopia
es la copia auténtica
a la vista
13 AGO 1987
MILY CORREA
NOTARIA SEGUNDA DE CALI

4539
0523

LUZ MERY CORREA
ELABORACION DE ESCRITURAS
Notaría 3ª de Cali

Se agregan comprobantes. Derechos \$30.000.00 Decreto 1681 de Septiembre 16 de 1996. Reformado mediante Resolución No. 5839 de Diciembre 27 del año 2.000 y Decreto 1326 de Julio 06 del año 2.001. Se corrió en la hoja número AA 3286877. ENMIENDA- DO "OCTUBRE" VALE.

En rep. de TECNOQUIMICAS S.A.



Dr. JORGE ENRIQUE CAICEDO ZAMORANO

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -



RECIBO DE CAJA

No. 12 - 0089494

Bogotá D.C., Agosto 30 de 2012 - 16:09:46

RECIBIDO DE : REYES Y REYES ABOGADOS NI 900.048.328

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	144638793	29/08/2012	945.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-04 PRESENTACION DE OBSERVACIONES A SOLICITUDES	12 MARCAS Y LEMAS COMERCIALES	315.000.00	315.000.00
				\$315.000.00
=====				

SON: **TRESCIENTOSQUINCE MIL PESOS MONEDA CORRIENTE***

Responsable:

Recibo de Caja Aplicado al Expediente No.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-091445- -00010-0000

Fecha: 2012-08-30 17:01:39 Dep. 2020 DIR.NUEVASCR
Tra 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 33

Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C.- Colombia

Web: www.sic.gov.co e-mail: info@sic.gov.co Conmutador: (571) 5870000 Fax: (571) 5870284 Línea: 018000-910165 Call Center: (571) 6513240

Agosto 30 de 2012 - 16:09:47

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -



RECIBO DE CAJA

No. 12 - 0089497

Bogotá D.C., Agosto 30 de 2012 - 16:10:24

RECIBIDO DE : REYES Y REYES ABOGADOS NI 900.048.328

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	206068365	29/08/2012	300.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-08 PRORROGA DE TERMINOS	1609 S.DISTINTIVOS PRORROGA DE TERMINOS O PLAZO ADICIONAL	100.000.00	100.000.00
				\$100.000.00
=====				

SON: **Cien MIL PESOS MONEDA CORRIENTE***

Responsable

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-091445- -00010-0000

Fecha: 2012-08-30 17:01:39 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 400 OPOSIOBSERVTER Folios: 33

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Agosto 30 de 2012 - 16:10:24