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SUPERINDUSTRIA Y COMERCIO
Radicación: 01000346 00000002 Folios: 42
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Trámite: 002 PATENTES D 1 REGISTRO/ 444 AEROLISTA
Resolución: 2000 NIT-0170 AC MEXICO REGISTRAR

Referencia : Expediente No. 01.050.546
Solicitante : BAYER CORPORATION
Asunto : Solicitud de patente para: "INTERLEUQUINA 2 ESTABILIZADA"
Fecha de la solicitud : 26 de junio de 2001

TRAMITE:02 - EVENTO:01 - ACTUACION:444

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al oficio número 2198, notificado por fijación en lista del 26 de julio de 2001.

2. Mediante el oficio número 2198 se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Atiendo a las observaciones de la manera siguiente:

2.1. Documento de cesión del derecho a la patente. Presento junto con este escrito el documento de cesión de los inventores al solicitante debidamente legalizado.

2.2. Copia Certificada. Presento con este escrito copia certificada de la solicitud de patente estadounidense número 09/605,577 del 28 de junio de 2000, en la cual se basa la reivindicación de la prioridad.

2.3. Poder. Por medio de este escrito me permito aclarar que el poder presentado junto con la solicitud no es el correcto, debido a que el domicilio de la sociedad solicitante no es Elkhart, Estado de Indiana. En

consecuencia presento junto con este memorial el poder correcto, en donde el domicilio de la sociedad Bayer Corporation es Pittsburgh, Estado de Pennsylvania, Estados Unidos de América.

Ruego a Usted reconocerme personería como apoderado de la solicitante.

2.4. Recibos. Presento junto con este escrito, el recibo de pago No.01-44,622 por valor de \$73.440.00 por concepto de modificación en solicitud pendiente.

3. Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

Bogotá, 1 AGO. 2001
Señora Jefe,


EMILIO FERRERO
T.P.A. No. 31.929

COLO 11996-801-001-5
JJC-ID-6

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

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2. has been signed by T. Lawrence

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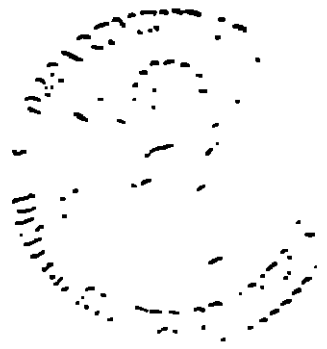
5. at Washington, D.C.

6. the twenty-first of June, 2001

7. by Assistant Authentication Officer, United States Department of State

8. No. 01020740-2

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10. Signature:

Patrick O. Hatchett
Patrick O. Hatchett

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June 19, 2001

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T. Lawrence

**T. LAWRENCE
Certifying Officer**

3936

FORM PTO-1000
(Rev. 6-92)
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RECORD

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Tab settings: 000 6280

To the Honorable Commissioner of Patents and

original documents or copy the

1. Name of conveying party(ies):

Wei Wang
Rajiv Nayar
Michael A. Shearer

Additional name(s) of conveying party(ies) attached? ☐ Yes ☒ No

2. Name and address of receiving party(ies)

Name: Bayer Corporation

Internal Address:

Street Address: P. O. Box 1986

800 Dwight Way

City: Berkeley State: CA ZIP: 94701

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of conveyance:

☒ Assignment

☐ Merger

☐ Security Agreement

☐ Change of Name

☐ Other

Execution Date:

4. Application number(s) or patent number(s):

0960557

If this document is being filed together with a new application, the execution date of the application is: June 24, 2000

A. Patent Application No.(s)

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: James A. Gibling

Internal Address: Bayer Corporation

Street Address: P. O. Box 1986

800 Dwight Way

City: Berkeley State: CA ZIP: 94701

6. Total number of applications and patents involved:

1

7. Total fee (37 CFR 3.41): \$ 40.00

☐ Enclosed

☒ Authorized to be charged to deposit account

8. Deposit account number:

03-4000

(Attach duplicate copy of this page if paying by deposit account)

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

James A. Gibling

Name of Person Signing

James A. Gibling

Signature

June 24, 2000

Date

Total number of pages including cover sheet, attachments, and document:

3

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents & Trademarks, Box Assignments
Washington, D.C. 20231

PATENT

REEL: 010979 FRAME: 0776

ASSIGNMENT

WHEREAS, WE, WEI WANG, RAJIV NAYAR and MICHAEL A. SHEARER, have invented certain new and useful improvements in STABILIZED INTERLEUKIN 2 for which Application for United States Letters Patent was executed by US on JUNE 28, 2006.

WHEREAS, Bayer Corporation, a corporation organized and existing under the laws of the State of Indiana, having a principal place of business at 800 Dwight Way, Berkeley, California 94710, is desirous of acquiring said invention and application and any and all Letters Patent that may be obtained therefor or thereupon;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN, be it known that, for and in consideration of the sum of One Hundred Dollars (\$100.00) and other good and valuable consideration, to US in hand paid by Bayer Corporation, the receipt of which is hereby acknowledged, WE have sold, assigned, transferred and set over and by these presents do sell, assign, transfer and set over unto Bayer Corporation, its successors and assigns, the full and exclusive right, title and interest in and to said invention and application and any and all divisions and continuations thereof, and in and to any and all United States Letters Patent and any and all reissues and extensions thereof that may be issued for or upon said invention and applications, to have and to hold for its own use and behoof and the use and behoof of its successors and assigns as fully and entirely as the same might be enjoyed by US if this sale and assignment had not been made;

AND WE HEREBY AUTHORIZE and request the Commissioner of Patents and Trademarks to issue said Letters Patent and any reissues and extensions thereof to Bayer Corporation, as assignee of the entire right, title and interest in and to said invention and application and any and all Letters Patent issued therefor or thereupon;

AND WE HEREBY AGREE to execute all instruments and documents within OUR power which may be necessary for the carrying out of this assignment in full; and to execute any divisional or continuation application which may be necessary or proper to obtain full protection on the invention hereby assigned; and to execute any and all supplemental oaths, preliminary statements and other papers should the same be proper and necessary in the prosecution of said application and any divisions and continuations thereof;

AND WE HEREBY FURTHER ASSIGN unto Bayer Corporation, its successors and assigns, the whole right, title and interest in and to the invention disclosed in said application throughout all countries foreign to the United States and do hereby ratify any acts of Bayer

Patent
MSA-7267

Corporation, its successors and assigns, in applying for patents therefor in its own name in countries where such application is proper and do agree to execute applications for said invention in the several countries where it is necessary that the same be executed by the inventor, and to execute assignments of such applications and the patents to be obtained thereupon to Bayer Corporation, its successors and assigns;

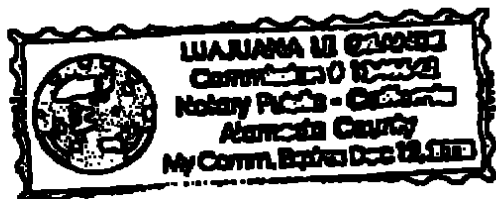
AND WE HEREBY AUTHORIZE Bayer Corporation, and its successors and assigns, to claim the priority of the aforesaid United States Patent Application in accordance with the provisions of each and every international convention or treaty in connection with each and every corresponding application which it may file or cause to be filed in each and every country which is a party to any such convention or treaty.

IN WITNESS WHEREOF, WE have hereunto set OUR hands this 29 day of June, 2000.

STATE OF CALIFORNIA)
) ss:
COUNTY OF ALAMEDA)

Wei Wang
Rajiv Nayar
Michael A. Shearer

On this 29 day of June, 2000, before me personally appeared WEI WANG, RAJIV NAYAR and MICHAEL A. SHEARER to me known to be the persons described in and who executed the foregoing instrument and acknowledge that THEY executed the same as THEIR free act and deed in their authorized capacity.



Luquana L. Hancock
Notary Public

RECORDED: 06/28/2000

PATENT
REEL: 010979 FRAME: 0777

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

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2. has been signed by E. Bornett

3. acting in the capacity of Certifying Officer

4. bears the seal/stamp of Patent and Trademark Office

Certified

5. at Washington, D.C.

6. the twentieth of June, 2001

7. by Assistant Authentication Officer, United States Department of State

8. No. 01020626-5

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10. Signature:

Patrick O. Hatchett
Patrick O. Hatchett

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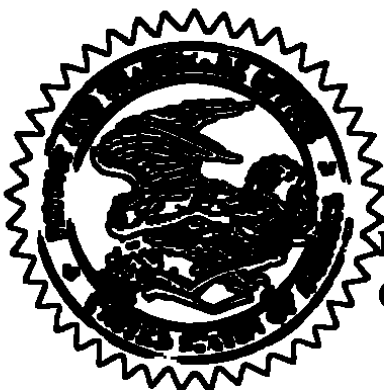
United States Patent and Trademark Office

June 18, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 09/605,577

FILING DATE: June 28, 2000



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

E. Bornett

**E. BORNETT
Certifying Officer**

AS
41

09/605577
Jc044 U. N. PTD

06/26/00

TRANSMITTAL LETTER

EXAMINER: Unassigned

ART UNIT: Unassigned

Title: STABILIZED INTERLEUKIN 2

Sir:

Transmitted herewith for filing are the following:

1. Application for United States Patent, including a Title page, 14 pages of Specification and Claims, an Abstract (1 page) and 2 sheets of drawings of Applicants: WEI WANG, RAJIV NAYAR and MICHAEL A. SHEARER for: Stabilized Interleukin 2.
2. An Assignment document is enclosed, together with a Request for Recordation.
3. Fee calculation:

Basic Fee				\$690.00
Total Claims	10	0	--	
Independent Claims	2	0	--	
Multiple Dependent Claims				0
TOTAL				\$ 690.00

48
42

PATENT
MSR-7267

4. Enclosed with this Transmittal Letter is a Deposit Account Order Form authorizing payment of the filing fee from Deposit Account No. 03-4000.
5. An executed Declaration for Patent Application is enclosed.
6. Address all future communications to:

James A. Giblin
Patent Department
Bayer Corporation
800 Dwight Way
P.O. Box 1986
Berkeley, CA 94701

Respectfully submitted,

Dated:

June 28, 2000

James A. Giblin

James A. Giblin
Attorney for Applicants
Reg. No. 25,772
Bayer Corporation
800 Dwight Way
P.O. Box 1986
Berkeley, CA 94701
(510) 705-7910

008230-22350300

APPLICATION FOR UNITED STATES PATENT

Title: STABILIZED INTERLEUKIN-2

Inventors: Wei Wang, Rajiv Nayar, Michael A. Shearer

0980577-DE2800

STABILIZED INTERLEUKIN 2

Related Application: WO 99/60128 (PCT/US99/10643 filed May 13, 1999, published November 25, 1999)

BACKGROUND OF THE INVENTION

Field: The invention is generally related to the field of pharmaceutical formulations. More specifically, the invention is directed to a stabilized, therapeutically active Interleukin-2 formulation capable of selectively activating T cells (PHA-blasts) and, very preferably, including an IL-2 mutein demonstrating reduced activation of Natural Killer ("NK") cells. The stabilized compositions having the preferred properties include variants of IL-2 described below.

Background: As discussed in a related application PCT/US 99/10643 published November 25, 1999, Interleukin 2 (IL-2) is a potent immune stimulator, activating diverse cells of the immune system, including T cells, B cells, and monocytes. IL-2 is also a potent and critical growth factor of T cells. It was by virtue of these activities that IL-2 was tested for its ability to treat cancer. Human IL-2 is a FDA approved drug for the treatment of metastatic renal carcinoma and metastatic melanoma. The use of IL-2 in eligible patients is restricted due to the severe toxicity associated with IL-2 therapy; it is estimated that at best only 20% of eligible patients actually receive therapy. The toxicities associated with IL-2 therapy include severe fever, nausea, vomiting, vascular leak and serious hypotension. Despite these toxicities, however, IL-2 is effective for its approved indications. Variants of IL-2 having reduced toxicity are the subject matter of application WO 99/60128.

Significant information on stabilization of IL-2 and other therapeutic protein formulations is available. The currently approved Human IL-2 preparation (Proleukin® IL-2, Chiron Corporation) is a freeze-dried preparation which includes mannitol, sodium dodecyl sulfate (SDS) and a phosphate buffer. Other formulated therapeutic proteins, including IL-2, are described in the following references.

Fernandes et al., 1986, Pharmaceutical compositions of microbially produced interleukin-2 (US patent No. 4,604,377) describes a freeze-dried formulation containing a stabilizer (mannitol) and a solubilizing agent such as sodium dodecyl sulfate or sodium deoxycholate sulfate at about 100 to about 250 ug per mg of IL-2. The formulation for the currently available Proleukin® IL-2 product is believed to be described in this reference.

Patel, 1994 Stabilization of protein formulations (US patent No. 5,358,708) describes aqueous formulations of an interferon, a granulocyte-macrophage colony-stimulating factor or an interleukin having extended storage lifetimes by incorporating methionine, histidine or mixtures thereof. Although reference is made to several interleukins, including IL-2, in work done with an IL-4 formulation the patentees found histidine to be less effective as a stabilizer than methionine, under conditions of the stabilizer test used.

Shaked, et al., 1991, Pharmaceutical compositions of recombinant interleukin-2 and formulation processes (US patent No. 5,037,644) describes formulations which are either in freeze-dried or liquid form. The excipients of the formulation include a non-ionic polymeric detergent such as Triton X405, Triton X305, PEG (4000) monostearate, Tween 80 and Tween 20 at concentrations of about 0.001% to about 5%, a bulking/stabilizing agent such as sucrose, fructose, dextrose, maltose, glucose, dextran, mannitol, sorbitol, inositol, galactitol, xylitol, lactose, trehalose, human serum albumin and bovine serum albumin, and a buffering agent such as glycine, citrate, or phosphate in a concentration range from about 10 mM to about 50 mM with a pH ranging from about 3 to about 7. The concentration (wt/vol) of the polyol sugar bulking agent ranges from about 0.025% to about 10%.

Roekam et al., 1993, Drugs containing a glycosylated interleukin-2 (US patent No. 5,417,970) describes a freeze-dried formulation containing hydrolyzed gelatin (or human serum albumin) and alanine with a pH 6.5.

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acceptable formulation for biologically active and useful Human IL-2. Our discovery is based on addressing what we believe is the basic mechanism responsible for stability, as described below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph illustrating the optimum pH range for an aqueous IL-2 solution.

Figure 2 compares the stabilizing effect of histidine to acetate and citrate on IL-2 aggregation induced by heating the protein solution at 1 C°/min from 25 °C to 95 °C.

SUMMARY OF INVENTION

The present invention is a pharmaceutical composition or formulation of IL-2 or variants (mutants) thereof stabilized with histidine. Preferably, the composition comprises a mixture resulting in a solution of low ionic strength (e.g. <0.1) and includes other stabilizers such as sugars and amino acids, preferably sucrose and glycine. The formulation may include from 0 to 0.9 wt. % NaCl. The composition is albumin-free and the formulation in lyophilized form can be rapidly reconstituted (< 1 minute) with water. The composition solubilizes under physiologically acceptable pH conditions, preferably at a pH ranging from about 5.0 to about 6.5, without the use of surfactants such as sodium dodecyl sulfate. The reconstituted solution is near isotonicity and can be administered both subcutaneously and intravenously. In a very preferred embodiment, the IL-2 of the composition is a mutant having a single amino acid substitution, preferably the N88R variant described in WO 99/60128.

The very preferred composition has a protein concentration of 1-5 mg/ml and comprises the following in aqueous form (on a wt/wt basis):

IL-2	0.1-0.5 wt%
Histidine	0.08-1.6 wt%
NaCl	0-0.9 wt%
Sucrose	1-10 wt%
Glycine	0-3 wt%, at a
pH of	5 to 6.5.

Details of our formulation and how it was discovered are described below.

SPECIFIC EMBODIMENTS

As used herein, the term IL-2 includes both active wild type IL-2 and its biologically active variants or muteins such as those described in WO 99/60128. In the examples below we used an IL-2 known as IL-2(N88R) which is a recombinant mutein of human IL-2, with asparagine (N) at amino acid position 88 mutated to arginine (R). This mutein was expressed from Chinese hamster ovary (CHO) cells and comprised a mixture of both glycosylated and non-glycosylated forms. It is described in WO 99/60128 cited above as a related application.

The objective that led to this invention was the need to identify a lyophilized dosage form for the preferred IL-2 mutein that was albumin-free with acceptable stability. Since, the bioassay for IL-2 was an insensitive measure of stability, we used quantitation of soluble IL-2 by reverse phase HPLC as the stability indicating assay. As used herein, the terms "stable" or "stabilized" mean reduction of soluble IL-2 quantity by reverse-phase HPLC to no less than 90% of original soluble quantity after storage for four months at 40 °C (see tables 3 and 4 below). Additional stability-indicating assays employed included Aggregation Index, a measure of aggregation by UV/VIS spectrophotometry and determination of soluble aggregates by size-exclusion HPLC. In addition to a stable product, rapid reconstitution (less than 1 minute) of the lyophilisate is highly preferred.

Finally, the formulation in a lyophilized dosage form with acceptable stability which could be easily lyophilized in production freeze dryers was desired.

Aqueous stability studies carried out during preformulation investigations indicated that IL-2(N88R) readily aggregates in the liquid state and the aggregation was pH-dependent. Two preformulation stability studies were conducted: a pH profile of IL-2(N88R) and stability in the presence of different buffer excipients. The objectives of these studies were to identify a suitable pH range for IL-2(N88R) and a suitable buffering excipient for aqueous stability (reduced aggregation potential). In generating the pH profile, IL-2 solutions were prepared with different pH conditions and stored under accelerated temperature conditions (40 °C). Samples were analyzed at different time intervals and rates of aggregation calculated. As shown in Figure 1, the optimal pH range for low aggregation rates was identified between pH 5.0 and 6.5. Histidine, acetate, and citrate were identified as pharmaceutical buffering agents for IL-2(N88R) in this pH range and were evaluated at a concentration of 20 mM in IL-2 (N88R) solutions containing 1 mg/ml IL-2 (N88R) and 150 mM (0.9 wt%) NaCl. These samples were heated from 25 °C to 95 °C at 1 °C per minute and precipitation was monitored by UV spectrophotometry at 350 nm. As shown in figure 2, to our surprise, histidine significantly stabilized IL-2(N88R) over the other buffer excipients at pH 5.5 as indicated by an increase in the onset temperature of precipitation. The onset temperatures in the presence of citrate, acetate, and histidine were 62 °C, 64 °C, and 70 °C, respectively. Studies such as these demonstrated that histidine could be used not only as a buffering agent but also a stabilizer for IL-2 under aqueous conditions.

For development of a lyophilized dosage form, other excipients that are used by those familiar with the art were investigated. These included bulking agents and cryoprotectants such as glycine, sucrose, and mannitol. Two surfactants were also evaluated as stabilizers for IL-2(N88R), since aggregation was one of the instability mechanisms for the molecule during the aqueous stability studies. The results of these studies are summarized in the examples below. They show that to our surprise histidine has selective stabilizing effects on IL-2 over other excipients, such as citrate for example.

EXAMPLE 1

The stability of liquid IL-2(N88R) at different pHs was examined at 40°C. The results showed that IL-2(N88R) precipitation rate was lowest between pH 5.0 to 6.5 (Figure 1). Therefore, pH 5.5 was chosen as the optimal formulation pH in liquid state and for preparation of lyophilized formulations.

EXAMPLE 2

We examined the potential effect of different buffering agents on the stability of IL-2(N88R). The buffering agents we examined included citrate, acetate, and histidine. These buffering agents were used at 20 mM in IL-2(N88R) solutions containing 1 mg/ml IL-2(N88R) and 150 mM (0.9 wt%) NaCl. These stability samples were heated from 25 °C to 95 °C at 1 °C per minute while being monitored by UV/VIS spectrophotometry. Histidine significantly stabilized IL-2(N88R) by increasing the precipitation temperature and by decreasing the precipitation rate in comparison with acetate and citrate (Figure 2). Table 1 shows the onset precipitation temperatures of IL-2(N88R) in the presence of these three buffering agents. The onset precipitation temperature was arbitrarily defined as the temperature at which the optical density at 350 nm (OD_{350}) reaches a certain level (0.2 and 1.0 in the case of OD_{350}). The precipitation temperature of IL-2(N88R) in the presence of histidine was several degrees higher than those in the presence of the other two buffering agents. This example demonstrated that histidine may be a specific stabilizer in addition to being used as a buffering agent for IL-2(N88R) in the liquid state.

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Table 1: Precipitation Temperature ($OD_{350}=0.2$ and $OD_{350}=1.0$) of IL-2(N88R) in Different Buffers

Buffering Agents	Precipitation Temperature, °C (when $OD_{350} = 0.2$)	Precipitation Temperature, °C (when $OD_{350} = 1.0$)
Citrate	62 °C	64 °C
Acetate	64 °C	66 °C
Histidine	70 °C	76 °C

EXAMPLE 3

In an effort to evaluate further the stabilizing effect of histidine, lyophilized IL-2(N88R) was prepared from different aqueous formulations (see Table 2). Most of the formulations contained 2 wt% glycine as a bulking agent and 1 wt% sucrose as a stabilizer. Mannitol at 5 wt% was used in a formulation as a comparator to Proleukin®, a commercialized product of IL-2. Two surfactants, Tween 80 and Pluronic F68 both at 0.1 wt%, were evaluated for prevention of protein surface adsorption and aggregation. All the formulations contained either histidine or citrate as a buffering agent with a pH adjusted to 5.5. Citrate was included to distinguish the stabilizing effect of histidine from that of citrate at pH 5.5. These lyophilized formulations were stored at 40°C and were analyzed by a number of analytical methods that included UV/VIS spectrophotometry, SEC-HPLC, and RP-HPLC.

Table 3 shows the stability of IL-2(N88R) in a number of formulations as assessed by UV/VIS spectrophotometry for aggregation, amount of soluble aggregates by size-exclusion HPLC (SEC-HPLC), and percent recovery of the protein by reverse-phase HPLC (RP-HPLC). Samples were analyzed after lyophilization and stored at an accelerated storage temperature of 40°C for four months. The lyophilization process did not change the net aggregation index of IL-2(N88R) from the pre-lyophilization state, suggesting that IL-2(N88R) tolerated the lyophilization process with respect to protein

aggregation/precipitation. After storage at 40°C for four months, a significant increase in net aggregation index was observed for the formulation containing citrate (B), Pluronic F-68 (D) or mannitol (E). These results indicated that inclusion of either citrate or mannitol, Tween-80 or Pluronic F-68 did not offer any protection of IL-2(N88R) aggregation in the solid state during storage. Formulations A and F did not show a significant change in the aggregation index, suggesting that inclusion of histidine, glycine, and sucrose with 1 or 5 mg/ml IL-2(N88R) resulted in a stable product.

However, significant amounts of soluble aggregates were found in the formulation containing Tween-80 even before lyophilization (Table 3), indicating that Tween-80 promotes formation of soluble IL-2(N88R) aggregates, although formation of insoluble aggregates may be inhibited. Since Pluronic F-68 (formulation D) also caused significant formation of soluble aggregates, surfactants may not be compatible with IL-2(N88R). Only the formulations that contained 2% glycine, 1% sucrose, and 20 mM (0.31 wt%) histidine (A, F) did not show any detectable formation of soluble aggregates after storage of the lyophilized formulation at 40°C for four months, suggesting again that IL-2(N88R) was stabilized by histidine.

The total recovery of soluble IL-2(N88R) after lyophilization and storage was determined by RP-HPLC (Table 3). The recovery of IL-2(N88R) after lyophilization was greater than about 96% for all formulations except for the formulation containing mannitol. After storage of these formulations at 40°C for 4 months, approximately 92% of IL-2(N88R) was recovered in formulations A and F containing 2% glycine, 1% sucrose and 20 mM (0.31 wt%) histidine containing 1 and 5 mg/ml IL-2(N88R). These data (greater than 90% recovery of IL-2 (N88R) for formulations A and F) again suggest that histidine stabilizes IL-2(N88R) while surfactants destabilize the protein.

Table 2: Composition of Lyophilized IL-2(N88R) Formulation

Formulation ID	A	B	C	D	E	F
IL-2(N88R), mg/mL	1	1	1	1	1	5
Glycine, wt% (w/w)	2	2	2	2	0	2
Sucrose, wt% (w/w)	1	1	1	1	0	1
Mannitol, wt% (w/w)	0	0	0	0	5	0
Sodium Citrate, wt% (w/w)	0	0.6	0	0	0	0
Histidine, wt% (w/w)	0.31	0	0.31	0.31	0.31	0.31
Tween 80, wt% (v/w)	0	0	0.1	0	0	0
Pluronic F68, wt% (w/w)	0	0	0	0.1	0	0
pH	5.5	5.5	5.5	5.5	5.5	5.5

Table 3: Stability of IL-2(N88R) during Lyophilization and Storage of the Lyophilized Formulation at 40 °C for 4 months.

Formulation ID	A	B	C	D	E	F
Aggregation Index (%)						
Before lyophilization	4.4	5.2	1.5	4.7	5.6	1.6
After lyophilization	5.2	5.1	0.7	1.5	3.8	1.6
4 months at 40 °C	2.9	13.9	2.4	14.3	20.8	3.3
Soluble Aggregates (%) by SEC-HPLC						
Before lyophilization	ND ^a	ND	4.4%	ND	ND	NA ^b
After lyophilization	ND	ND	7.2%	ND	ND	NA
4 months at 40 °C	ND	4.2%	32.9%	15.9%	12.2%	NA
Recovery (%) by RP-HPLC						
Before lyophilization	100	100	100	100	100	100
After lyophilization	96.5	95.7	96.4	99.4	91.7	97.8
4 months at 40 °C	92.5	82.9	71.9	84.3	79.1	91.7

^aND = not detectable^bNA = not available

EXAMPLE 4

Wild-type IL-2 was also lyophilized from an aqueous formulation of the same composition as formulation A. The stability data for the wild-type IL-2 at 40 °C was comparable to those for IL-2(N88R) (Table 4).

Table 4: Stability of Wild-type IL-2(N88R) Formulation during Lyophilization and Storage of the Lyophilized Formulation

Conditions	Aggregation Index (%)	Soluble Aggregates (%)	Recovery (%)
Before lyophilization	4.2	ND*	100
After lyophilization	5.1	ND	93.6
4 months at 40 °C	4.3	ND	93.0

*ND = not detectable

DISCUSSION

Human IL-2 has 133 amino acids that form six helical structures (A-F). Four of these helices form what is termed a tetra-helix bundle motif. The intramolecular disulfide bond between cys²⁸ and cys¹⁰⁸ is located on the extended loops between the helices. The free cys¹²⁹ is located on helix F that incorporates amino acids 117-133.

The surprise finding that histidine is a specific stabilizer of IL-2 suggests that histidine may interact with IL-2 in a specific manner which results in stabilizing the molecule in both the aqueous and lyophilized states. One of the major mechanisms of instability of IL-2 is aggregation that results from the formation of oligomers due to thiol-disulfide exchange reactions. Hence, one can hypothesize that histidine may in fact inhibit or reduce the thiol-disulfide exchange reactions in IL-2. Since wild type IL-2, IL-2(N88R) and possibly other IL-2 variant molecules that have one disulfide bond and a free cysteine (Cys¹²⁹), the free -SH group on Cys¹²⁹ could easily react with the disulfide bond via the thiol/disulfide exchange pathway thereby resulting in aggregation/precipitation events.

The mechanism of thiol/disulfide exchange has been described recently by Bulaj et al. (Ionization-reactivity relationships for cysteine thiols in polypeptides. *Biochemistry* 1998 Jun 23;37(25):8965-72). In studies of model peptides and proteins, the reaction rate has been shown to be sensitive to electrostatic forces as well as to the secondary structure of the proteins. The electron distribution around a sulfur atom in a thiol can be influenced by the presence of nearby charges and through-bond inductive effects which can alter the pK_a of the thiol. For example, increased reactivity of the thiols in the thiol/disulfide exchange can be attributed to the lowering of its pK_a due to the presence of either nearby positive charges or peptide dipole contributions from a nearby alpha-helical structure. In contrast, negative charges near the thiol group can raise the pK_a of the thiol and lead to lower thiol/disulfide reaction rates. This proposed mechanism is also supported by other studies, where stabilization of the highly reactive thiolate ions by neighboring positively-charged residues has been demonstrated for protein tyrosine phosphatase (Zhang and Dixon, 1993 Active site labeling of the Yersinia protein tyrosine phosphatase: the determination of the pK_a of the active site cysteine and the function of the conserved histidine 402. *Biochemistry* 1993 Sep 14;32(36):9340-5) and protein disulfide isomerase (Kortemme et al., Electrostatic interactions in the active site of the N-terminal thioredoxin-like domain of protein disulfide isomerase. *Biochemistry* 1996 Nov 19;35(46):14503-11).

Based on these properties of the thiol/disulfide exchange reaction, one can envision a stabilization mechanism where histidine plays either a kinetic or a thermodynamic role in the stabilization of IL-2(N88R). There are five glutamic acid residues (57, 60, 61, 62, and 106) near the disulfide bridge Cys⁵⁸ - Cys¹⁰³. Specific binding of histidine to these negatively-charged residues could lead to a kinetic barrier for the thiol/disulfide exchange reaction or the binding of histidine near the disulfide bridge could thermodynamically stabilize the IL-2 molecule into a conformation that is less prone to aggregation reactions. In addition, the His-Glu ionic interactions may also create steric hindrance that would further reduce the rate-determining step in the thiol/disulfide exchange which is the formation of an intermediate transition state between the three participating sulfur atoms. The accessibility of histidine to the

glutamic acid residues is very likely because the disulfide bond is located on an extended loop on the protein surface.

Given the above examples it is expected that variations of the inventions disclosed herein will occur to those skilled in the art. Accordingly, it is intended that the above examples should be construed as illustrative only and that the inventions disclosed herein should be limited only by the following claims.

00505577-032800

WE CLAIM

1. A stable pharmaceutical composition comprising human interleukin-2 or a variant thereof stabilized with histidine.
2. The preparation of claim 1 in lyophilized form which can be rapidly reconstituted in water.
3. The preparation of claim 1 in aqueous form having a pH ranging from about 5.0 to about 6.5.
4. The preparation of claim 1 including glycine.
5. The preparation of claim 1 including sucrose.
6. The preparation of claim 1 including glycine and sucrose.
7. The preparation of claim 1 where the IL-2 is a mutein.
8. The preparation of claim 7 where the IL-2 is a mutein having a single amino acid substitution, N88R.
9. A stable, lyophilized composition which, upon aqueous reconstitution, comprises the following:

IL-2	0.1-5 mg/ml
Histidine	0.08-1.6 wt%
NaCl	0-0.9 wt%
Sucrose	1-10 wt% and
Glycine	0-3 wt% at a
pH of	5 to 6.5

10. The preparation of claim 9 where the IL-2 is a mutein having a single amino acid substitution, N88R.

ABSTRACT OF THE DISCLOSURE

A stable pharmaceutical preparation comprising Human interleukin-2 or a variant thereof and a stabilizing amount of histidine. A preferred formulation includes glycine and sucrose and a variant of IL-2 having a single mutation, N88R. The preferred formulation is in lyophilized form which, on reconstitution with an aqueous diluent, results in a solution having a pH ranging from about 5.0 to 6.5.

63

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)
MSB-7267

59

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled STABILIZED INTERLEUKIN 2, the specification of which

is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Claimed

<u>NONE</u>			☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

<u>NONE</u>			
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)	
(Application Number)	(Filing Date)	(Status - patented, pending, abandoned)	

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the

Patent and Trademark Office connected therewith: Michael A. Beck, Reg. No. 40,807
James A. Giblin, Reg. No. 25, 772
Melissa A. Shaw, Reg. No. 38,301Address all telephone calls to James A. Giblin at telephone number (510) 705-7810Address all correspondence to James A. Giblin
Bayer Corporation
800 Dwight Way
Berkeley, CA 94701

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name) Wei WangInventor's signature [Signature] Date 6/28/2000Residence Alameda, California Citizenship USPost Office Address 823 Linc Street
Alameda, California 94502Full name of second joint inventor, if any (given name, family name) Rajiv NayerSecond inventor's signature [Signature] Date 6/28/2000Residence Richmond, California Citizenship CanadianPost Office Address 5357 Coach Drive
Richmond, California 94803

Additional inventors are being named on separately numbered sheets attached hereto.

DECLARATION FOR PATENT APPLICATION

Docket Number (Optional)
MSB-7267

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled STABILIZED INTERLEUKIN 2, the specification of which

is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number or PCT International Application Number _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Claimed

NONE

☐ Yes ☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes ☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes ☐ No

(Number)

(Country)

(Day/Month/Year Filed)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

NONE

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

(Application Number)

(Filing Date)

(Status - patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Michael A. Beck, Reg. No. 40,907
James A. Gible, Reg. No. 25,772
Melissa A. Shaw, Reg. No. 38,301Address all telephone calls to James A. Gible at telephone number (510) 708-7810Address all correspondence to James A. Gible
Bayer Corporation
800 Dwight Way
Berkeley, CA 94701

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of third joint inventor (given name, family name) Michael A. ShearerThird inventor's signature [Signature] Date 6/25/00Residence Fairfield, California Citizenship U.S.Post Office Address 2402 Homestead Court
Fairfield, California 94533

Full name of fourth joint inventor (given name, family name) _____

Fourth inventor's signature _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

☐ Additional inventors are being named on separately numbered sheets attached hereto.

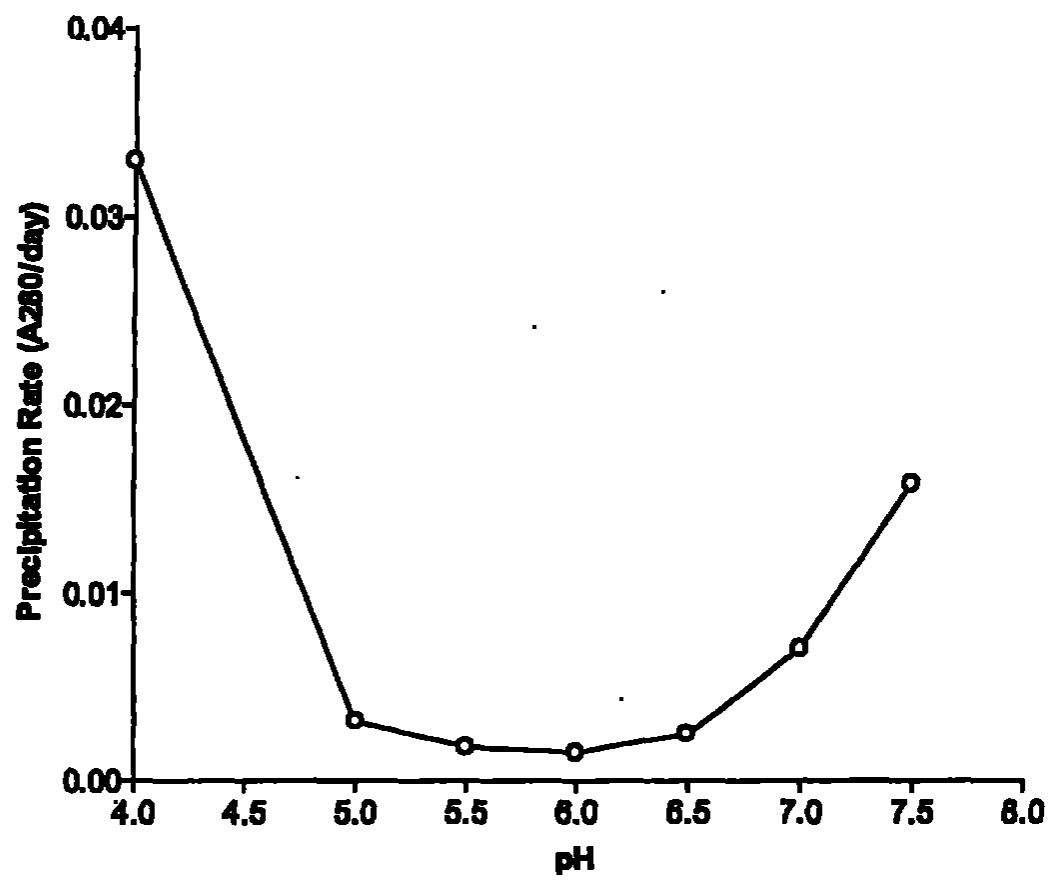


Figure 1.

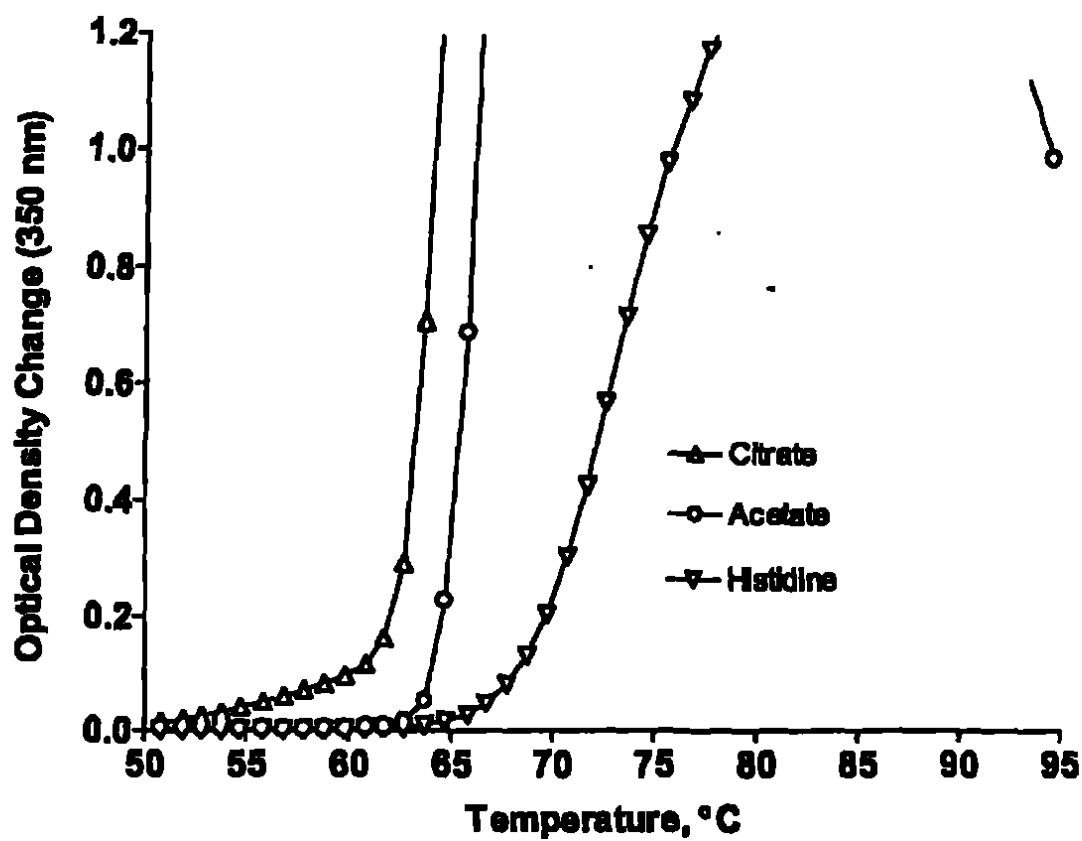


Figure 2.



CONSULADO DE COLOMBIA
WASHINGTON, D C

9597
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No. 1040

EL CONSULADO DE COLOMBIA
CERTIFICA

Que es auténtica la firma de PATRICK O HATCHETT
quien el día 19 DE ABRIL DEL 2000
desempeñaba las funciones de OFICIAL DE AUTENTICACIONES DEPARTAMENTO DE ESTADO
la cual aparece al pie de los documentos anexos relacionados con REPRESENTACION Y PODER.

Dado en Washington, D.C. a los VEINTICINCO (25) días del mes de
ABRIL del año dos mil (2000)

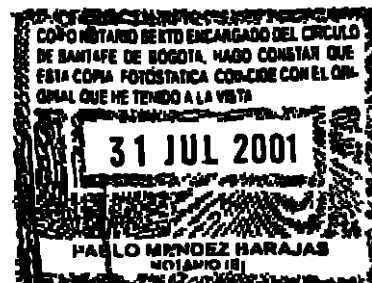
Pagado Impuesto de Timbre

Pagado Derecho Consular

Total

(El Consulado no asume ninguna
responsabilidad por el contenido de los
documentos anexos)

Jaqueline Espitia Arias
JAQUELINE ESPITIA ARIAS
Vicecónsul



NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2000 JUN -7 A 8 43

JEFE DE SECCIONES
SANTAFE DE BOGOTA D.C.



MINISTERIO DE DEFENSA
EXTERIOR

2706352

JARUQUE ESPITA
COMANDANTE EN JEFE
REGIMIENTO DE INFANTERIA
LAS FUERZAS ARMADAS

COMO REPRESENTANTE ENCARGADO DEL CUAL
DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE
ESTA COPIA FOTOSTATICA COINCIDE CON EL ORIGINAL QUE ME FUE ENTREGADO A LA VISTA

31 JUL 2001

PABLO MENDEZ BARRALAS



64

STATE OF CONNECTICUT
COUNTY OF New Haven

} ss. West Haven

I, Deborah Collins Asst., Clerk of the Town of West Haven

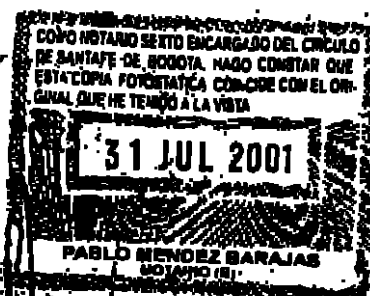
in said County, do hereby certify that Elaine Porter
by and before whom the foregoing (or annexed) acknowledgment was taken, was at the time of taking the same
a notary public (or other officer) residing (or authorized to act) in said County, and was authorized by the laws
of said State to take and certify acknowledgments in said State, and, further, that I believe that the signature to
the certificate of acknowledgment is genuine.

IN TESTIMONY WHEREOF, I have hereunto set my hand and affixed the seal of the State and Town this

13th day of April 1901 2000

Deborah Collins
Asst. Town Clerk

ADKINS FORM 223



00034295-4
United States of America

4517
65 5726
28



DEPARTMENT OF STATE

To all to whom these presents shall come, Greetings



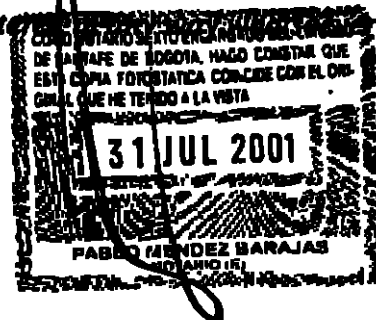
I Certify That the document hereunto annexed is under the Seal of the State(s) of Connecticut, and that such Seal(s) is/are entitled to full faith and credit.*

In testimony whereof, I, Strobe Talbott, Acting Secretary of State, have hereunto caused the seal of the Department of State to be affixed and my name subscribed by the Assistant Authentication Officer, of the said Department, at the city of Washington, in the District of Columbia, this nineteenth day of April, 2000.

Strobe Talbott
Acting Secretary of State
By Pablo Mendez Barajas
Assistant Authentication Officer,
Department of State

Issued pursuant to CHOIV, State of
Sept. 15, 1789, 1 Stat. 68-69; 22
USC 2657; 22 USC 2651a; 5 USC
301; 28 USC 1733 et. seq.; 8 USC
1443(f); RULE 44 Federal Rules of
Civil Procedure.

*For the contents of the annexed document, the Department assumes no responsibility
This certificate is not valid if it is removed or altered

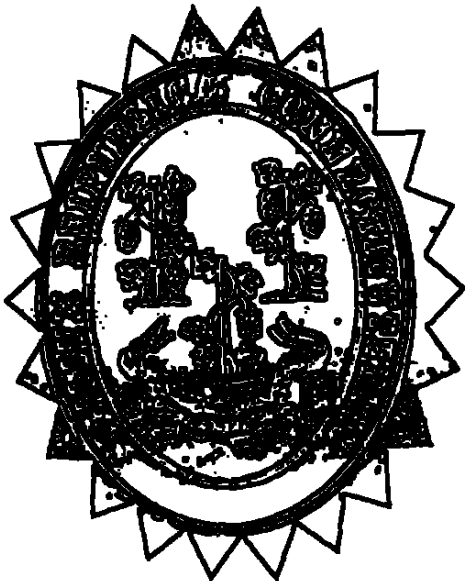


#66

**STATE OF CONNECTICUT
OFFICE OF THE SECRETARY OF THE STATE**

I, **SUSAN BYSIEWICZ**, Secretary of the State of Connecticut and keeper of the seal thereof,

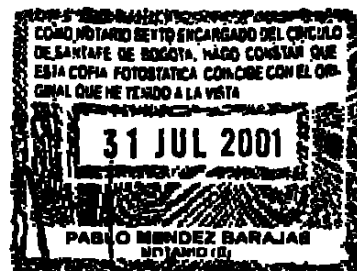
DO HEREBY CERTIFY, that **DEBORAH COLLINS**, was duly elected or appointed to the office of **ASSISTANT CITY AND TOWN CLERK**, for the town of **WEST HAVEN** in the County of **NEW HAVEN** in said state, for the term of **DECEMBER 5, 1999 to DECEMBER 2, 2001**;



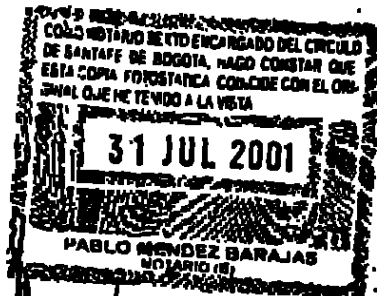
and that to his/her acts and attestations as such, full faith and credit are and ought to be given in and out of Court.

In Testimony Whereof, I have hereunto set my hand and affixed the Great Seal of the State of Connecticut, at Hartford, on April 14, 2000.

Susan Bysiewicz
Secretary of the State



75
67



Subscribed and sworn to before me this 10th day of April, 2001.

Elaine Porter
ELAINE PORTER
NOTARY PUBLIC
MY COMMISSION EXPIRES JUNE 30, 2001



CAVELIER

ABOGADOS

EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTÁ 8, COLOMBIA

For patents, utility models, industrial designs, trademarks, commercial names, trade emblems, slogans.

Para patentes, modelos de utilidad, diseños industriales, marcas de fábrica, nombres comerciales, enseñas, lemas comerciales.

REPRESENTACION Y PODER

REPRESENTATION AND POWER OF ATTORNEY

I (we), the undersigned/Yo (nosotros), abajo firmado (a)

BAYER CORPORATION

of/domiciliado (a) en **100 BAYER ROAD, PITTSBURGH, PENNSYLVANIA 15205,**

UNITED STATES OF AMERICA

por el presente nominamos a EMILIO FERRERO, tpa. 31929; GONZALO PERDOMO, tpa. 831; LUZ CLEMENCIA DE PAEZ, tpa. 23555 y GERMAN CAVELIER, tpa. 832, domiciliados en la Carrera 4ª N° 72-35, Bogotá, Colombia, en obediencia a lo dispuesto en el párrafo 1º del Artículo 543 del Código de Comercio, el primero como principal y los demás como suplentes, en su orden, como mis (nuestros) representantes en todos los asuntos de propiedad industrial con facultad para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales. El primer representante suplente reemplazará al principal en caso de ausencia o impedimento temporal o definitivo de aquél. La misma regla se aplicará cuando el segundo representante suplente haya de reemplazar al primero, o el tercero al segundo. En tales casos, el representante principal, o el primero, o segundo suplente, en su caso, o ambos, declararán su intención de no ejercer la representación mediante declaración autenticada por Notario Público en Colombia, o por Notario u otro Funcionario Público, o Consol de Colombia, en el extranjero; y al se tratare de impedimento, con el certificado respectivo. Si alguno de los nombrados faltare definitivamente, el siguiente tomará su lugar. Cuando cesare la ausencia o impedimento del representante principal, o de su primero o segundo suplentes, en su caso, podrán ejercer la representación, en su orden, uno u otros según el caso, esumiéndola por el solo hecho de ejercerla, cesando en ese momento el ejercicio de la representación por el primero, segundo o tercer suplente en su caso.

Además, confiero (conferimos) poder a los abogados arriba nombrados, para obtener la protección de mis (nuestras) propiedad industrial y contra la competencia desleal, a cuyo efecto autorizo (amos) a los dichos apoderados para que me (nos) representen ante cualesquiera entidades o personas, ejerzan o no jurisdicción, especialmente ante las del orden administrativo, contencioso-administrativo y judicial, así como los tribunales de arbitramento, en todo lo concerniente a los siguientes asuntos: a) Obtención de patentes de invención, modelos de utilidad y diseños industriales; el registro de marcas de fábrica, colectivas, defensivas, de servicio, lemas comerciales, nombres comerciales, enseñas, variedades vegetales y denominaciones de origen; su renovación, traspaso, cambio de nombre o domicilio, cancelación voluntaria; y el registro de licencias de uso de esos derechos; b) Las acciones de competencia desleal, protección del consumidor, oposiciones u observaciones, cancelación administrativa y judicial, nulidad, medidas cautelares, concesión de licencias, la tutela, la protección de secretos industriales, y en general los juicios civiles, penales, administrativos o contencioso-administrativos relacionados con estos derechos; acciones y procesos que promovamos o se nos promuevan. Y para que en todos los asuntos arriba determinados, puedan suscribir este mandato, transcribir, conciliar, admitir los hechos del proceso, desistír, cancelar, recibir, renunciar, y ratificar los actos de agencias oficiales.

In due compliance with the terms of paragraph 1st. of article 543 of the Commercial Code, do hereby appoint EMILIO FERRERO, tpa. 31929; GONZALO PERDOMO, tpa. 831; LUZ CLEMENCIA DE PAEZ, tpa. 23555 and GERMAN CAVELIER, tpa. 832, domiciled at Carrera 4ª N° 72-35, Bogotá, Colombia, the first as principal and the others as alternates, in the order of their appointment, as our representatives for all industrial property matters with faculty to receive notifications and to appoint attorneys in and out of Court. The first alternate shall replace the principal in case of his absence or of temporal or definitive impediment. The same rule will apply when the second alternate representative is to replace the first one, or when the third is to replace the first or second alternates in their turn, or both of them, second. In such cases, the principal representative, or the first or second alternates in their turn, or both of them, shall state their intention not to act as representatives through a declaration given before a Notary Public in Colombia, or before a Notary or another Public Official or a Colombian Consul abroad; and in case of impediment, the appropriate certificate shall suffice. If any of the appointees were to be definitely absent, the following alternate shall take his place. When the absence or impediment of the principal representative, or of the first or second alternate in their turn ceases, the representation can be taken again in their sequence, by the principal, the first or second alternate depending on case, and the mere fact of its exercise will suffice. The exercise of the representation by the first, second or third alternates in their turn shall end at such time.

In addition, we grant a Power of Attorney in favor of the above-named attorneys-at-law so that they may obtain the protection of my (our) industrial property and against the unfair competition, for which purpose I (we) authorize the said attorneys to represent me (us) before any authorities or persons, with or without jurisdiction, specially those administrative, administrative contentious and judicial, as well as before Arbitration Court, in all matters concerning the following: a) Obtainment of patents, utility models and industrial designs; the registration of trademarks, collective, defensive and service marks, slogans, commercial names, trade emblems, vegetal varieties and denominations of origin; their renewal, transfer, change of name or domicile, voluntary cancellation, and the registration of use of the above rights; b) Actions of unfair competition, consumer, consumer, oppositions or observations, cancellation administrative, nullity, infringement, granting of licenses, the action for unfair competition, the protection of trade secrets, and in general the civil, penal and administrative suits relating to those rights; actions and processes that we promote or against me (us). And that in all the above matters, they may subscribe this Power of Attorney, compromise, transcribe, conciliate, admit the facts, desist, cancel, receive, resign, and ratify the acts of official agencies with a power of attorney.

Given and signed this/Dado y firmado hoy 11th day of April, 2000.

In/en West Haven, Connecticut, United States of America

Signature

Name:

Title:

James M. Garman

Vice President, Patents and Licensing

Subscribed and sworn to before me this 11th day of April, 2000.

• CAVELIER ABOGADOS
(Minuta N° 12.1.1.2.6.USA)

Elaine Foster
ELAINE FOSTER
NOTARY PUBLIC
MY COMMISSION EXPIRES JUNE 30, 2001

32

CERTIFICADO NOTARIAL (INDIVIDUAL)

A los _____ días del mes de _____ de 19__

ante mí, Notario Público de _____

compareció(eron) personalmente _____

a quien(es) doy fe de conocer y me consta que es(son) la(s) persona(s) firmante(s) del documento que precede, y quien(es) tiene(n) capacidad legal para otorgarlo.

Notario Público _____

* Los documentos deben identificarse con fecha y origen.

CERTIFICADO (PARA SOCIEDAD)

De conformidad con las leyes de Estados Unidos de America, yo soy el funcionario que tiene la facultad legal de certificar acerca de la existencia y representación de la Sociedad/Compañía.

denominada BAYER CORPORATION

Certifico:

1. Que he visto los siguientes documentos:

Ver ANEXO

2. Que dichos documentos prueban lo siguiente:

2.1. Que BAYER CORPORATION

es una Sociedad/Compañía legalmente constituida y existente de acuerdo con las leyes del Estado de Indiana,

Estados Unidos de América

2.2. Que el domicilio de dicha Sociedad/Compañía es

100 Bayer Road, Pittsburgh, Pennsylvania15205, Estados Unidos de América

2.3. Que el poder otorgado por el presente otorga el presente poder según los fines y para los fines de la Sociedad de la mencionada Sociedad/Compañía.

2.4. Que el Sr. Jeffrey M. Greenman

está(n) autorizado(s) para otorgar el presente Poder.

Ciudad: West HavenEstado: ConnecticutHoy: 12 Abril de 2000

Signature: _____

Nombre del funcionario:

Cargo:

Firma: _____

* Los documentos deben identificarse con fecha y origen.

1. Articles of Incorporation fechados 6 Marzo, 1996 en Indianapolis, Indiana - E.U.U.2. Articles of Merger de Bayer USA, Inc. y Bayer Corporation4 Miles Financial Services a Miles Inc. yCambio de nombre a Bayer Corporation, certificadopor el Secretario de Estado de Indiana en Indianapolisel 10 Abril 1995.3. Estadutos de Bayer Corporation de 10 Abril 19954. Resolución de la Junta Directiva de Miles Inc.de una reunión de la Juntafechada 22 Nov. 1991 autorizando funcionarios parafirmar documentos legales en nombre de laSociedad

NOTARIAL CERTIFICATE (INDIVIDUAL)

On this _____ day of _____, 19__

before me, a Notary Public of _____

personally appeared _____

to me known, and known to me to be the person(s) who executed that foregoing document, and who has(have) legal capacity to execute it.

Notary Public _____

* Documents must be identified with date and origin.

CERTIFICATE (FOR CORPORATION)

According to the laws of the United States of America, I am the official who has the legal function to certify about the existence and representation of Corporation/Company

named BAYER CORPORATION

I Certify:

1. That I have seen the following documents:

- See below -

2. That the above documents duly prove the following:

2.1. That BAYER CORPORATION

is a Corporation/Company legally constituted and in existence in accordance with the laws of the State of Indiana,

United States of America

2.2. That the domicile of said Corporation/Company is

100 Bayer Road, Pittsburgh, Pennsylvania 15205,United States of America

2.3. That the purpose of purposes for which the present Power of Attorney is granted are comprised within the corporation purposes set forth in the charter of the mentioned Corporation/Company.

2.4. That Mr. (Messrs.) Jeffrey M. Greenman

is(are) authorized to grant the present Power of Attorney.

City West HavenState ConnecticutThis 12th day of April, 2000.

Signature: _____

Name of Attesting Officer: George J. LykosPosition held: Vice President, Assistant GeneralCounsel, and Assistant Secretary

Signature: _____

* Documents must be identified with date and origin.

1. Articles of Incorporation of Miles Inc., filed and approved 6 March 1996,Indianapolis, Indiana, U.S.A.2. Articles of Merger of Bayer U.S.A. Inc., and Bayer Corporation,and Miles Financial Services Inc., into Miles Inc., and changeof name of the survivor to Bayer Corporation; certified by theSecretary of State of the State of Indiana, at Indianapolis, Indiana,10 April 1995.3. Bylaws of Bayer Corporation effective as of April 1, 1995.4. Resolution of the Board of Directors of Miles Inc., now Bayer Corporation,at a meeting of the Board held 22 November 1991, authorizing corporateofficers to execute legal instruments on behalf of the corporation.firmar documentos legales en nombre de laSociedad

TRADUCCIÓN OFICIAL N° 0806001 del inglés al español de los sellos y autenticaciones que aparecen en el documento de poder otorgado por BAYER CORPORATION, preparada por Jaime Armand Garavito Burgos, Traductor Oficial debidamente reconocido por el Ministerio de Relaciones Exteriores y aprobado por el Ministerio de Justicia de la República de Colombia mediante Resolución N° 034 de enero 11 de 1983

Documento escrito en inglés y español por medio del cual BAYER CORPORATION, otorga poder a favor de EMILIO FERRERO, GONZALO PERDOMO, LUZ CLEMENCIA DE PÁEZ y GERMÁN CAVELIER. Dado y firmado el 11 de abril del 2000 en West Haven, Connecticut, Estados Unidos de América (firmado) Jeffrey M. Greenman

Nombre: Jeffrey M. Greenman

Cargo: Vicepresidente, Patentes y Licenciamiento

Suscrito y juramentado en mi presencia hoy 11 de abril del 2000. (firmado) ELAINE PORTER, Notario Público

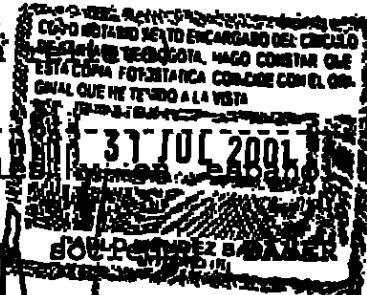
(Sello notarial en tinta y sello notarial en relieve)

CERTIFICACIÓN NOTARIAL (SOCIEDAD) en inglés

referente a la existencia legal de la BAYER CORPORATION y su representante autorizado Jeffrey M. Greenman, expedida el 12 de abril del 2000 en West Haven, Connecticut, Estados Unidos de América

Suscrito y juramentado en mi presencia hoy 10 de abril del 2000. (firmado) ELAINE PORTER, Notario Público

(Sello notarial en tinta y sello notarial en relieve)



7/10

(Aparece escudo impreso)

ESTADO DE CONNECTICUT

]

declaración juramentada

West Haven

CONDADO DE New Haven

Yo, Deborah Collins, Subsecretario de la ciudad de West Haven en dicho condado, por medio del presente certifico que Elaine Porter por quien ante quien se hizo el reconocimiento anterior (anexo), era en el momento de hacerlo un notario público (u otro funcionario) residente (o autorizado para actuar) en dicho Condado y estaba autorizado por las leyes de dicho Estado para hacer y certificar reconocimientos en dicho Estado. Certifico además que creo que la firma en dicho certificado de reconocimiento es auténtica.

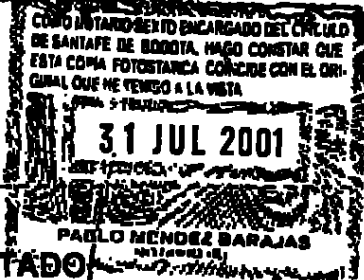
EN TESTIMONIO DE LO CUAL, he estampado mi firma y el sello del estado y ciudad hoy 13 de abril del 2000.

(firmado) Deborah Collins, Subsecretaria del Poblado

(Aparece sello en relieve)

ADAMS FORMATO 223

ESTADO DE CONNECTICUT
OFICINA DEL SECRETARIO DE ESTADO



Yo, SUSAN BYSIEWICZ, Secretario del Estado de Connecticut y guardián de su sello,

POR MEDIO DEL PRESENTE CERTIFICO que DEBORAH COLLINS, fue debidamente electa o nombrada al cargo de SUBSECRETARIA DE CIUDAD Y DE PUEBLO, para la población de WEST HAVEN en el Condado de NEW HAVEN en dicho estado, por el período comprendido entre DICIEMBRE 5, 1999 y DICIEMBRE 2, 2001;

(Aparece adherido sello dorado)

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y que se da y deberá darse plena fe y crédito a sus actos y testimonios como tales, tanto procesal como extraprocesalmente. En testimonio de lo cual, he estampado mi firma y el Gran Sello del Estado de Connecticut en Hartford el 14 de abril del 2000.
(firmado) Susan Bysiewicz, Secretaria del Estado

N° 00034295-4

Estados Unidos de America
(Aparece escudo)

DEPARTAMENTO DE ESTADO

A todos aquellos a quienes los presentes llegaren, Saludos:

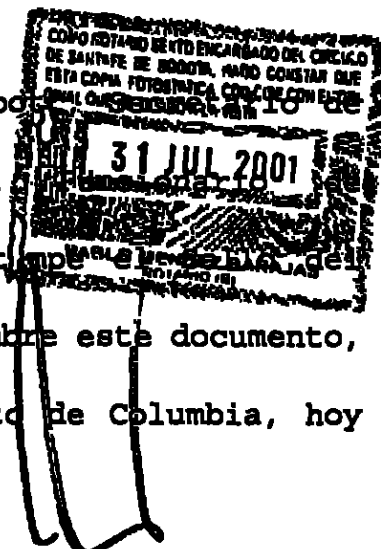
Certifico que el documento adjunto al presente se encuentra bajo el(los) Sello(s) del Secretario de Estado del Estado de Connecticut y que debe darse plena fe y crédito a dicho(s) Sello(s).*

En testimonio de lo cual, yo, Strobe Talbot, Secretario de Estado Encargado, he hecho que el Departamento de Estado estampe en el Departamento de Estado y suscriba en mi nombre este documento, en la ciudad de Washington, en el Distrito de Columbia, hoy diecinueve de abril del 2000.

(firmado) Strobe Talbott, Secretario de Estado

(Firmado) Patrick O. Hatchett, Funcionario Asistente de Autenticaciones Departamento de Estado.

*El Departamento no asume ninguna responsabilidad por el contenido del documento anexo.



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Este certificado no es válido si se le remueve o altera cualquier forma.

(Sello en relieve del Departamento de Estado de los Estados Unidos de América)

(El formulario detalla las disposiciones legales bajo las cuales fue emitido e impreso)

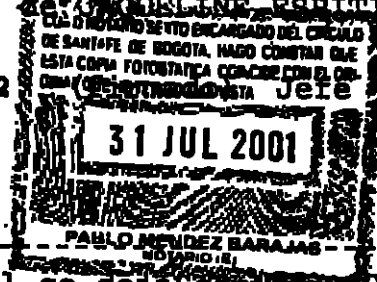
N° 1040. EL CONSULADO DE COLOMBIA en Washington, D.C., Estados Unidos de América, certifica las funciones y firma de PATRICK O. HATCHETT, el 25 de abril del 2000. Pagado impuesto de timbre y derechos consulares. (firmado) JAQUELINE ESPITIA ARIAS, Vicecónsul

EL MINISTERIO DE RELACIONES EXTERIORES en Santa Fe de Bogotá, D.C. certifica la firma y las funciones de JAQUELINE ESPITIA, el 07 de junio del 2000. N° 276352 Jefe de Legalizaciones.

Es traducción fiel y completa de la cual se deja copia en los archivos de esta oficina para su confrontación y a la cual me remito.

Santa Fe de Bogotá, D.C., junio 08 del 2000

COLO: 9597-801-014-8
WPCL9597 - ID # 156



Jaime A. Garavito B.
JAIMÉ A. GARAVITO B.
TRADUCTOR E INTERPRETE OFICIAL
Bo. 034 de Enero 11 de 1983
MINISTERIO DE JUSTICIA

MINISTERIO DE RELACIONES
EXTERIORES

278511

JAI ME GARAYITO
CUYA FIRMA APARECE EN LA
PRIMERA PÁGINA DESEMPLEA
LAS INSTRUCCIONES ANTERIORES

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2000 JUN -01 13:12:54

DE FIC DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

COMO NOTARIO SEXTO DEL CIRCULO DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE LA(S) ANTERIOR(ES) FIRMA(S) PUESTA(S) POR

Jaime Garayito

IDENTIFICADOS CON C.C. No. (n)

ERON AUTENTICAS)

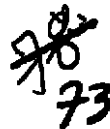
SANTAFE DE BOGOTA, 09 JUN 2000

NOTARIA SEXTO
PABLO MENDEZ
BARAJAS

COMO NOTARIO SEXTO ENCARGADO DEL CIRCULO DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE ESTA COPIA FOTOSTATICA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA

31 JUL 2001

PABLO MENDEZ BARAJAS
NOTARIO DEL



Carrera 13 No. 27-00 Pisos 5, 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail: Info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

2020
Bogotá DC

Folio 70

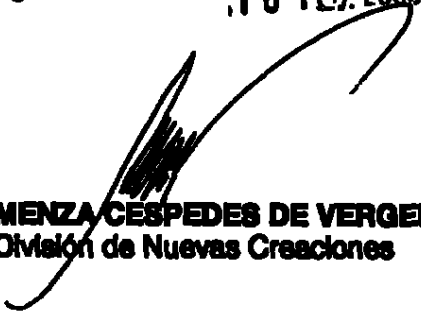
Doctor(a)
EMILIO FERRERO.
Apoderado(a)

REFERENCIA:	Radicación No.	01-50548
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No.	321
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

Cúmplase

Dado en Bogotá DC, a los **18 FEB. 2003**


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

202048

529

Carrera 13 No. 27-00 Piso 5 y 10
Conmutador: 334 12 21-2-3-4
Fax: 281 31 25 e-mail: info@sic.gov.co
Santa Fe de Bogotá D.C. - Colombia