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DIRECCION DE NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Trámite : **011** Evento : **378** Actuación : **449**

Referencia : Expediente No. **10-091445**
Asunto : Solicitud de patente para PREPARACIÓN
ESTABLE QUE CONTIENE ANTICUERPOS, QUE
COMPRENDE ARGININA Y METRONINA
Solicitante : CHUGAI SEIYAKU KABUSHIKI KAISHA y F.
HOFFMANN-LA ROCHE AG
Opositor : TECNOQUÍMICAS S.A.

I. ALEGATOS CONTESTACIÓN OPOSICIÓN

Yo, Luz Clemencia de Páez, en mi condición de apoderada de las sociedades solicitantes CHUGAI SEIYAKU KABUSHIKI KAISHA y F. HOFFMANN-LA ROCHE AG, por el presente escrito doy respuesta a la oposición formulada a nombre de la sociedad TECNOQUÍMICAS S.A. contra la solicitud de patente para la invención que se tramita en el expediente de la referencia.

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

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II. CONTESTACIÓN DENTRO DEL TÉRMINO LEGAL

El Oficio N° 22122, que dispone el traslado de la oposición formulada fue notificado por fijación en lista el 11 de diciembre de 2012, por lo que el término concedido de 60 días para presentar el correspondiente escrito de defensa venció el 12 de marzo de 2013 (teniendo en cuenta que la Superintendencia de Industria y Comercio suspendió términos los días 24 y 31 de diciembre de 2012); sin embargo, dado que el día 5 de marzo solicité una prórroga del término, esta expira el 17 de junio de 2013 (teniendo en cuenta que la Superintendencia de Industria y Comercio suspendió términos los días 26 y 27 de marzo de 2013), de manera que la oposición está siendo contestada en término legal.

III. RESPUESTA A LA PETICIÓN

Me opongo a las peticiones presentadas a nombre de la sociedad TECNOQUÍMICAS S.A., y en consecuencia solicito se declare infundada la oposición formulada en su nombre y se otorgue el privilegio de patente para la invención denominada PREPARACIÓN ESTABLE QUE CONTIENE ANTICUERPOS, QUE COMPRENDE ARGININA Y METRONINA tramitada bajo el expediente 10-091445.

IV. FUNDAMENTO FÁCTICO DE LA OPOSICIÓN

Fundamenta el apoderado de la sociedad TECNOQUÍMICAS S.A. su petición, en los siguientes argumentos principales:

4.1. La invención solicitada carece de los requisitos de NOVEDAD y NIVEL INVENTIVO, teniendo en cuenta la materia revelada "entre otros", en los siguientes documentos:

- WO 2003/072060 del 4 de septiembre de 2003
- WO 2005/049078 del 2 de junio de 2005
- US 2003/190316 del 9 de octubre de 2003

4.2. La solicitud tampoco cumple el requisito 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."

4.3. En opinión del apoderado de la sociedad opositora "la solicitud colombiana en trámite no presenta pleno sustento en la descripción."

4.4. Adicionalmente señala que, algunas de las reivindicaciones no se refieren a un método o procedimiento sino sobre una función.

4.5. Finalmente señala que una persona versada en la materia podría haber llegado al resultado de la presente invención, con base en las enseñanzas de los documentos citados como fundamento de la oposición.

V. RESPUESTA A LOS HECHOS Y ALEGATOS

Tal y como se demostrará en desarrollo de las presentes alegaciones, los argumentos de la sociedad opositora para que se niegue el privilegio de la patente solicitada por mis representadas en el presente asunto carecen de sustento fáctico y jurídico válido, por lo que la oposición formulada en su nombre no tiene vocación de prosperar.

En efecto, en primer lugar considero pertinente llamar la atención de este Despacho sobre el hecho que el día 25 de febrero de 2013 presenté a nombre de mis poderdantes un nuevo capítulo reivindicatorio conformado por 18 reivindicaciones.

Tal y como este Despacho podrá constatar de conformidad con la revisión de las piezas procesales que obran en el expediente de la referencia, la oposición formulada a nombre de la sociedad TECONQUÍMICAS S.A. fue radicada el 30 de agosto de 2012; así, es evidente de entrada que, tal y como fueron expuestos los argumentos de la parte opositora en este asunto, estos no resultan aplicables teniendo en cuenta el nuevo capítulo reivindicatorio presentado a consideración de este Despacho, como se ha destacado en el párrafo anterior.

En segundo lugar considero pertinente destacar que, tal y como se mencionó en la solicitud de examen de patentabilidad presentada el 21 de septiembre de 2012, siendo corroborado en el escrito de defensa presentado el 25 de febrero de 2013 en contra de la oposición formulada a nombre de la sociedad LABORATORIOS SYNTHESIS S.A.S., la Oficina

Europea de Patentes profirió una decisión de intención de concesión de la patente solicitada en el expediente de la referencia; como prueba de este hecho, se presentó copia de la respectiva publicación EP 2 238 985 BI.

Conviene destacar al respecto, que la decisión de concesión de la patente ya fue proferida, y una copia de la misma se está adjuntando al presente memorial como prueba para que sea tomada en cuenta al momento de ser tomada una decisión en este asunto.

Sobre el particular, llamo respetuosamente la atención de este Despacho sobre el hecho que el capítulo reivindicatorio que fue concedido por la Oficina de Patentes Europea, es el mismo que se ha puesto a consideración de este Despacho en este asunto, y en el cual sólo se han eliminado aquellas reivindicaciones que no se consideran procedentes conforme a la práctica de esta Superintendencia, esto es, las reivindicaciones 14-16.

Por lo tanto comedidamente solicito que, en aplicación de las disposiciones pertinentes, sea tenido en cuenta el antecedente anteriormente mencionado como prueba de la patentabilidad de la invención de mis representadas, y por lo tanto se otorgue el privilegio solicitado en el expediente de la referencia.

Lo anterior, teniendo en cuenta además los argumentos que tuvieron ocasión de exponerse en el memorial de defensa presentado el 25 de febrero de 2013 en contra de la oposición

formulada a nombre de la sociedad LABORATORIOS SYNTHESIS S.A.S., al cual se ha hecho referencia con anterioridad, y que resultan perfectamente aplicables al caso que nos ocupa, en el cual claramente se señaló lo siguiente:

“La presente invención hace referencia a una formulación líquida que contiene anticuerpos, que comprende de 40 a 1000 mM de arginina y de 10 a 200 mM de metionina.

Esta invención también hace referencia a un método para inhibir la dimerización de las moléculas de un anticuerpo en una formulación líquida que contiene el mismo, incluyendo la adición de arginina y metionina a la formulación líquida.

La presente invención es novedosa porque nada en el estado de la técnica revela o sugiere una formulación farmacéutica líquida que contenga un anticuerpo como ingrediente activo y que además contenga tanto arginina como metionina o un método para inhibir la dimerización de las moléculas de un anticuerpo en una formulación líquida por adición de arginina y metionina a la formulación líquida.

Adicionalmente, la invención solicitada por mis representadas cumple con el requisito de nivel inventivo, ya que proporciona un efecto técnico inesperado, y describe un efecto sinérgico ejercido por la presencia de los dos aminoácidos.

Tal y como se describe en el capítulo del “Problema que se resolverá por medio de la invención”, la presente

invención está dirigida a la provisión de una formulación líquida que contiene un anticuerpo, en donde se inhibe la dimerización y la desamidación de las moléculas del anticuerpo durante el almacenamiento a largo plazo.

Los inventores realizaron una serie de pruebas de aceleración de calor y luz para evaluar la estabilidad de almacenamiento de una formulación líquida que contiene un anticuerpo, y obtuvieron unos resultados en donde la arginina es un estabilizador efectivo contra la dimerización de las moléculas del anticuerpo y que la arginina combinada con metionina ejerce un efecto sinérgico.

Adicionalmente, el uso combinado de estos dos aminoácidos como aditivos a la formulación líquida que contiene anticuerpos se describe como un segundo aspecto de la presente invención.

En el capítulo descriptivo se revelan rangos específicos de concentración de aminoácidos, como por ejemplo "...En los casos donde la formulación líquida que contiene anticuerpos de acuerdo con la presente invención contiene arginina y metionina, la concentración total de arginina y metionina es preferiblemente 50 a 1200 mM, por ejemplo, preferiblemente, la concentración de arginina es 40 a 1000 mM y la concentración de metionina es 10 a 200 mM, más preferiblemente, la concentración de arginina es 50 a 700 mM y la concentración de metionina es 10 a 100 mM ...". Los rangos de concentración en las reivindicaciones corresponden con las modalidades descritas en la descripción.

Adicionalmente, el efecto sinérgico se muestra a partir de los resultados del Ejemplo 1 y el Ejemplo 3 en la descripción."

Por los anteriores hechos, es evidente que no existe anterioridad alguna en el estado del arte, "que revele o sugiera que la arginina actúa para estabilizar en contra de la dimerización y la desamidación de las moléculas del anticuerpo, y mucho menos que la arginina, combinada con la metionina, ejerce algún efecto sinérgico."

Así las cosas se puede concluir sin lugar a dudas, que la concesión de la patente que se solicita en este asunto resulta procedente conforme a los parámetros previstos en los artículos 16 y 18 de la Decisión 486 de la Comisión de la Comunidad Andina, en tanto la invención solicitada por mi representada en este asunto no está comprendida en ninguno de los documentos citados como existentes en el estado del arte, y tampoco habría podido ser derivada de manera obvia y evidente de las enseñanzas de dicho documentos.

Precisamente en relación con el requisito de NOVEDAD, considero pertinente citar las siguientes consideraciones del Tribunal Andino en la sentencia de interpretación prejudicial del Proceso 66-IP-2012:

"(...) para que una invención sea novedosa se requiere que no esté comprendida en el estado de la técnica, es decir, que antes de la fecha de presentación de la solicitud de patente o de la

prioridad reconocida, ni la innovación ni los conocimientos técnicos que de ella se desprenden hayan sido accesibles al público, entendiendo que la difusión de la información que se menciona debe ser detallada y suficiente para que una persona versada en la materia pueda utilizar esa información a fin de explotar la invención.”

Tal y como este Despacho podrá advertir, la opositora no efectuó ningún análisis que permita demostrar que en efecto la invención solicitada por mis representadas “esté comprendida” en los documentos citados del estado del arte previo.

Tampoco demostró que la invención solicitada ni los conocimientos técnicos que de ella se desprenden hubieran sido accesibles al público, ni que información detallada y suficiente de la misma hubiera sido revelada para que una persona versada en la materia la hubiera explotado.

Adicionalmente, es de tener en cuenta que dentro de los parámetros que esta Superintendencia ha fijado para analizar la novedad de una invención, “no se puede combinar elementos de más de un documento del estado de la técnica.”

En efecto, se requiere un análisis de las características técnicas de la invención, en comparación con las de por lo menos uno de los documentos citados del arte previo, que permita llevar al examinador al convencimiento que lo revelado por la invención solicitada ya se encontraba

en el estado del arte, o que por lo menos información detallada y suficiente de la misma ya había sido revelada.

Adicionalmente, no quedó plenamente demostrado ninguno de los presupuestos que exige la norma para determinar si una invención es patentable o no por cumplir o carecer de nivel inventivo, esto es:

- ✓ Haber demostrado que una persona normalmente versada en la materia técnica correspondiente hubiese podido llegar a la invención de mis representadas,
- ✓ Haber demostrado que la invención hubiese resultado obvia y evidente del estado de la técnica

Es más, de acuerdo igualmente con los parámetros que esta Superintendencia ha fijado para analizar el nivel inventivo de una invención, "El máximo de documentos que puede combinarse para examinar nivel inventivo, es dos", y que "En caso de que haya varios documentos relacionados, el estado de la técnica cercano es el documento que menciona una función, propósito, problema a resolver o actividad semejante al de la invención."

Así, conforme los anteriores parámetros, se demuestra claramente que no pueden ser consideradas como prueba idónea y suficiente las afirmaciones de la sociedad opositora en el sentido que la invención solicitada carece de nivel inventivo.

En efecto, se requiere demostrar que al menos uno de los documentos citados haya revelado información tal que permita que una persona normalmente versada en la materia técnica correspondiente hubiese podido llegar a la misma invención, o que de la información del documento o documentos citados se hubiese podido derivar la invención de manera **obvia y evidente**.

Sobre el particular, vale la pena destacar lo señalado en el Manual para la solicitud de patentes de invención de esta Superintendencia, según el cual:

“La invención no tiene nivel inventivo cuando la combinación de los elementos que proveen la solución está revelada en el estado de la técnica, es decir, no basta con la existencia de los elementos de manera aislada o individualmente considerados para concluir la falta de nivel inventivo, sino que ellos en su totalidad e integridad enseñen de manera evidente la manera de llegar a la solución del problema técnico.”

Finalmente, en cuanto al argumento de falta de unidad de invención que señala el opositor en su escrito, me permito respetuosamente llamar la atención de este Despacho sobre el hecho que cualquier persona versada en la materia sabe que la arginina y la metionina son aminoácidos y los mismos tienen unas propiedades físico-químicas muy similares; precisamente por el hecho de compartir esas características pueden configurar un efecto técnico muy similar y por consiguiente si hay unidad de invención en la patente solicitada por mi representada en este asunto.

Por lo tanto, ha quedado así demostrado que la oposición presentada a nombre de la sociedad TECNOQUÍMICAS S.A. deberá ser declarada infundada.

VI. PETICIONES

Por los anteriores hechos, solicito respetuosamente a este Despacho proceder a:

- 6.1. Declarar infundada la oposición presentada a nombre de la sociedad TECNOQUÍMICAS S.A.
- 6.2. Reconocer que la invención denominada PREPARACIÓN ESTABLE QUE CONTIENE ANTICUERPOS, QUE COMPRENDE ARGININA Y METRONINA cuyo privilegio de patente se solicita en el expediente de la referencia, cumple los requisitos de NOVEDAD, NIVEL INVENTIVO y APLICACIÓN INDUSTRIAL.
- 6.3. Conceder el privilegio de patente para la invención denominada PREPARACIÓN ESTABLE QUE CONTIENE ANTICUERPOS, QUE COMPRENDE ARGININA Y METRONINA, a nombre de mis representadas, las sociedades CHUGAI SEIYAKU KABUSHIKI KAISHA y F. HOFFMANN-LA ROCHE AG, que se tramita en el presente asunto.

VII. PRUEBAS

Comendidamente solicito se tengan como pruebas en este asunto, todos y cada uno de los documentos que obran en el expediente de la referencia, y adicionalmente la siguiente:

- ✓ Copia de la decisión de la Oficina Europea de Patentes, por la cual se concede el privilegio de patente de la invención cuya solicitud se tramita en este asunto.

VIII. ANEXOS

Me permito adjuntar al presente escrito, copia de la decisión de la Oficina Europea de Patentes por la cual se concede el privilegio de patente de la invención solicitada en el expediente de la referencia.

IX. NOTIFICACIONES

Mi representada y yo recibiremos notificaciones en la Secretaría de su Despacho o en mi oficina de abogado ubicada en la carrera 4ª N° 72 - 35 de esta ciudad de Bogotá, D.C.

Señor Director,

LUZ CLEMENCIA DE PÁEZ
LUZ CLEMENCIA DE PÁEZ
C.C. 35.456.344
T.P.A. 23.555

COLO

COLO 17753-841-001-6

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SCT/SCT



(11) **EP 2 238 985 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

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(54) **HIGH CONCENTRATION ANTIBODY-CONTAINING LIQUID FORMULATION**
HOCHKONZENTRIERTE ANTIKÖRPERHALTIGE FLÜSSIGE FORMULIERUNG
PRÉPARATION DE SOLUTION CONTENANT UN ANTICORPS À HAUTE CONCENTRATION

- | | |
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RO SE SI SK TR</p> <p>(30) Priority: 27.12.2007 JP 2007336310</p> <p>(43) Date of publication of application:
13.10.2010 Bulletin 2010/41</p> <p>(73) Proprietors:
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EP-A1- 1 977 763 WO-A1-02/13860
WO-A1-2004/091658 WO-A2-03/072060
JP-A- 2007 204 498 JP-T- 2005 527 503
JP-T- 2007 511 566 US-A1- 2004 191 243</p> |
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Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 2 238 985 B9

EP 2 238 985 B9

Description

TECHNICAL FIELD

5 [0001] The present invention relates to an antibody-containing formulation, and particularly, to a stable liquid formulation containing a high concentration of an antibody.

BACKGROUND ART

10 [0002] In recent years, various antibody formulations have been developed and used in practice. Many such antibody formulations are used in intravenous injection. However, due to needs of a clinical site, there is an increasing demand for development of an antibody-containing formulation that can be administered as a self-injectable subcutaneous injection.

[0003] In designing an antibody-containing formulation for subcutaneous injection, since a dose of an antibody per administration is large (about 100 mg to 200 mg) and an amount of an injection solution is generally limited in subcutaneous injection, it is necessary to increase a concentration of an antibody in a liquid to be administered. In view of this, in many cases, high concentration formulations are used, which are prepared by the lyophilization-concentration technique, in which a lyophilized formulation is reconstituted in water having a volume smaller than that before lyophilization. However, a strong demand exists for a liquid formulation which does not require reconstitution, and which is easy to handle. Although an increase in a viscosity of a formulation due to addition of a cryoprotective agent such as a sugar in the production process of the lyophilized formulation is not preferred for formulations for subcutaneous injection, it is surmised that this problem could be avoided if the formulation were a liquid formulation.

[0004] Solutions containing a high concentration of an antibody tend to form solutions having a high viscosity due to macromolecular properties of proteins, and due to the intermolecular interactions of proteins. Further, in cases where a protein is stored in a form of a solution having a high concentration, problematic degradation occurs, which includes a generation of insoluble and/or soluble aggregates; and it is necessary to prevent such degradation. Especially, in antibody formulations, associations are likely to be formed and insoluble aggregates are likely to be generated in a liquid state. In cases where a liquid formulation is stored for a long time, a problem exists in that a bioactivity of antibody molecules is lost due to deamidation of amino acid residues such as asparagine residues.

30 [0005] There have been proposed various ideas for providing a stabilized formulation, in which loss of an active component is small even after the formulation is stored for a long period of time. Such formulations are produced by dissolving an active component and various additives in a buffer solution. However, for liquid formulations containing a high concentration of an antibody, there does not yet exist a technology that is sufficient to prevent dimerization and deamidation.

35 WO 2004/091658 and US 2004/0191243 disclose highly concentrated stable antibody formulations comprising e.g. arginine. WO 2007/074880 (see also EP 1977763) discloses antibody-containing lyophilized formulations comprising e.g. arginine to inhibit formation of dimers.

[0006] A need to provide a high concentration antibody-containing formulation exists, in which dimerization and deamidation during long-term storage are inhibited, and which is both stable and suitable for use in subcutaneous administration.

DISCLOSURE OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

45 [0007] An object of the present invention is to provide a high concentration antibody-containing liquid formulation, in which dimerization and deamidation during long-term storage are inhibited, and which is stable and suitable for use in subcutaneous administration.

50 MEANS FOR SOLVING THE PROBLEM

[0008] The present inventors conducted intensive study with a view to attaining the above object, and as a result, discovered that a stable high concentration antibody-containing liquid formulation can be provided by adding an amino acid, arginine or a salt thereof, as a stabilizer, to thereby complete the present invention.

55 [0009] That is, the present invention provides the following:

- (1) A stable antibody-containing liquid formulation, characterized by comprising 40 to 1000 mM arginine and 10 to 200 mM methionine.

(2) A method for inhibiting dimerization of molecules of an antibody in a liquid formulation containing the antibody, comprising adding arginine and methionine to the liquid formulation.

ADVANTAGES OF THE INVENTION

[0010] By the present invention, a liquid formulation containing a high concentration of an antibody is provided, with which reformulation by concentration by lyophilization is not necessary, and hence does not require reconstitution. The antibody-containing liquid formulation according to the present invention can be stored in a liquid state for a long time. Since the antibody-containing liquid formulation according to the present invention can be produced by a process not including a lyophilization step, addition of a sugar or the like as a cryoprotective agent is not necessary.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

Fig. 1 shows a typical chromatogram of Example 1.
 Fig. 2 shows evaluation results of the gel permeation chromatography (SEC) in Example 1.
 Fig. 3 shows evaluation results of the gel permeation chromatography (SEC) in Example 1.
 Fig. 4 shows a typical chromatogram of Example 2.
 Fig. 5 shows evaluation results of the ion exchange chromatography (IEC) in Example 2.
 Fig. 6 shows evaluation results of the ion exchange chromatography (IEC) in Example 2.
 Fig. 7 shows evaluation results of the gel permeation chromatography (SEC) in Example 3.
 Fig. 8 shows evaluation results of the ion exchange chromatography (IEC) in Example 3.

BEST MODE FOR CARRYING OUT THE INVENTION

[0012] The present invention will now be described in detail.

[0013] In the present invention, "antibody-containing liquid formulation" means a liquid formulation containing an antibody as an active component, which is prepared such that it can be administered to an animal such as human, and which is preferably produced by a process not including a lyophilization step.

[0014] The antibody-containing liquid formulation according to the present invention is a liquid pharmaceutical formulation containing an antibody at a high concentration, which preferably has an antibody concentration of not less than 50 mg/mL, more preferably not less than 100 mg/mL, still more preferably not less than 120 mg/mL, and yet more preferably not less than 150 mg/mL. It should be noted that a liquid formulation containing antibody at a concentration of 120 mg/mL or higher, or preferably 150 mg/mL or higher, has not been developed for commercial use. Namely, the present invention allows for the first time to put to use a liquid formulation containing antibody at this high concentration.

[0015] Further, considering the manufacturing process, the highest concentration of antibody in the liquid formulation according to the present invention may be typically 300 mg/mL, preferably 250 mg/mL and more preferably 200 mg/mL. Therefore, the antibody-containing liquid formulation according to the present invention preferably has an antibody concentration of from 50 to 300 mg/mL, more preferably from 100 to 300 mg/mL, still more preferably from 120 to 250 mg/mL, and yet more preferably from 150 to 200 mg/mL.

[0016] The antibody to be used in the present invention is not restricted as long as it binds to a desired antigen. The antibody can be either a polyclonal antibody or a monoclonal antibody, although a monoclonal antibody is preferred because an antibody having uniform properties can be produced stably.

[0017] A monoclonal antibody which can be used in the present invention includes not only monoclonal antibodies originated from an animal such as human, mouse, rat, hamster, rabbit, sheep, camel or monkey, but also includes artificially modified recombinant antibodies such as chimeric antibody, humanized antibody and bispecific antibody. The immunoglobulin class of the antibody is not restricted, and can be any of the classes including IgGs such as IgG1, IgG2, IgG3 and IgG4, IgA, IgD, IgE and IgM. Among these classes, IgG and IgM are preferred.

[0018] The antibody which can be used in the present invention includes not only whole antibodies, but also antibody fragments such as Fv, Fab and F(ab)₂; and low molecular weight antibodies such as single chain Fv (scFv, sc(Fv)₂, diabodies such as scFv dimer) having one or more specificities, prepared by binding the variable regions of an antibody through a linker such as a peptide linker.

[0019] The above-described antibodies which can be used in the present invention can be prepared by methods well known to those skilled in the art.

[0020] A hybridoma producing a monoclonal antibody can be prepared as follows basically utilizing a known technique. That is, the hybridoma can be prepared by immunizing an animal with a desired antigen or cells expressing the desired antigen as a sensitizing antigen by a standard method; fusing the obtained immunocytes with known parent cells by a

standard cell-fusion method; and screening a monoclonal antibody-producing cell (hybridoma) by a standard screening method. Preparation of a hybridoma can be carried out by, for example, the method according to the method by Milstein et al (Kohler, G. and Milstein, C., Methods Enzymol. (1981) 73: 3-46). In cases where the immunogenicity of the antigen is low, the antigen can be bound to an antigenic macromolecule such as albumin, and the resulting conjugate can be used as an immunogen.

[0021] Recombinant antibodies can be employed, which are prepared by the genetic recombination technique in which an antigen gene is cloned from a hybridoma, incorporating the gene into an appropriate vector, introducing the vector into a host, and making the host produce the antibody (see, for example, Carl, A. K. Borrebaeck, James, W. Larrick, THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990). More specifically, a cDNA encoding the variable region (V region) in the antibody is synthesized from the mRNA of a hybridoma using a reverse transcriptase. If a DNA encoding the V region of the desired antibody is obtained, the DNA is then ligated to a DNA encoding the constant region (C region) of a desired antibody, and the resulting ligated DNA is introduced into an expression vector. Alternatively, a DNA encoding the V region of the antibody can be incorporated into an expression vector containing the DNA encoding the C region of the antibody. The DNA is incorporated into the expression vector such that the DNA is expressed under the control of an expression-controlling region such as enhancer or promoter. Host cells are then transformed with the resulting expression vector, and the antibody can be expressed by the host cells.

[0022] In the present invention, recombinant antibodies artificially modified for the purpose of reducing the heteroantigenicity to human, such as chimeric antibodies and humanized antibodies can be used. These modified antibodies can be produced by known methods. A chimeric antibody is an antibody comprising variable regions in the heavy chain and light chain in an antibody of an animal other than human, such as mouse, and constant regions in the heavy chain and light chain in an antibody of human, and can be obtained by ligating a DNA encoding the variable region in the mouse antibody with a DNA encoding the constant region in the human antibody, incorporating the obtained DNA into an expression vector, introducing the expression vector into a host, and making the host produce the antibody.

[0023] Humanized antibody is also called reshaped human antibody, and is obtained by transplanting the CDR (complementarity determining region) of, for example, a mouse antibody to the complementarity determining region of a human antibody. A standard genetic recombination technique for preparing the humanized antibody is also known. Specifically, a DNA designed such that the CDR of the mouse antibody and the framework region (FR) of the human antibody are ligated is synthesized by PCR method from several oligonucleotides prepared so as to have overlapping regions at their terminals. The obtained DNA is ligated to a DNA encoding the constant region of a human antibody, and the resulting DNA is introduced into an expression vector. The expression vector is introduced into a host, and the host is made to produce the humanized antibody (see EP 239400 A and WO 96/02576). As the FR of the human antibody to be ligated through CDR, one of which complementarity determining region forms a good antigen-binding site is selected. As required, an amino acid(s) in the complementarity determining region can be substituted so that the complementarity determining region of the reshaped human antibody forms an appropriate antigen-binding site (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

[0024] Methods for obtaining a human antibody are known in the art. For example, a desired human antibody having a binding activity to a desired antigen can be obtained by sensitizing, *in vitro*, human lymphocytes with the desired antigen or with the cells expressing the desired antigen; fusing the sensitized lymphocytes with human myeloma cells, for example, U266 cells; and obtaining the antibody from the cells (see JP 1-59878 B). The desired human antibody can also be obtained by immunizing a transgenic animal having all repertoires of human antibody genes with the antigen (see WO 93/12227, WO 92/03918, WO 94/02602, WO 94/25585, WO 96/34096 and WO 96/33735). Further, a technique by which a human antibody is obtained by panning using a human antibody library is also known.

For example, a variable region of a human body is expressed in the form of a single chain antibody (scFv) on the surface of a phage by use of a phage display method, and the phage which binds to the antigen can be selected. By analyzing the gene of the selected phage, the DNA sequence coding for the variable region of the human antibody which binds to the antigen can be determined. If the DNA sequence of the scFv which binds to the antigen is determined, an appropriate expression vector containing the sequence is constructed, and the humanized antibody can be obtained. These methods are well known, and WO 92/01047, WO 92/20791, WO 93/06213, WO 93/11236, WO 93/19172, WO 95/01438 and WO 95/15388 can be referred to.

[0025] In cases where an antibody gene is once isolated, and the gene is introduced into an appropriate host so as to prepare the antibody, appropriate combinations of the host and expression vector can be used. In cases where eukaryotic cells are used as the host, animal cells, plant cells and fungal cells can be used. Known animal cells include (1) mammalian cells, for example, CHO, COS, myeloma, BHK (baby hamster kidney), Hela and Vero; (2) amphibian cells, for example, Xenopus oocytes and (3) insect cells, for example, sf9, sf21 and Tn5. Known plant cells include cells originated from plants belonging to the genus *Nicotiana*, for example, *Nicotiana tabacum*, and the cells can be subjected to callus culture. Known fungal cells include the cells originated from yeasts, for example, those belonging to the genus *Saccharomyces* such as *Saccharomyces cerevisiae*; and filamentous bacteria, for example, those belonging to the

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genus *Aspergillus* such as *Aspergillus niger*. In cases where prokaryotic cells are used, there are production systems using bacterial cells. Known bacterial cells include *E. coli* cells and *Bacillus subtilis* cells. The antibody is obtained by introducing a desired antibody gene into these cells by transformation, and culturing the transformed cells *in vitro*.

5 [0026] Antibodies in the form of antibody fragments, low molecular weight antibodies and modified antibodies can also be employed as the antibody in the present invention. Examples of the antibody fragments and low molecular weight antibodies include Fab, F(ab')₂, Fv, and single chain Fv (scFv, sc(Fv)₂ and the like) having one or more specificities, prepared by ligating the Fvs in the H-chain and L-chain through an appropriate linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883). Specifically, an antibody is treated with papain or pepsin to generate antibody fragments, or a gene encoding these antibody fragments is constructed, and the gene is expressed in appropriate host cells after introducing the gene into an expression vector (see, for example, Co, M. S. et al., T. Immunol. (1994) 152, 2968-2976; Better, M. and Horwitz, A. H., Methods Enzymol. (1989) 178, 476-496; Pluckthun, A. and Skerra, A., Methods Enzymol. (1989) 178, 497-515; Lamoyi, E., Methods Enzymol. (1986) 121, 652-663; Rousseaux, J. et al., Methods Enzymol. (1986) 121, 663-669; Bird, R. E. and Walker, B. W., Trends Biotechnol. (1991) 9, 132-137.

10 [0027] Antibodies bound to various molecules such as polyethylene glycol (PEG) can also be used as modified antibodies. The term "antibody" used in the present invention also includes these modified antibodies. These modified antibodies can be obtained by chemically modifying an obtained antibody. Methods for carrying out the modifications are established in the art.

[0028] Examples of the antibody contained in the formulation according to the present invention include, but not limited to, anti-tissue factor antibodies, anti-IL-6 receptor antibodies, anti-IL-6 antibodies, HM1.24 antigen monoclonal antibodies, anti-parathyroid hormone-related peptide antibodies (anti-PTHrP antibodies), anti-glypican-3 antibodies, anti-ganglioside GM3 antibodies, anti-TPO receptor antagonist antibodies, factor VIII-substituting antibodies, anti-CD3 antibodies, anti-CD20 antibodies, anti-GPIIb/IIIa antibodies, anti-TNF antibodies, anti-CD25 antibodies, anti-EGFR antibodies, anti-Her2/neu antibodies, anti-RSV antibodies, anti-CD33 antibodies, anti-CD52 antibodies, anti-IgE antibodies, anti-CD11a antibodies, anti-VEGF antibodies, anti-VLA4 antibodies, anti-AXL antibodies, and so on.

25 [0029] Preferred examples of the reshaped human antibodies used in the present invention include humanized anti-interleukin (IL-6) receptor antibodies (hPM-1 or MRA) (see WO 92-19759), humanized anti-HM1.24 antigen monoclonal antibodies (see WO 98-14580), humanized anti-parathyroid hormone-related peptide antibodies (anti-PTHrP antibodies) (see WO 98-13388), humanized anti-tissue factor antibodies (see WO 99-51743) and anti-glypican-3 humanized IgG1 κ antibodies (see PCT/JP05/013103). The humanized antibodies especially preferred in the present invention are humanized anti-IL-6 receptor antibodies.

30 [0030] As the human IgM antibodies, anti-ganglioside GM3 recombinant human IgM antibodies (see WO 05-05636) and the like are preferred.

[0031] As the low molecular weight antibodies, anti-TPO receptor antagonist diabodies (see WO 02-33072), anti-CD47 agonist diabodies (see WO 01-66737) and the like are preferred.

35 [0032] To evaluate the shelf stability of the high concentration antibody-containing liquid formulation, the present inventors studied the effects of various additives by conducting heat acceleration tests and light acceleration tests. As a result, it was found that in solutions in which a high concentration of antibody was dissolved in a buffer solution containing the amino acid arginine, the amount of generated dimer was smaller than that in solutions to which arginine was not added. From these results, it was found that arginine is effective as a stabilizer for inhibiting dimerization. Further, in solutions in which a high concentration of antibody was dissolved in a buffer solution containing arginine and methionine, the inhibitory effect against dimerization was observed at a total concentration of arginine and methionine which is lower than the concentration of arginine alone needed for attaining the same inhibitory effect. From these results, it was found that a synergistic effect is obtained by the addition of arginine and methionine in combination. Further, it was found that deamidation of the antibody molecules is inhibited by the addition of arginine. These results are exemplified as test results obtained for a sample containing a humanized anti-IL-6 receptor antibody at a concentration of 180mg/ml.

40 [0033] Thus, by adding arginine as a stabilizer, a stable antibody formulation can be provided, in which dimerization of the antibody is reduced and deamidation of the antibody is prevented.

[0034] Further, as described above, an antibody-containing liquid formulations of the present invention additionally contains methionine in the solution, with a synergistic effect being obtained by use of arginine and methionine in combination. Therefore, an aspect of the present invention is characterized by adding arginine and methionine to a solution, whereby dimerization in particular, of the antibody molecules is inhibited in the resulting antibody-containing liquid formulation. Accordingly, an embodiment as a stable antibody-containing liquid formulation is characterized in that it contains an antibody, arginine and methionine in a buffer solution.

50 [0035] As the arginine used in the present invention, any of the arginine compound *per se*, derivatives thereof and salts thereof can be used. L-arginine and salts thereof are preferred. As the methionine used in the present invention, any of the methionine compound *per se*, derivatives thereof and salts thereof can be used. L-methionine and salts thereof are preferred.

55 [0036] In cases where the antibody-containing liquid formulation according to the present invention contains arginine

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and methionine, the total concentration of arginine and methionine is preferably 50 to 1200 mM; more preferably, the arginine concentration is 50 to 700 mM and the methionine concentration is 10 to 100 mM; and still more preferably, the arginine concentration is 100 to 300 mM, and the methionine concentration is 10 to 50 mM

[0037] The buffer solution is prepared using a buffering agent which is a substance for maintaining a pH of the solution. In a high concentration antibody-containing liquid formulation according to the present invention, a pH of the formulation is preferably 4 to 8, more preferably 5.0 to 7.5, still more preferably 5.5 to 7.2, and still more preferably 6.0 to 6.5. A buffering agent which can be used in the present invention is one which can adjust the pH in this range and which is pharmaceutically acceptable. Such a buffering agent is known by those skilled in the art, and examples thereof include inorganic salts such as phosphoric acid salts (sodium or potassium) and sodium hydrogen carbonate; organic acid salts such as citric acid salts (sodium or potassium), sodium acetate and sodium succinate; and acids such as phosphoric acid, carbonic acid, citric acid, succinic acid, malic acid and gluconic acid. Further, Tris buffers, Good's buffers such as MES, MOPS and HEPES, histidine (e.g., histidine hydrochloric acid salt) and glycine can also be used. In the high concentration antibody-containing liquid formulation according to the present invention, the buffer is preferably a histidine buffer or glycine buffer, and a histidine buffer is especially preferred. The concentration of the buffer solution is generally 1 to 500 mM, preferably 5 to 100 mM, still more preferably 10 to 20 mM. In cases where a histidine buffer is used, the buffer solution contains histidine at a concentration of preferably 5 to 25 mM, more preferably 10 to 20 mM.

[0038] For the "stable" high concentration antibody-containing liquid formulation according to the present invention, significant change is not observed when it is stored at a refrigeration temperature (2 to 8°C) for at least 12 months, preferably for 2 years, and more preferably, for 3 years; or when it is stored at room temperature (22 to 28°C) for at least 3 months, preferably 6 months, and more preferably 1 year. For example, sum amount of dimers and degradation products in the formulation when it is stored at 5°C for 2 years is 5.0% or lower, preferably 2% or lower, and more preferably 1.5% or lower; or sum amount of dimers and degradation products in the formulation when it is stored at 25°C for 6 months is 5.0% or lower, preferably 2% or lower, and more preferably 1.5% or lower.

[0039] The formulation according to the present invention can further contain a surfactant.

[0040] Typical examples of the surfactant include nonionic surfactants, for example, sorbitan fatty acid esters such as sorbitan monocaprylate, sorbitan monolaurate and sorbitan monopalmitate; glycerin fatty acid esters such as glycerol monocaprylate, glycerol monomyristate and glycerol monostearate; polyglycerol fatty acid esters such as decaglycerol monostearate, decaglycerol distearate and decaglycerol monolinoleate; polyoxyethylene sorbitan fatty acid esters such as polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylenesorbitan monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene sorbitan trioleate and polyoxyethylene sorbitan tristearate; polyoxyethylene sorbitol fatty acid esters such as polyoxyethylene sorbitol tetrastearate and polyoxyethylene sorbitol tetra oleate; polyoxyethylene glycerin fatty acid esters such as polyoxyethylene glyceryl monostearate; polyethylene glycol fatty acid esters such as polyethylene glycol distearate; polyoxyethylene alkyl ethers such as polyoxyethylene lauryl ether; polyoxyethylene polyoxypropylene alkyl ethers such as polyoxyethylene polyoxypropylene glycol ether, polyoxyethylene polyoxypropylene propyl ether and polyoxyethylene polyoxypropylene cetyl ether; polyoxyethylene alkyl phenyl ethers such as polyoxyethylene nonylphenyl ether; polyoxyethylene hardened castor oils such as polyoxyethylene castor oil and polyoxyethylene hardened castor oil (polyoxyethylene hydrogenated castor oil); polyoxyethylene bees wax derivatives such as polyoxyethylene sorbitol bees wax; polyoxyethylene lanolin derivatives such as polyoxyethylene lanolin; surfactants having an HLB of 6 to 18 such as polyoxyethylene fatty acid amides, for example, polyoxyethylene octadecanamide; anionic surfactants, for example, alkyl sulfate salts having a C₁₀-C₁₈ alkyl group, such as sodium cetyl sulfate, sodium lauryl sulfate and sodium oleyl sulfate; polyoxyethylene alkyl ether sulfate salts in which the average number of moles of the added ethylene oxide units is 2 to 4 and the number of carbon atoms of the alkyl group is 10 to 18, such as polyoxyethylene sodium lauryl sulfate; alkyl sulfosuccinate salts having a C₈-C₁₈ alkyl group, such as sodium lauryl sulfosuccinate; natural surfactants such as lecithin and glycerophospholipids; sphingophospholipids such as sphingomyelin; and sucrose esters of C₁₂-C₁₈ fatty acids. These surfactants can be added to the formulation of the present invention individually, or two or more of these surfactants can be added in combination.

[0041] Preferred surfactants are polyoxyethylene sorbitan fatty acid esters and polyoxyethylene polyoxypropylene alkyl ethers, and especially preferred are polysorbates 20, 21, 40, 60, 65, 80, 81 and 85, and Pluronic type surfactants, and most preferred are polysorbates 20 and 80, and Pluronic F-68 (Poloxamer 188).

[0042] The amount of the surfactant(s) to be added to the antibody formulation according to the present invention is generally 0.0001 to 10% (w/v), preferably 0.001 to 5%, more preferably 0.005 to 3%.

[0043] In another aspect of the present invention, the formulation according to the present invention is preferably substantially composed of the following components:

- A) anti-IL-6 receptor antibody;
- B) arginine and methionine, and additional other amino acid(s) (e.g., tryptophan) as an optional additional component (s);
- C) buffering agent(s); and

D) surfactant(s).

[0044] The term "substantially composed of" herein means that a component other than the components usually added to formulations is not contained, the components usually added to formulations being the optional additive components described below, such as suspending agents, solubilizing agents, isotonic agents, preservatives, adsorption inhibitors, diluents, vehicles, pH-adjusters, soothing agents, sulfur-containing reducing agents and antioxidants.

[0045] The above-described "B) arginine and methionine, and additional other amino acid(s) (e.g., tryptophan) as an optional additional component(s)" is meant to include the cases where the formulation contains arginine and methionine and further include the cases where the formulation additionally contains other amino acid(s). Preferred example of the other amino acid(s) is tryptophan. As the tryptophan, any of the tryptophan compound *per se*, derivatives thereof and salts thereof can be used. L-tryptophan and salts thereof are preferred.

[0046] As required, a suspending agent, solubilizing agent, isotonic agent, preservative, adsorption inhibitor, diluent, vehicle, pH-adjuster, soothing agent, sulfur-containing reducing agent, antioxidant and the like can be added to the formulation according to the present invention.

[0047] Examples of the suspending agent include methyl cellulose, polysorbate 80, hydroxyethyl cellulose, gum arabic, powdered tragacanth, sodium carboxymethylcellulose and polyoxyethylene sorbitan monolaurate.

[0048] Examples of the solubilizing agent include, polyoxyethylene hydrogenated castor oil, polysorbate 80, nicotina-mide, polyoxyethylene sorbitan monolaurate, macrogol and castor oil fatty acid ethyl ester.

[0049] Examples of the isotonic agent include sodium chloride, potassium chloride and calcium chloride.

[0050] Examples of the preservative include methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, sorbic acid, phenol, cresol and chlorocresol.

[0051] Examples of the adsorption inhibitor include human serum albumin, lecithin, dextran, ethyleneoxide-propylene oxide copolymer, hydroxypropylcellulose, methyl cellulose, polyoxyethylene hydrogenated castor oil and polyethylene glycol.

[0052] Examples of the sulfur-containing reducing agent include the compounds having a sulfhydryl group(s), such as N-acetylcysteine, N-acetyl homocysteine, thiocetic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, glutathione and C₁-C₇ thioalkanes.

[0053] Examples of the antioxidant include erythorbic acid, dibutylhydroxytoluene, butylated hydroxyanisole, α -tocopherol, tocopherol acetate, L-ascorbic acid and salts thereof, L-ascorbyl palmitate, L-ascorbyl stearate, sodium hydrogen sulfite, sodium sulfite, triamyl gallate, propyl gallate, and chelating agents such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate and sodium metaphosphate.

[0054] The antibody-containing liquid formulation according to the present invention is usually administered through a parenteral route, for example, by injection (subcutaneous, intravenous, intramuscular injections or the like), percutaneous, transmucosal, transnasal or pulmonary administration, but it can also be administered orally. In subcutaneous injection, the dose of antibody per administration is large (about 100 to 200 mg) while the amount of the injection solution is limited, so that the formulation according to the present invention is especially suited for subcutaneous injection.

[0055] The osmotic pressure ratio of the antibody-containing liquid formulation according to the present invention is preferably about 0.5 to 4, more preferably about 0.7 to 2, and still more preferably about 1.

[0056] The viscosity of the antibody-containing liquid formulation according to the present invention is preferably about 2 to 15 mPa·s, more preferably about 4 to 10 mPa·s. It should be noted that the viscosity described herein is measured by a rotation viscometer method using a cone-plate type viscometer, in accordance with 2.53 Viscosity Determination / General Tests, the Japanese Pharmacopoeia, 15th edition.

[0057] As can be seen from the results of the examples described below, a stable liquid formulation can be obtained, in which dimerization and deamidation of the antibody during long-term storage are small, by adding to the formulation arginine alone, or arginine and methionine, or methionine alone.

[0058] As still another aspect of the present invention, a method for inhibiting dimerization of antibody in antibody-containing liquid formulations is provided, the method comprising adding to the formulation arginine and methionine.

[0059] In the above-described two methods, the antibody is preferably an anti-IL-6 receptor antibody, which is a humanized antibody or human antibody.

[0060] The present invention will now be described in more detail by way of the examples given below. However, the scope of the present invention is not restricted thereto.

[Examples]

Antibody Sample

[0061] The humanized anti-IL-6 receptor antibody was the humanized antibody prepared in accordance with the method described in Reference Example 2 in JP 8-99902 A using the human elongation factor 1 α promoter described

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in Example 10 in WO 92/19759. This antibody will occasionally be referred to as "MRA" in the tables in Examples.

EXAMPLE 1

Stabilizing Effects by Combination of Arginine and Methionine

[0062] Liquid formulations containing anti-IL-6 receptor humanized antibody were evaluated for an influence on stabilization of the formulations obtained by use of a combination of arginine and methionine.

[0063] In this study, to evaluate the effects by the combination of arginine and methionine, evaluation samples numbered A1 to A9 were prepared. Prescriptions for the evaluation samples were as follows:

[0064]

[Table 1-1]

[Prescriptions]						
Sample No.	Antibody mg/mL	Arg mM	Met mM	Polysorbate 80 mg/mL	Histidine buffer mM	pH
A1	180	-	-	0.5	20	6.0
A2	180	50	-	0.5	20	6.0
A3	180	100	-	0.5	20	6.0
A4	180	150	-	0.5	20	6.0
A5	180	200	-	0.5	20	6.0
A6	180	300	-	0.5	20	6.0
A 7	180	100	10	0.5	20	6.0
A8	180	100	30	0.5	20	6.0
A9	190	100	50	0.5	20	6.0

[0065] Samples A1 to A6 are comparative examples.

[0066] To evaluate stability of the liquid formulations, each sample was subjected to a heat acceleration test (stored at 40°C for 3 months and at 25°C for 6 months, respectively). The purity of the antibody before and after the heat acceleration test was evaluated by gel permeation chromatography (SEC). The analytical conditions were as follows:

[Gel Permeation Chromatography]

[0067] The sample was used as the solution to be measured as it was.

[0068] One microliter of the solution to be measured was subjected to liquid chromatography, and the peak areas of the peaks of dimer, monomer and low molecular weight degradation products (LMW) were measured by an automatic analytical method, and the amounts thereof (%) were determined.

[Table 1-2]

Analytical Conditions

[0069]

Column: TSKgel G3000SWx1 7.8 mm I. D. x 30 cm (TOSOH)
Mobile Phase: phosphate buffer, pH 7.0 (50 mmol/L phosphate buffer, pH 7.0, containing 300 mmol/L of sodium chloride and 0.05% sodium azide)
Amount of Injected Sample: about 180 µg in terms of humanized anti-IL-6 receptor antibody
Flow Rate: 1 mL/min
Detection Wavelength: 280 nm

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[Formula 1]

Calculation Equation

[0070]

Total Area of All Peaks = Peak Area of Monomer + Peak Area of Dimer + Peak
Area of Low Molecular Weight Degradation Products (LMW)

Amount of Dimer (%)= (Peak Area of Dimer/Total Area of All Peaks) x 100

Amount of Low Molecular Weight Degradation Products (LMW) (%) = (Peak Area
of Low Molecular Weight Degradation Products/ Total Area of All Peaks) x 100

A typical chromatography is shown in Fig. 1.

[0071] The evaluation results obtained by the gel permeation chromatography (SEC) are shown in Table 1 and Figs. 2 and 3. As shown, the amount of dimer in the samples (Sample Nos. A2 to A6) to which arginine was added, after the acceleration at 40°C for 3 months and at 25°C for 6 months, respectively, was smaller than that in the sample (Sample No. A1) to which arginine was not added; and accordingly, the inhibitory effect of arginine against dimerization was confirmed. It was also confirmed that the amount of dimer was reduced proportionally to the amount of the arginine added. On the other hand, the amount of dimer in the samples (Sample Nos. A7 to A9) to which arginine (100 mM) and methionine were added, after the acceleration at 40°C for 3 months and at 25°C for 6 months, respectively, was smaller than that in the samples (Sample Nos. A3 and A4) containing 150 mM of arginine, which concentration was about the same as the total concentration of the stabilizers; and the amount of dimer was about the same as in the sample (Sample No. A6) having an arginine concentration of 300 mM. These results are considered to indicate that a synergistic effect in the inhibition of dimerization is obtained by combining arginine and methionine.

[0072] Influence of arginine and methionine on the amount of low molecular weight degradation products was not observed.

[0073] [Table 1-3]

Table 1

	40°C-3months		25°C-6months	
	Dimer (%)	LMW (%)	Dimer (%)	LMW (%)
A1	2.70	1.25	1.88	0.48
42	2.19	1.24	1.41	0.47
A3	2.00	1.34	1.33	0.49
A4	1.85	1.38	1.19	0.49
A5	1.62	1.37	1.09	0.49
A6	1.53	1.46	0.99	0.50
A7	1.58	1.29	1.11	0.45
A8	1.52	1.21	1.07	0.47
A9	1.48	1.32	1.03	0.47

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EXAMPLE 2 (not according to the invention)

Inhibitory Effect by Arginine against Deamidation

[0074] Liquid formulations containing anti-IL-6 receptor humanized antibody were evaluated for influence on the deamidation by arginine.

[0075] In this study, evaluation samples numbered A10 to A15 and numbered A16 to A18, containing different amounts of arginine and methionine, respectively, were prepared. Prescriptions for the evaluation samples were as follows:

[0076]

[Table 2-1]

[Prescriptions]						
Sample No.	Antibody mg/mL	Arg mM	Met mM	Polysorbate 80 mg/mL	Histidine buffer mM	pH
A10	180	-	-	0.5	20	6.0
A11	180	50	-	0.5	20	6.0
A12	180	100	-	0.5	20	6.0
A13	180	150	-	0.5	20	6.0
A14	180	200	-	0.5	20	6.0
A15	180	300	-	0.5	20	6.0
A16	180	-	10	0.5	20	6.0
A17	180	-	30	0.5	20	6.0
A18	180	-	50	0.5	20	6.0

[0077] To evaluate the stability of the liquid formulations, each sample was subjected to a heat acceleration test (stored at 40°C for 3 months and at 25°C for 6 months, respectively). The purities of the antibody before and after the heat acceleration test were evaluated by ion-exchange chromatography (IEC). The analytical conditions were as follows:

[Ion-exchange Chromatography]

[0078] To each sample, purified water was added to adjust the amount of the humanized anti-IL-6 receptor antibody to about 1 mg in 1 mL of the sample, and the resulting sample was used as the sample to be measured.

[0079] Thirty microliters of the sample solution was subjected to liquid chromatography, and the peak areas of the peaks of MRA Pre, MRA Main, MRA Sub-1, MRA Sub-2, MRA R-1, 1Q(H)-MRA, 2Q(H)-MRA and other related substances (Others) were measured by an automatic analytical method, and the amounts thereof (%) were determined by an area percentage method.

[0080] MRA Pre indicates the total of the peaks of the substances each eluted after a retention time shorter than that of the main component, and a plurality of degradation products, mainly deamidation products of humanized anti-IL-6 receptor antibody, was included. When the production amount of this Pre peak was small, inhibition of deamidation of the antibody is indicated.

[Table 2-2]

Analytical Conditions

[0081]

Column: ProPac WCX-10 4 x 250 mm (DIONEX)
Mobile Phase: Solution A: 25 mmol/L MES buffer solution, pH 6.1
Mobile Phase: Solution B: 25 mmol/L MES buffer solution, pH 6.1 (containing 250 mmol/L of sodium chloride)
Amount of Injected Sample: about 30 µg in terms of humanized anti-IL-6 receptor antibody
Flow Rate: 0.5 mL/min
Detection Wavelength: 280 nm

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[0082]

[Formula 2]

Calculation Equation

Total Area of All Peaks = Grand Total of Total Area of MRA Pre Peaks + Peak
Area of MRA Main + Peak Area of MAR Sub-1 + Peak Area of MAR Sub-2 + Peak Area of
MAR Sub-3 + Peak Area of MAR R-1 + Total Area of 1Q(H)-MRA Peaks + Total Area of
2Q(H)-MRA Peaks + Peak Area of Others

Amount of MRA Pre (%) = (Total Area of MRA Pre Peaks/Total Area of All Peaks)
x 100

A typical chromatography is shown in Fig. 4. MRA Pre indicates the total of the peaks of the substances appearing earlier than that of the main component.

[0083] Evaluation results of the ion-exchange chromatography are shown in Table 2 and Figs. 5 and 6. As shown, the amount of Pre peaks in the samples (Sample Nos. A11 to A15) to which arginine was added, after the acceleration at 40°C for 3 months and at 25°C for 6 months, respectively, was smaller than that in the sample (Sample No. A10) to which arginine was not added; and accordingly, the inhibitory effect of arginine against the generation of Pre peaks was confirmed. It was also confirmed that the amount of Pre peaks was reduced proportionally to an amount of arginine added. On the other hand, the amount of Pre peaks in the samples (Sample Nos. A16 to A18) to which methionine was added, after the acceleration at 40°C for 3 months and at 25°C for 6 months, respectively, was similar to the sample (Sample No. A10) to which arginine was not added; and accordingly, influence of the addition of methionine was not observed.

[0084] [Table 2-3]

Table 2

	Pre peak (%)	
	40°C-3months	25°C-6months
A10	56.2	32.3
A11	51.3	30.3
A12	50.7	29.3
A13	49.0	28.7
A14	47.8	28.5
A15	47.0	27.9
A16	55.7	31.2
A17	55.0	31.2
A18	55.3	31.4

EXAMPLE 3

Stabilizing Effects by Combination of Arginine and Methionine (2)

[0085] As in Example 1, liquid formulations containing anti-IL-6 receptor humanized antibody were evaluated for

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influence on stabilization of the formulations obtained by use of a combination of arginine and methionine.
[0086] In this study, to evaluate effects of the combination of arginine and methionine, evaluation samples numbered A19 to A27 were prepared. Prescriptions for the evaluation samples were as follows:
[0087]

[Table 3-1]

[Prescriptions]						
Sample No.	Antibody mg/mL	Arg mM	Met mM	Polysorbate 80 mg/mL	Histidine buffer mM	pH
A19	180	-	-	0.5	20	6.0
A20	180	50	-	0.5	20	6.0
A21	180	100	-	0.5	20	6.0
A22	180	150	-	0.5	20	6.0
A23	180	200	-	0.5	20	6.0
A24	180	300	-	0.5	20	6.0
A25	180	100	10	0.5	20	6.0
A26	180	100	30	0.5	20	6.0
A27	180	100	50	0.5	20	6.0

Samples A19 to A24 are comparative examples.
[0088] To evaluate the stability of the liquid formulations, each sample was subjected to a light acceleration test (total illuminance 1,200,000 lux and total near-ultraviolet radiation energy: 200 W·h/m²). The purities of the antibody before and after the light acceleration test were evaluated by gel permeation chromatography (SEC) and ion exchange chromatography (IEC) as in Examples 1 and 2.
[0089] The evaluation results by the gel permeation chromatography (SEC) are shown in Table 3 and Fig 7. As shown, the amount of dimer in the samples (Sample Nos. A20 to A24) to which arginine was added, after the light acceleration test was smaller than that in the sample (Sample No. A19) to which arginine was not added; and accordingly, the inhibitory effect of arginine against dimerization was confirmed. It was also confirmed that the amount of dimer was reduced proportionally to an amount of arginine added. On the other hand, the amount of dimer in the samples (Sample Nos. A25 to A27) to which arginine (100 mM) and methionine were added, after the light acceleration test was smaller than that in the sample (Sample No. A22) containing 150 mM of arginine, which concentration was about the same as the total concentration of the stabilizers; and the amount of dimer was smaller than in the samples (Sample Nos. A23 and A24) having arginine concentrations of 200 mM and 300 mM, respectively. These results are thought to indicate that a synergistic effect in the inhibition of dimerization is obtained by combining arginine and methionine.
[0090] Influence of arginine and methionine on the amount of low molecular weight degradation products was not observed.
[0091] [Table 3-2]

Table 3

	1,200,000lux + 200 W -h/m ²	
	Dimer (%)	LMW (%)
A19	6.95	0.22
A20	6.75	0.24
A21	5.78	0.21
A22	5.08	0.19
A23	4.73	0.18
A24	4.13	0.18
A25	5.27	0.19

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(continued)

	1,200,000lux + 200 W · h/m ²	
	Dimer (%)	LMW (%)
A26	4.05	0.17
A27	3.84	0.16

[0092] Next, the evaluation results by the ion exchange chromatography (IEC) are shown in Table 4 and Fig 8.

[0093] As shown, the amount of Pre peak in the samples (Sample Nos. A20 to A24) to which arginine was added, after the light acceleration test was smaller than that in the sample (Sample No. A19) to which arginine was not added; and accordingly, the inhibitory effect of arginine against formation of Pre peak was confirmed. Further, it was confirmed that as the amount of arginine increases, the production amount of Pre peak decreases proportionately. On the other hand, the amount of dimer after the light acceleration test in the samples (Sample Nos. A25 to A27) to which methionine was further added to arginine (100 mM) was smaller than that in the sample (Sample No. A22) containing 150 mM of arginine, which concentration was about the same as the total concentration of the stabilizers; and it was smaller than in the samples (Sample Nos. A23 and A24) having arginine concentrations of 200 mM and 300 mM, respectively. These results are thought to indicate that a synergistic effect in the inhibition of formation of Pre peak by the combination of arginine and methionine.

[0094]

[Table 4]

	Pre peak (%)
	1,200,000lux + 200 W · h/m ²
A19	39.2
A20	38.6
A21	36.7
A22	35.7
A23	34.9
A24	34.9
A25	36.8
A26	35.0
A27	33.8

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Claims

1. A stable antibody-containing liquid formulation comprising 40 to 1000 mM arginine and 10 to 200 mM methionine.
2. The formulation of claim 1 further comprising a histidine buffering agent.
3. The formulation of claim 2 further comprising a surfactant.
4. The formulation according to claim 3 containing the antibody in an amount of at least 50 mg/ml.
5. The formulation according to claim 3 containing the antibody in an amount of at least 100 mg/ml.
6. The formulation according to claim 3 containing the antibody in an amount of at least 120 mg/ml.
7. The formulation according to any one of claims 4 to 6, wherein the antibody is an anti-IL-6 receptor antibody.

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8. The formulation according to any one of claims 4 to 7 having the pH in the range from 4 to 8.
9. The formulation according to claim 8, wherein the antibody is a humanized antibody or human antibody.
- 5 10. The formulation according to claim 8, wherein the antibody is a humanized anti-IL-6 receptor antibody, the amount of surfactant is 0.0001 to 10% (w/v), the concentration of the histidine buffer solution is 1 to 500 mM, and the concentration of the antibody is 50 to 300 mg/ml.
- 10 11. The formulation according to claim 8, wherein the antibody is the humanized anti-IL-6 receptor antibody MRA, the concentration of arginine is 50 to 700 mM, the concentration of methionine is 10 to 100 mM, the amount of polysorbate 80 as the surfactant is 0.005 to 3% (w/v), the concentration of the histidine buffer solution is 5 to 100 mM, and the concentration of the antibody is 100 to 300 mg/ml.
- 15 12. The formulation according to claim 10 or 11, further comprising tryptophane.
13. The formulation according to claim 10 or 11 having a viscosity of from 2 to 15 mPa·s.
14. The formulation according to claim 10 or 11, which is stable at 22-28°C for at least 6 months.
- 20 15. The formulation according to claim 10 or 11, wherein the dimerization of antibody molecules is inhibited.
16. The formulation according to claim 10 or 11, wherein the deamidation of antibody molecules is inhibited.
- 25 17. The formulation according to claim 10 or 11, which is for subcutaneous administration.
18. The formulation according to claim 10 or 11 which has not been subjected to lyophilization during preparation of the formulation.
- 30 19. A method for inhibiting dimerization of molecules of an antibody in a liquid formulation containing the antibody, comprising adding arginine and methionine to the liquid formulation.
20. The method of claim 19, wherein the antibody is a humanized anti-IL-6 receptor antibody, and the formulation comprises 50 to 300 mg/ml of the antibody, 1 to 500 mM of a histidine buffering agent, and 0.0001 to 10% (w/v) of a surfactant, and has a pH of 4 to 8, and wherein the arginine is added to the formulation in the amount of 40 to 1000 mM. and the methionine in the amount of 10 to 200 mM.
- 35 21. The method of claim 19, wherein the antibody is the humanized anti-IL-6 receptor antibody MRA, and the formulation comprises 100 to 300 mg/ml of the antibody, 5 to 100 mM of a histidine buffering agent, and 0.005 to 3% (w/v) of polysorbate 80, and has a pH of 4 to 8, and wherein the arginine is added to the formulation in the amount of 50 to 700 mM, and the methionine in the amount of 10 to 100 mM.
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Patentansprüche

- 45 1. Stabile, Antikörper-enthaltende flüssige Zubereitung umfassend 40 bis 1000 mM Arginin und 10 bis 200 mM Methionin.
2. Zubereitung nach Anspruch 1, weiter umfassend ein Histidin-Puffermittel.
- 50 3. Zubereitung nach Anspruch 2, weiter umfassend ein Tensid.
4. Zubereitung nach Anspruch 3, die den Antikörper mit einem Gehalt von mindestens 50 mg/ml beinhaltet.
5. Zubereitung nach Anspruch 3, die den Antikörper mit einem Gehalt von mindestens 100 mg/ml beinhaltet.
- 55 6. Zubereitung nach Anspruch 3, die den Antikörper mit einem Gehalt von mindestens 120 mg/ml beinhaltet.
7. Zubereitung nach einem der Ansprüche 4 bis 6, wobei der Antikörper ein Anti-IL6-Rezeptor-Antikörper ist.

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8. Zubereitung nach einem der Ansprüche 4 bis 7 mit einem pH-Wert im Bereich von 4 bis 8.
9. Zubereitung nach Anspruch 8, wobei der Antikörper ein humanisierter Antikörper oder ein menschlicher Antikörper ist.
- 5 10. Zubereitung nach Anspruch 8, wobei der Antikörper ein humanisierter Anti-IL6-Rezeptor-Antikörper ist, der Gehalt des Tensids 0.0001 bis 10% (w/v) ist, die Konzentration der Histidin-Pufferlösung 1 bis 500 mM ist und die Konzentration des Antikörpers 50 bis 300 mg/ml ist.
- 10 11. Zubereitung nach Anspruch 8, wobei der Antikörper der humanisierte Anti-IL6-Rezeptor-Antikörper MRA ist, die Konzentration von Arginin 50 bis 700 mM ist, die Konzentration von Methionin 10 bis 100 mM ist, der Gehalt an Polysorbat 80 als Tensid 0.005 bis 3% (w/v) ist, die Konzentration der Histidin-Pufferlösung 5 bis 100 mM ist und die Konzentration des Antikörpers 100 bis 300 mg/ml ist.
12. Zubereitung nach Anspruch 10 oder 11 weiter umfassend Tryptophan.
- 15 13. Zubereitung nach Anspruch 10 oder 11 mit einer Viskosität von 2 bis 15 mPa.s.
14. Zubereitung nach Anspruch 10 oder 11, die bei 22-28°C für mindestens 6 Monate stabil ist.
- 20 15. Zubereitung nach Anspruch 10 oder 11, wobei die Dimerisierung des Antikörpermoleküls gehemmt ist.
16. Zubereitung nach Anspruch 10 oder 11, wobei die Desamidierung des Antikörpermoleküls gehemmt ist.
17. Zubereitung nach Anspruch 10 oder 11 zur subkutanen Verabreichung.
- 25 18. Zubereitung nach Anspruch 10 oder 11, die während der Herstellung der Zubereitung nicht lyophilisiert wurde.
19. Verfahren zur Hemmung der Dimerisierung von Antikörpermolekülen in einer flüssigen Zubereitung, die den Antikörper beinhaltet, umfassend die Zugabe von Arginin und Methionin zu der flüssigen Zubereitung.
- 30 20. Verfahren nach Anspruch 19, wobei der Antikörper ein humanisierter Anti-IL6-Rezeptor-Antikörper ist, und die Zubereitung 50 bis 300 mg/ml des Antikörpers, 1 bis 500 mM eines Histidin-Puffermittels und 0.0001 bis 10% (w/v) eines Tensids umfasst, und einen pH-Wert von 4 bis 8 hat, und wobei das Arginin mit einem Gehalt von 40 bis 1000 mM und das Methionin mit einem Gehalt von 10 bis 200 mM zu der Zubereitung gegeben wird.
- 35 21. Verfahren nach Anspruch 19, wobei der Antikörper der humanisierte Anti-IL6-Rezeptor-Antikörper MRA ist, und die Zubereitung 100 bis 300 mg/ml des Antikörpers, 5 bis 100 mM eines Histidin-Puffermittels, und 0.005 bis 3% (w/v) Polysorbat 80 umfasst, und einen pH-Wert von 4 bis 8 hat, und wobei das Arginin mit einem Gehalt von 50 bis 700 mM und das Methionin mit einem Gehalt von 10 bis 100 mM zu der Zubereitung gegeben wird.
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Revendications

- 45 1. Formulation liquide contenant un anticorps stable comprenant 40 à 1000 mM d'arginine et 10 à 200 mM de méthionine.
2. Formulation selon la revendication 1, comprenant en outre un agent tampon histidine.
3. Formulation selon la revendication 2, comprenant en outre un surfactant.
- 50 4. Formulation selon la revendication 3, contenant l'anticorps en une quantité d'au moins 50 mg/ml.
5. Formulation selon la revendication 3, contenant l'anticorps en une quantité d'au moins 100 mg/ml.
- 55 6. Formulation selon la revendication 3, contenant l'anticorps en une quantité d'au moins 120 mg/ml.
7. Formulation selon l'une quelconque des revendications 4 à 6, dans laquelle l'anticorps est un anticorps anti-récepteur à l'IL-6.

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8. Formulation selon l'une quelconque des revendications 4 à 7, ayant un pH dans l'intervalle allant de 4 à 8.
9. Formulation selon la revendication 8, dans laquelle l'anticorps est un anticorps humanisé ou un anticorps humain.
- 5 10. Formulation selon la revendication 8, dans laquelle l'anticorps est un anticorps humanisé anti-récepteur à l'IL-6, la quantité de surfactant est de 0.0001 à 10% (p/v), la concentration de solution tampon histidine est de 1 à 500 mM, et la concentration de l'anticorps est de 50 à 300 mg/ml.
- 10 11. Formulation selon la revendication 8, dans laquelle l'anticorps est l'anticorps MRA humanisé anti-récepteur à l'IL-6, la concentration d'arginine est de 50 à 700 mM, la concentration de méthionine est de 10 à 100 mM, la quantité de polysorbate 80 en tant que surfactant est de 0.005 à 3% (p/v), la concentration de solution tampon histidine est de 5 à 100 mM, et la concentration de l'anticorps est de 100 à 300 mg/ml.
- 15 12. Formulation selon la revendication 10 ou 11, comprenant en outre du tryptophane.
13. Formulation selon la revendication 10 ou 11 ayant une viscosité de 2 à 15 mPa.s.
14. Formulation selon la revendication 10 ou 11, qui est stable à 22-28°C pendant au moins 6 mois.
- 20 15. Formulation selon la revendication 10 ou 11, dans laquelle la dimérisation des molécules d'anticorps est inhibée.
16. Formulation selon la revendication 10 ou 11, dans laquelle la désamidation de molécules d'anticorps est inhibée.
- 25 17. Formulation selon la revendication 10 ou 11, qui est destinée à une administration sous-cutanée.
18. Formulation selon la revendication 10 ou 11 qui n'a pas fait l'objet d'une lyophilisation durant la préparation de la formulation.
- 30 19. Méthode pour inhiber la dimérisation de molécules d'un anticorps dans une formulation liquide contenant l'anticorps, comprenant l'ajout d'arginine et de méthionine à la formulation liquide.
- 35 20. Méthode selon la revendication 19, dans laquelle l'anticorps est un anticorps humanisé anti-récepteur à l'IL-6, et la formulation comprend 50 à 300 mg/ml de l'anticorps, 1 à 500 mM d'un agent tampon histidine, et 0.0001 à 10% (p/v) d'un surfactant, et a un pH de 4 à 8, et dans laquelle l'arginine est ajoutée à la formulation en une quantité de 40 à 1000 mM, et la méthionine en une quantité de 10 à 200 mM.
- 40 21. Méthode selon la revendication 19, dans laquelle l'anticorps est l'anticorps MRA humanisé anti-récepteur à l'IL-6, et la formulation comprend 100 à 300 mg/ml de l'anticorps, 5 à 100 mM d'un agent tampon histidine, et 0.005 à 3% (p/v) de polysorbate 80, et a un pH de 4 à 8, et dans laquelle l'arginine est ajoutée à la formulation en une quantité de 50 à 700 mM, et la méthionine en une quantité de 10 à 100 mM.
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- 50
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Fig. 1

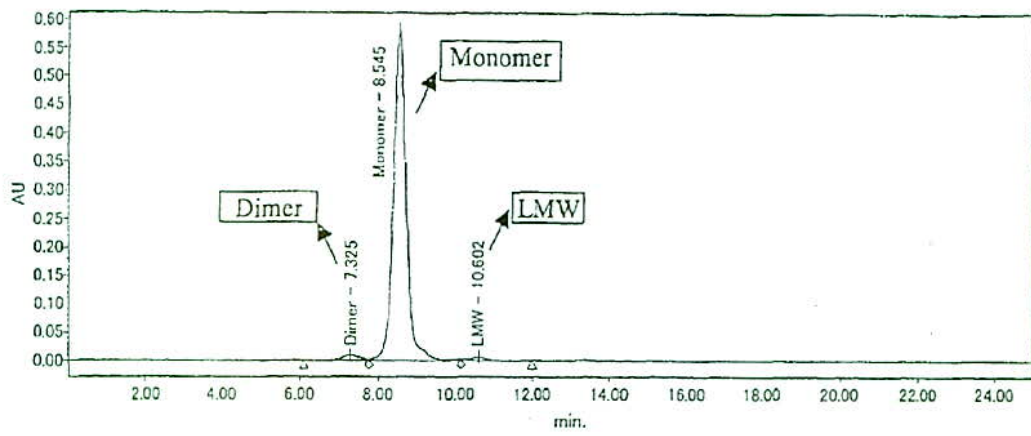


Fig. 2

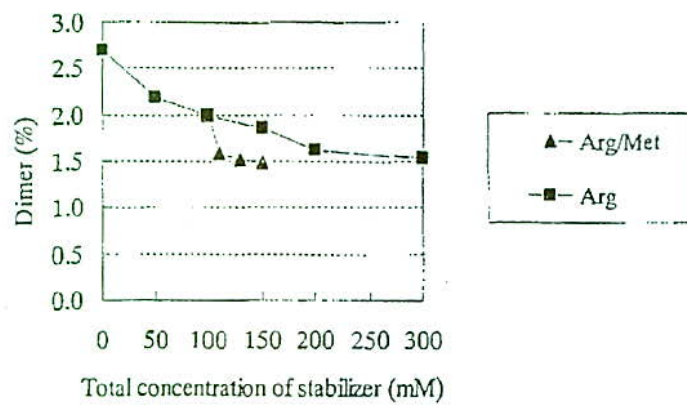


Fig. 3

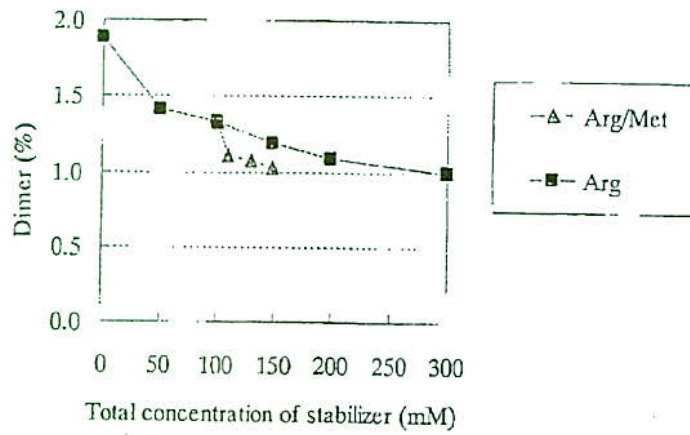


Fig. 4

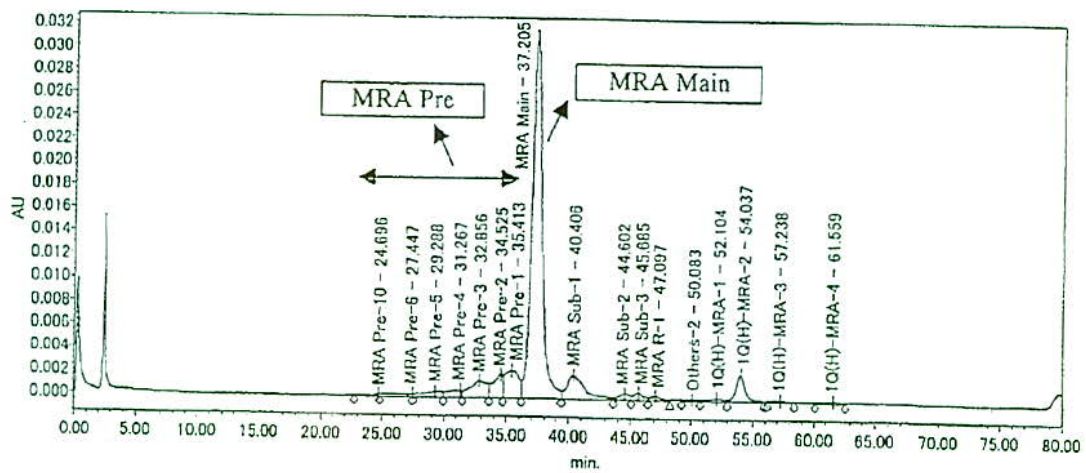


Fig. 5

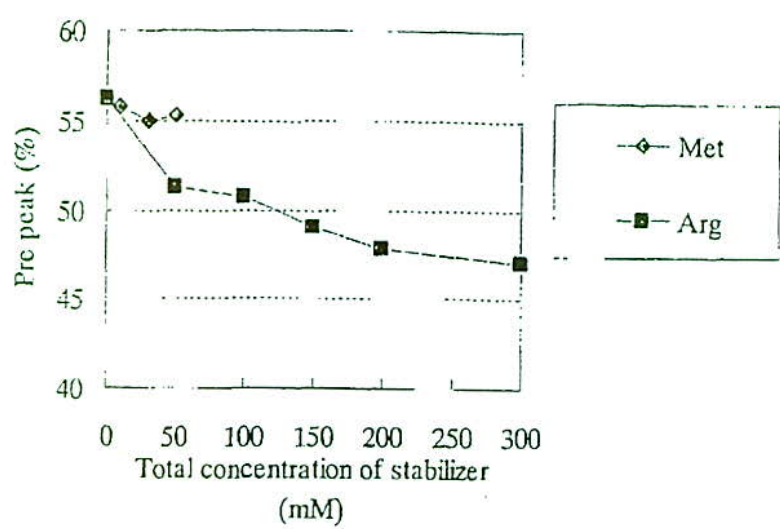


Fig. 6

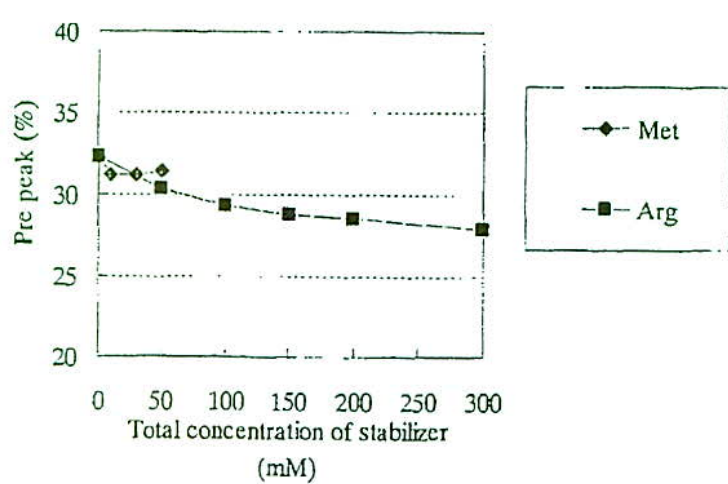


Fig. 7

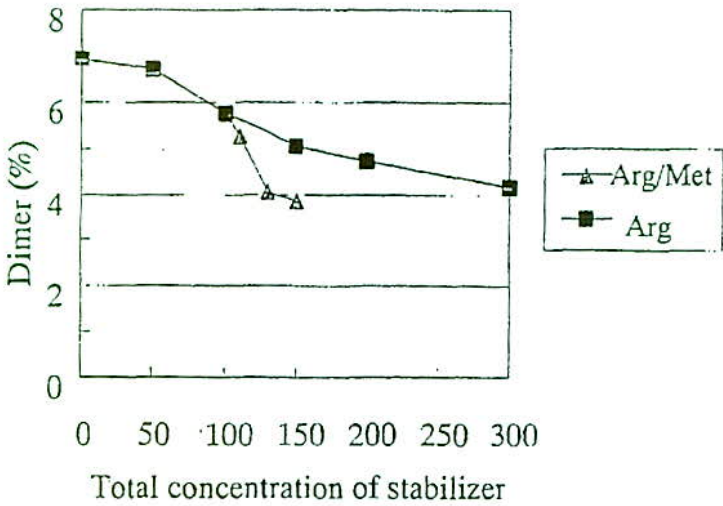
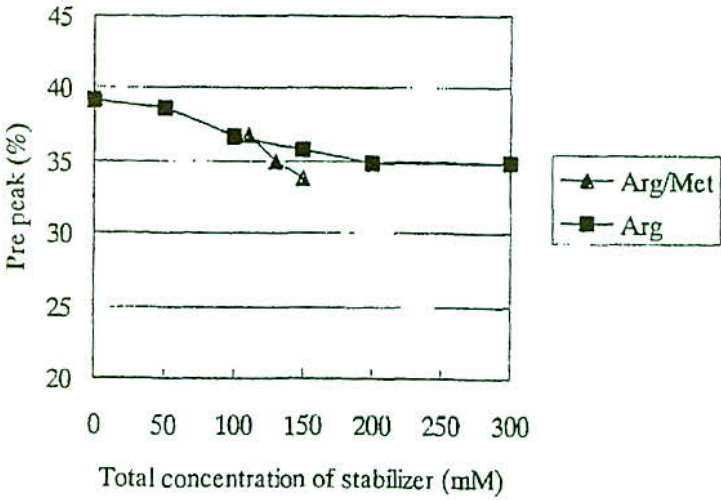


Fig. 8



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