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(54) Title: PHARMACEUTICAL FORMULATIONS OF CNTF (57) Abstract Pharmaceutical formulations of ciliary neurotrophic factor (CNTF) are described which are useful for therapeutic treatment of damage to the peripheral nervous system. Formulations of CNTF ideally suited for intrathecal administration are provided.		

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PHARMACEUTICAL FORMULATIONS OF CNTF

TECHNICAL FIELD OF THE INVENTION

5 This invention relates to pharmaceutical formulations of ciliary neurotrophic factor suitable for therapeutic treatment of damage to the peripheral nervous system.

10 BACKGROUND OF THE INVENTION

 The peripheral nervous system consists of those nerve cells that extend axonal processes outside the spinal cord and brain. The principle nerve cell types in the peripheral nervous system are primary motor
15 neurons innervating skeletal muscle and controlling movement, autonomic neurons (both sympathetic and parasympathetic) innervating the cardiovascular system and other internal organs and regulating their function, and sensory neurons innervating sensory
20 receptors throughout the body and conveying sensations including pain and proprioception.

 Conditions that compromise the survival and proper function of one or more of these types of peripheral nerve cells cause peripheral nerve damage. Such nerve
25 damage may occur from a wide variety of different causes. Nerve damage may occur through physical injury, which causes the degeneration of the axonal processes and/or nerve cell bodies near the site of injury. Nerve damage may also occur because of
30 temporary or permanent cessation of blood flow to parts of the nervous system, as in stroke. Nerve damage may also occur because of intentional or accidental exposure to neurotoxins, such as the cancer and AIDS
 chemotherapeutic agents cisplatin and dideoxycytidine
35 (ddC), respectively. Nerve damage may also occur because of chronic metabolic diseases, such as diabetes or renal dysfunction. Nerve damage may also occur because of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and

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Amyotrophic Lateral Sclerosis (ALS), which result from the degeneration of specific neuronal populations.

Neurotrophic factors are naturally occurring proteins that promote the survival and functional activities of nerve cells. Neurotrophic factors have been found in the target cells to which an innervating nerve cell connects. Such target-derived neurotrophic factors regulate the number of contacts formed between innervating nerve cells and the target cell population, and are necessary for the survival and maintenance of these nerve cells.

Neurotrophic factors are also found in cells that are not innervated. An example of such a neurotrophic factor is CNTF. Human CNTF and the gene encoding human CNTF are described in detail in U. S. patent numbers 4,997,929, and 5,141,856, which are specifically incorporated herein by this reference.

Although the biological role of CNTF has not been conclusively established, CNTF appears to be released upon injury to the nervous system and may limit the extent of injury or neuronal damage. Highly-purified CNTF has been shown to support the survival in cell cultures of chick embryonic parasympathetic, sympathetic, sensory, and motor neurons. There is significant evidence to support that CNTF is a neurotrophic factor for peripheral primary neurons in vivo and in vitro. U. S. patent application serial number 07/735,538 filed July 23, 1991, specifically incorporated herein by reference, shows the surprising effectiveness of systemically administered CNTF to accelerate local recovery at the site of peripheral nerve damage.

The ability of CNTF to protect motor neurons from lesion-induced death may also make it effective in preventing nerve cell degeneration associated with such neurodegenerative diseases as Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis

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(ALS), and Spinal Muscular Atrophy (SMA). U.S. patent application serial number 08/015,218 filed February 8, 1993 and U.S. patent application serial number 08/116,440 filed September 3, 1993, both of which are
5 entitled Methods for Treating Amyotrophic Lateral Sclerosis With CNTF, are specifically incorporated herein by reference. These patents present evidence demonstrating the effectiveness of treating amyotrophic lateral sclerosis in humans with CNTF. In addition,
10 Sendtner et al. (1990) Nature 345:440-441, showed that local application of CNTF prevents lesion-induced death of motor neurons in the rat facial brain stem nucleus. Oppenheim et al. (1991) Science 251:1616-1618, showed that CNTF promoted the in vivo survival of chick spinal
15 motor neurons.

A major problem with the delivery of a therapeutically effective amount of CNTF to a patient for treatment or prevention of peripheral nerve damage is the instability of CNTF in solution. CNTF in
20 solution rapidly precipitates when agitated or subjected to thermal incubation. To the extent that any of the protein is denatured, the effective amount of biologically active CNTF is diminished. Protein integrity must therefore be maintained during
25 manufacture and storage as well as during administration. Proteins are particularly prone to degradation at elevated temperatures. Excipients known to physically stabilize proteins in solutions appear to negatively affect the thermal stability of CNTF.

30 In addition, CNTF is subject to loss from solution by nonspecific adsorption to the surface areas of storage containers and dispensing devices. Such nonspecific binding may occur to a variety of materials including glass and plastics, for example, polyethylene
35 or polypropylene. These materials may be in the form of vials, tubing, syringes, implantable infusion devices, or any other surface which may come in contact

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with CNTF during its manufacture, storage or administration.

For certain applications for the treatment or prevention of peripheral nerve damage in humans it is desirable to administer the CNTF, in formulation, intrathecally. Intrathecal administration necessitates that CNTF must be maintained in an aqueous solution for relatively long periods of time at elevated temperatures. In certain such administrations the CNTF formulations will be maintained at room temperature, while other administrations will actually require that the CNTF formulation will be held at body temperature. These are obviously relatively harsh conditions for a sensitive protein such as CNTF.

In addition to the problems associated with the administration of CNTF, there are also problems associated with its long term storage from the time of manufacture to administration.

Lyophilization is one method of enabling the long term storage of biological proteins, impeding degradation, aggregation, and/or nonspecific adsorption. However, the lyophilization process itself often presents difficulties. As the volume of liquid decreases during the freezing process, the effective salt concentration increases dramatically, which tends to denature the protein, reducing effective therapeutic activity upon reconstitution. In addition, formation of ice crystals during the freezing process may cause denaturation and also decrease the effective amount of bioactive CNTF available. The most desired formulation for CNTF must be able to prevent loss of bioactivity of the CNTF during the lyophilization process.

The present invention includes formulations of CNTF which physically and thermally stabilize CNTF against degradation and precipitation under a wide variety of storage and administration regimes.

This invention further includes formulations of

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CNTF in which bioactivity is maintained after lyophilization and reconstitution, and methods of storing biologically active CNTF in solution.

5 BRIEF SUMMARY OF THE INVENTION

The present invention includes therapeutically useful formulations of CNTF which greatly reduce the prior art problems of loss of CNTF from solutions due to chemical degradation, precipitation, and adsorption.

10 The present invention includes three types of stabilized CNTF formulations, characterized as stabilized aqueous formulations, aqueous formulations suitable for intrathecal administration and lyophilization formulations suitable for lyophilization and subsequent reformulations. Also included within
15 the scope of this invention are CNTF containing mixtures that are the result of lyophilization of the lyophilization formulations of the present invention, as well as the same after reconstitution.

20 This invention provides physically and chemically stable aqueous formulations of CNTF. Preferred formulation embodiments comprise aqueous solutions of:

- (a) ciliary neurotrophic factor;
- (b) stabilizing agents;
- 25 (c) a sufficient amount of biologically acceptable salt to maintain isotonicity;
- (d) a buffer to maintain the pH of the formulation from about 4.5 to about 6.0; and
- (e) optionally, a biologically acceptable water
30 soluble carrier.

The pharmaceutical CNTF formulation embodiments of this invention suitable for intrathecal administration comprise aqueous solutions of:

- (a) ciliary neurotrophic factor;
- 35 (b) two or more stabilizing agents;
- (c) a sufficient amount of a biologically acceptable salt to maintain isotonicity; and

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(d) a buffer in an amount sufficient to maintain the pH of the formulation at about 6.0.

This invention further includes pharmaceutical CNTF formulation embodiments suitable for

5 lyophilization that comprise aqueous solutions of:

(a) ciliary neurotrophic factor;

(b) a biologically acceptable bulking agent;

(c) one or more stabilizing agent(s);

10 (d) a buffer in an amount sufficient to maintain the pH of the formulation at about 5.0 to about 7.6; and

(e) a biologically acceptable salt.

Further embodiments of this invention are the lyophilized formulations from which the water has been
15 substantially removed. Upon reconstitution with a reconstituting vehicle, optionally including a biologically acceptable carrier, the lyophilized formulations of the present invention are suitable for administration to patients in need thereof. Such
20 reconstituted solutions of CNTF are also included within the scope of this invention.

Another embodiment of the present invention is a method for the prevention and treatment of peripheral nerve damage by administering a therapeutically
25 effective amount of a human protein ciliary neurotrophic factor formulation as described herein to a patient in need thereof. In particular, the invention provides formulations for administering therapeutically effective amounts of CNTF in order to
30 prevent and treat peripheral nerve damage, including neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, ALS, and SMA. The present invention also includes methods for preventing and treating peripheral nerve damage by administering to a
35 patient in need thereof a therapeutically effective amount of CNTF formulated as described herein.

It is to be understood that both the foregoing

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general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

5 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

10 The present invention includes therapeutically useful formulations of CNTF for the prevention and treatment of peripheral nerve damage, including neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, ALS, and SMA.

15 The present invention includes the use of CNTF as a therapeutic agent by administering a therapeutic composition whose active ingredient consists of CNTF. Such therapeutic compositions include compositions suitable for use in a wide variety of administration regimes which are stable under a variety of storage and
20 administration conditions. When included in most standard prior art formulations, CNTF is chemically unstable even when stored at 4°C. The therapeutic use of CNTF in the treatment of peripheral nerve damage
25 requires formulations which are acceptable for rapid and easy administration to patients in need thereof, readily manufacturable, and stable for a prolonged period of time over a variety of storage conditions.

30 This application describes CNTF formulations suitable for certain situations. The aqueous CNTF formulations described herein are suitable for maintaining the stability and bioactivity of CNTF in aqueous solution. The aqueous formulations suitable for intrathecal administration are suitable for
35 maintaining the stability and bioactivity of CNTF in situations where the formulation is maintained at elevated temperatures for long periods of time and is

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contained in high surface area containers. The lyophilization formulations of CNTF are suitable for maintaining the stability and bioactivity of CNTF when subjected to lyophilization and subsequent
5 reconstitution. The most preferred embodiments of the present invention are aqueous formulations that are stable prior to lyophilization and that have retained their bioactivity following lyophilization, long term storage, and reconstitution.

10 According to the present invention, CNTF may be formulated for use as a therapeutic agent when included in solution with agents that are effective in stabilizing the CNTF against precipitation from solution, thermal degradation and adsorption. Such
15 agents include non-ionic surfactants such as polysorbate-80, as well as propylene glycol, polyethylene glycol, lysine, arginine, cysteine, glutathione, ethanol and other alcohols. The preferred formulations of the present invention also may include
20 other ingredients that function to improve the therapeutic capabilities of the CNTF. Such other ingredients include sodium chloride, glycerol, human serum albumin, sodium phosphate, and tris (hydroxymethyl) aminomethane.

25 As used in the specification, "pharmaceutically effective amount" means an amount of CNTF which is therapeutically effective in various administration regimes in the prevention and treatment of peripheral nerve damage. "Biologically acceptable" applies to
30 materials characterized by the absence of significant adverse biological effects in vivo. "Room temperature" is between about 22°C to about 25°C. "Body temperature" is between about 36°C to about 40°C. "Lyophilizable formulation" refers to an aqueous
35 formulation of CNTF which may be freeze dried to a moisture content of less than about 2% and which retains at least about 70% of the initial CNTF

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bioactivity upon reconstitution. "Isotonic" refers to a solution having approximately the same osmotic pressure as blood serum, about 300 mM per liter. A "carrier" is any biologically acceptable emulsifier, dispersing agent, surfactant, or protein which decreases adsorption of CNTF to a surface.

The preferred form of CNTF is human CNTF (hCNTF). The most preferred form of hCNTF is recombinant hCNTF (rhCNTF). Methods of obtaining CNTF suitable for use in the formulations of this invention are known to those skilled in the art. For example, suitable rhCNTF may be produced by the recombinant DNA procedures described in U.S. patent number 5,141,856, specifically incorporated herein by this reference. The CNTF should be at least 65% pure, and most preferably at least 98% pure. The purity of isolated CNTF for use in the formulations may be determined by silver-stained SDS-PAGE or other means known to those skilled in the art.

A. Aqueous CNTF formulations.

In the aqueous CNTF formulations provided, CNTF is present in therapeutically effective amounts. Preferably CNTF comprises from about 0.01 to about 8.0 mg/ml.

The aqueous CNTF formulations optionally include carriers. The presence of the carrier in the formulation reduces or prevents CNTF adsorption to various surfaces. The need for a carrier depends upon the concentration of CNTF in the aqueous formulation. Suitable carriers include, but are not limited to, polysorbates such as Tween® 80 and proteins such as serum albumin. Further, amino acids such as lysine and arginine have been shown to prevent adsorptive losses of CNTF. In one embodiment of the invention, human serum albumin (HSA) is particularly preferred. In another embodiment of the invention, Tween® is preferred. In yet another embodiment of the invention,

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a combination of lysine and arginine is preferred.

The CNTF formulations may also contain a sufficient amount of a chemically stabilizing agent such as glycerol. Glycerol has been shown to physically stabilize CNTF formulations from degradation resulting from repeated freezing and thawing cycles, and from degradation when exposed to high temperatures (37°C or above). In the absence of a physically stabilizing agent such as glycerol, CNTF in solution must be stored at -20°C to maintain biological activity.

The aqueous CNTF formulations further contain a biologically acceptable buffer to maintain solution pH. The preferred stable CNTF formulation is buffered with a biologically acceptable buffer to a pH between 4.5 to about 8.0, most preferably between about 5.0 and 7.6. Depending on the specific formulation, suitable buffers include citric acid, tris (hydroxymethyl) aminomethane, citrate, acetate, phosphate, tromethamine, and histidine. The preferred amount of buffer will vary depending on the type of buffer used and its buffering capacity. The buffer should be present in the formulation in sufficient quantity to maintain the preferred pH range. The preferred concentration of buffer for stable CNTF formulations is 1 to 50 mM.

In one embodiment of the aqueous CNTF formulation of the present invention, a non-ionic surfactant is added to stabilize CNTF in solution upon agitation. In one embodiment of formulated CNTF, the non-ionic surfactant is polysorbate-80 (Tween® 80). The preferred concentration of Tween® 80 was found to increase with increasing CNTF concentration. In one embodiment of the present invention, CNTF is present in a concentration range of between 0.01 - 8.0 mg/ml and the non-ionic surfactant is polysorbate-80 (Tween® 80) in the concentration range of between 0.1 - 1.0% weight/volume (w/v). In a more preferred embodiment of

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the present invention, the formulation further comprises glycerol in the concentration range between 4 - 20%. In a further preferred embodiment of the present invention, the formulation comprises 0.01 - 8.0 mg/ml CNTF, 0.2 - 0.8% (w/v) Tween® 80, 5 - 15% (w/v) glycerol, human serum albumin (HSA) in the concentration range of 0.01 - 5% (w/v), 1-50 mM sodium phosphate, pH 6-8, 1 - 50 mM tris (hydroxymethyl) aminomethane, and 100 - 400 mM sodium chloride.

In a second embodiment of the aqueous CNTF formulation of the present invention, propylene glycol is added to prevent precipitation of CNTF in solution. In one embodiment of the present invention, CNTF is present in the formulation in the concentration range of between 0.01 - 8.0 mg/ml and propylene glycol is present in the concentration range of between 1 - 30% (w/v). In a more preferred embodiment of the present invention, the formulation is further comprised of glycerol in the concentration range between 4 - 20% (w/v). In a further preferred embodiment of the present invention, the formulation is comprised of 0.01 - 8.0 mg/ml CNTF, 20 -25% (w/v) propylene glycol, 10 - 20% (w/v) glycerol, HSA in the concentration range of 0.01 - 5% (w/v), 1 - 50 mM sodium phosphate, pH 6-8, 1 - 50 mM tris (hydroxymethyl) aminomethane, and 100 - 400 mM sodium chloride.

In a third embodiment of the aqueous CNTF formulation of the present invention, an alcohol is added to stabilize CNTF in solution upon agitation. Such alcohols that may be used include ethanol, propanol, t-butanol, and other simply alkyl alcohols. In one embodiment of the formulation of the present invention, the alcohol is ethanol. In a preferred embodiment of the present invention, CNTF is present in the concentration range of between 0.01 - 8.0 mg/ml and ethanol is present in the concentration range of 0.1 - 20% (v/v). In a more preferred embodiment of the

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present invention, the formulation further comprises glycerol in the concentration range between 4 - 20% (w/v). In a further preferred embodiment of the present invention, the formulation is comprised of 0.01 - 8.0 mg/ml CNTF, 8 - 13% (v/v) ethanol, and 5 - 20% (w/v) glycerol, HSA in the concentration range of 0.01 - 5% (w/v), 1 - 50 mM sodium phosphate, pH 6-8, 1 - 50 mM tris (hydroxymethyl) aminomethane, and 100 - 400 mM sodium chloride.

10 B. Aqueous CNTF formulations suitable for Intrathecal Administration.

Several embodiments of the CNTF formulation of the present invention are useful for intrathecal delivery.

15 The intrathecal CNTF formulations of the present invention represent a major advance in solving the problems of CNTF instability and surface adsorption upon incubation and agitation. An intrathecal formulation requires chemical, physical, and thermal stability under all conditions associated with intrathecal delivery via an external or implantable pump. Further, biological activity must not be decreased through surface adsorption upon incubation.

20 CNTF formulations appropriate for intrathecal delivery must also contain a sufficient amount of biologically acceptable salt to maintain fluid tonicity. Preferably, the CNTF formulation contains sufficient salt to be isotonic, within physiologically acceptable limits, with human blood or cerebral spinal fluid. The preferred salt is sodium chloride (NaCl), but other biologically acceptable salts may be used, such as potassium chloride (KCl), calcium chloride (CaCl₂), and magnesium chloride (MgCl₂). The salt may be one salt or a combination of salts. A preferred formulation comprises 100 to 400 mM salt of the aqueous formulation.

35 One intrathecal formulation of the present

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invention is comprised of 0.01 - 8.0 mg/ml CNTF, 10-13% (v/v) ethanol, and 5% lysine. In a preferred embodiment, the intrathecal formulation is further comprised of 10 mM phosphate (pH 7.0), 150 mM NaCl, and 0.1 - 20% glycerol.

An additional intrathecal formulation is comprised of 0.01 - 8.0 mg/ml CNTF, 20-25% propylene glycol, and 10% glycerol. In a more preferred embodiment, the intrathecal formulation is further comprised of 3-5% lysine. In a preferred embodiment, the formulation is further comprised of 10 mM citrate acid (pH 6.0) and 150 mM NaCl.

An additional intrathecal formulation is comprised of 0.01 - 8.0 mg/ml CNTF, 4% polyethylene glycol 8000 (PEG 8000), and 10% glycerol. In a preferred embodiment, the formulation is further comprised of 5% lysine. In a more preferred embodiment, the formulation is further comprised of 10 mM citrate acid (pH 6.0) and 150 mM NaCl.

A final intrathecal formulation is comprised of 0.1 - 8.0 mg/ml CNTF, 2% PEG 8000, 10% propylene glycol, and 10% glycerol. The preferred embodiment is further comprised of 5% lysine, 10 mM citrate acid (pH 6.0), and 150 mM NaCl.

As described above, the therapeutic compositions of this invention may be administered intrathecally by continuous infusion from an implanted or an external pump. Other effective administration forms, such as parenterally slow-release formulations, parentally by injection, inhalant mists, orally active formulations, or suppositories, are also envisioned.

C. Lyophilizable CNTF formulation.

The most preferred embodiments of the CNTF formulation of the present invention are specifically formulated to remain stable and bioactive during and after lyophilization, and upon reconstitution of the

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lyophilized material. The aqueous lyophilized formulations of this invention are particularly useful for providing long term storage of CNTF. The lyophilizable formulations of this invention comprise
5 CNTF, a biologically acceptable bulking agent, a buffer to maintain the pH of the formulation from about 5.0 to about 7.5, biologically acceptable salt, and optionally a biologically acceptable water soluble carrier.

CNTF is present in the lyophilizable formulations
10 over the same concentration range as in the aqueous formulations described above. The bulking agent generally provides mechanical support by allowing the matrix to maintain its conformation during and after the freeze drying process. One or more sugars may be
15 used as the bulking agent. Sugars, as used herein, include but are not limited to, monosaccharides, oligosaccharides and polysaccharides. Examples of suitable sugars include fructose, glucose, mannose, ribose, xylose, maltose, lactose, sucrose, and dextran.
20 Sugar also includes sugar alcohols, such as mannitol, sorbitol, inositol, dulcitol, xylitol, and arabitol. Mixtures of sugars may also be used in accordance with this invention. In one embodiment of the lyophilization formulation of the invention,
25 polyethylene glycol of average molecular weight 3500 (PEG 3500) is preferred. The most preferred bulking agent of the present invention is sucrose.

Ideally, the choice of buffer takes into account potential pH shifts during lyophilization caused by
30 sequential crystallization of buffer components. For example, with phosphate buffers, the basic component has a higher eutectic point than the acidic component, hence it crystallizes out first and the pH drops. This behavior may be acceptable and perhaps even beneficial
35 in the formulations of the present invention. In one embodiment of the invention, citric acid buffer is preferred because it is thought that both buffer

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components have about the same eutectic point, resulting in very little pH fluctuation as the temperature drops. Other suitable buffers are the amino acids histidine and cysteine.

5 The lyophilizable formulations may also comprise a biologically acceptable salt. The salt, which may be selected from the same salts useful in the aqueous formulations described above, is present in the lyophilizable formulations at the same or reduced
10 concentrations as that in the aqueous formulations. Because the salt concentration may increase during lyophilization, it may be desirable to reduce the concentration of salt present in the lyophilizable formulations to prevent protein denaturation.

15 Reductions in salt concentration in the lyophilizable formulations may be compensated for during reconstitution so as to provide a final formulation sufficiently isotonic to be suitable for administration into an individual.

20 Optionally, the lyophilizable formulations comprise a biologically acceptable water soluble carrier. The carriers and the concentration of carriers which may be used in the lyophilizable formulations of this invention are the same as those
25 that are suitable for use in the aqueous formulations.

 The lyophilizable formulations of this invention are generally lyophilized to a residual moisture content of less than about 2%; however, formulations which retain CNTF biological activity at higher or
30 lower amounts of moisture content are also contemplated.

 An example of a formulation of CNTF which retains bioactivity after lyophilization and reconstitution is comprised of 0.5 mg/ml CNTF, 85 mg/ml sucrose, 2.0
35 mg/ml polysorbate 80 (Tween® 80), 4.0 mg/ml cysteine, 0.1 mg/ml disodium EDTA, and 2.0 mg/ml citric acid (pH 5.0).

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A further example of a formulation of CNTF which retains bioactivity after lyophilization and reconstitution is comprised of 0.5 mg/ml CNTF, 80 mg/ml polyethylene glycol 3350 (PEG 3350), 50 mg/ml lysine, 20 mg/ml arginine, 4 mg/ml cysteine, 0.58 mg/ml sodium chloride, and 1.6 mg/ml histidine (pH 7.5). Any higher molecular weight polyethylene glycol that is a solid at room temperature may be used in this formulation. In addition, glutathione may be used in place of cysteine, which may provide improved solution state stability.

The most preferred embodiment of the invention is a lyophilizable CNTF formulation comprised of CNTF, sucrose, polysorbate 80 (Tween®80), cysteine, disodium EDTA and tromethamine. A specific formulation that represents the most preferred embodiment is comprised of 0.5 mg/ml CNTF, 85 mg/ml sucrose, 2.0 mg/ml polysorbate 80 (Tween® 80), 4.0 mg/ml cysteine, 0.1 mg/ml disodium EDTA, and 2.0 mg/ml tromethamine, buffered to pH 7.6.

The lyophilized CNTF formulations of this invention are also useful as a component of a kit to provide a convenient and economical way of providing stable lyophilized CNTF in a form which may be rapidly and easily reconstituted in an appropriate vehicle for administration to a patient in need of treatment. In addition to the lyophilized CNTF formulation, the kits of this invention also comprise a reconstituting vehicle. The reconstituting vehicle may comprise sterile water and a sufficient amount of salt to make the final reconstituted formula essentially isotonic. The reconstituting vehicle may further comprise additional buffer. The total volume of reconstituting vehicle present in the kit should be sufficient to achieve a final CNTF concentration suitable for administration to an individual in need of treatment.

It is also contemplated that certain formulations containing CNTF are to be administered orally.

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Preferably, CNTF which is administered in this fashion is encapsulated. Preferably, the capsule is designed so that the active portion of the formulation is released at that point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of CNTF. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Regardless of the manner of administration, the specific dose of CNTF is calculated according to the approximate body weight or surface area of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the scope of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. These dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

It should be noted that the CNTF formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

General methods for determining the stability of CNTF-containing solutions upon agitation or heating are described in Example 1. The effect of sodium chloride on the chemical and thermal stability of CNTF is described in Example 2. Example 3 describes the CNTF formulation which contains Tween® 80. This composition is particularly suited to use in pulmonary dosing and in needleless jet injector guns. Example 4 describes

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the formulation of CNTF containing 1 - 25% propylene glycol. Besides loss through degradation and precipitation, CNTF can be lost from solution by adsorption to the surface areas of the container in which the solution is stored and to the surface areas of the dispensing device. Example 5 describes the effect of human serum albumin (HSA) and lysine on prevention of CNTF loss through adsorption. Example 6 describes the formulation of CNTF containing ethanol. Example 7 describes a formulation of CNTF specific for use intrathecally. Also described is the effect of the molecular weight and concentration of PEG species on the physical and thermal stability of CNTF. Example 9 describes experiments in which CNTF formulations were incubated for a period of days in catheter tubes or pump reservoirs. Example 10 describes the physical and chemical stability of the lyophilizable CNTF formulations of this invention under a variety of storage conditions. These experiments establish that the formulations of the present invention represent a major advance in solving the problems associated with the pharmaceutical administration of CNTF.

EXAMPLE 1. MATERIALS AND METHODS.

Material. The CNTF used in all of the Examples was rhCNTF prepared as described generally in U.S. patent number 5,141,856.

Physical Stability. The stability of CNTF compositions when agitated was tested by vortexing CNTF-containing solutions using a Vortex Genie 2 (Scientific Industries, Inc., Bohemia, NY 11716). Precipitation was determined by absorbance spectroscopy at OD 405 using a kinetic microplate reader (Molecular Devices) and a 96 well plate.

Thermal Stability. Thermal stability was determined by spectroscopy after incubating CNTF-containing solutions at room temperature or at 37°C.

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Identification and Quantification of CNTF Using Reverse Phase HPLC (RP-HPLC). Oxidative losses of CNTF under a variety of conditions were determined by RP-HPLC. CNTF was identified and quantified by analyzing 100 μ l samples with a reverse phase HPLC (Hewlett Packard HP 1090 Liquid Chromatograph) equipped with a 4.6 mm x 25 cm L Dynamax 300 Å 5 μ m Analytical Reversed-Phased column with a Dynamax 300 Å 5 μ m 4.6 mm x 1.5 cm guard column and a diode array UV detector.

Identification of CNTF was established by comparing its retention time in the sample with the respective retention time of freshly prepared calibrated standard CNTF solutions made from CNTF from the same lot. The quantity of CNTF in the samples was calculated by comparison to a standard curve obtained with serial dilutions of known concentrations.

Determination of CNTF Concentrations by Cation Exchange Chromatography (CEX). Deamination losses of CNTF under a variety of conditions were determined by CEX.

Determination of CNTF Concentrations by Size Exclusion Chromatography (SEC). Losses of CNTF through protein aggregation were determined by SEC.

EXAMPLE 2. EFFECT OF NaCl ON PHYSICAL AND CHEMICAL STABILITY.

The presence of a salt such as sodium chloride in formulations of this invention provides both fluid tonicity and acts to maintain CNTF in solution. Described herein are experiments determining the specific effect of NaCl on the physical and chemical stability of formulated CNTF.

NaCl was added in an amount equal to the tonicity of the human body. Table 1 shows the effects of varying the concentration of sodium chloride on the rate of precipitation when 1.0 mg/ml CNTF (pH 6.2) was vortexed in the presence of 25% propylene glycol and

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10% glycerol at room temperature (20°C).

No effect was observed on the rate of CNTF precipitation between 10 mM and 400 mM sodium chloride at room temperature. The effect of varying sodium chloride concentration from 10 - 400 mM on CNTF precipitation when 1.0 mg/ml solutions of CNTF (pH 6.2) were vortexed at 37°C is shown in Table 2. The 1.0 mg/ml solutions of CNTF also contained 25% propylene glycol and 10% glycerol.

Precipitation was lowest with 10 and 50 mM sodium chloride, intermediate with 400 mM sodium chloride, and highest with 100 and 200 mM sodium chloride.

EXAMPLE 3. CNTF COMPOSITION WITH POLYSORBATE-80.

The ability of the non-ionic surfactant polysorbate -80 (Tween® 80) to stabilize CNTF in solution against degradation and precipitation was studied. The amount of precipitate formed as a function of time when CNTF (1.0 mg/ml, pH 7.0) in solution was vortexed in the presence of 0.20 - 0.75% Tween® 80 and 10% glycerol is shown in Table 3.

The rate of precipitation formation was significantly delayed with increasing concentrations of Tween® 80, with the maximum inhibition of precipitation achieved with 0.75% Tween® 80. The inhibitory effect of Tween® 80 on precipitation resulting from agitation was seen with CNTF solutions of up to 4 mg/ml (data not shown). The effect of Tween® 80 on thermal stability of CNTF is shown in Table 4. CNTF solutions (1.0 mg/ml, pH 7.0; 10% glycerol) were incubated at room temperature up to 20 hours, and the amount of precipitate formed was measured. Generally, precipitate formation increased with increasing concentration of Tween® 80.

Taken together, the results shown in Tables 3 and 4 indicate that Tween® 80 stabilized CNTF against precipitation as a result of agitation while having a

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negligible negative effect on thermal stability.

EXAMPLE 4. CNTF COMPOSITION WITH PROPYLENE GLYCOL.

5 The ability of propylene glycol to stabilize CNTF solutions upon agitation was studied. Table 5 shows the effect of 25% propylene glycol on precipitate formation when a 1.0 mg/ml CNTF solution (pH 5.0, 5.75, or 6.0) (with 10% glycerol) was vortexed for up to 420 minutes.

10 25% propylene glycol significantly inhibited the formation of precipitate resulting from vortexing.

The ability of propylene glycol to thermally stabilize CNTF in solution was also studied. The effect of varying concentrations of propylene glycol on the amount of precipitate formed at room temperature with vortexing for a solution of CNTF (1.0 mg/ml) is shown in Table 6.

15 In the absence of propylene glycol, CNTF rapidly precipitated from solution. The presence of propylene glycol in the CNTF formulation significantly inhibits CNTF precipitation, with maximum inhibition reached with 25% propylene glycol.

20 For incubations at 37°C for periods of up to 4 weeks, propylene glycol concentrations of between 13 - 25% slightly decreased thermal stability, while 30% propylene glycol increased CNTF precipitation (Table 7).

30 Table 8 shows the effect of 25% propylene glycol on the amount of precipitate formed as a function of time when CNTF solutions (0.05 - 4.0 mg/ml) were vortexed at room temperature.

The precipitation seen in the 0.05 mg/ml CNTF solution in the absence of 25% propylene glycol was strongly inhibited in the presence of propylene glycol. 25% propylene glycol stabilized solutions of CNTF of up to 4 mg/ml.

The ability of propylene glycol to stabilize CNTF

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in solution relative to polyethylene glycols of different chain lengths was studied. Relative to no additions, or the addition of polyethylene glycol 400 (PEG 400), PEG 600, or PEG 1000 to a 1.0 mg/ml solution of CNTF, 25% propylene glycol (with 10% or 20% glycerol) significantly inhibited precipitation of CNTF resulting from vortexing at room temperature (Table 9).

25% propylene glycol was superior at thermally stabilizing 1.0 mg/ml CNTF at 37°C relative to PEG 400, PEG 600, and PEG 1000 (Table 10). Higher molecular weight polyethylene glycols will work better at lower concentrations, as high concentrations of polyethylene glycol can precipitate proteins. Therefore, as the molecular weight of the polyethylene glycol increases, the concentration must be decreased.

The effect of propylene glycol on thermal stability was most marked for incubations at 37°C for 2 weeks.

The ability of propylene glycol to stabilize CNTF (1.0 mg/ml, pH 6.0) in solutions vortexed at room temperature relative to PEG 300 is shown in Table 11.

Without additions, CNTF quickly precipitates out of solution upon vortexing. The addition of 25% PEG 300 resulted in a greater total amount of precipitate formed although the initial formation of precipitate was delayed. The addition of 12.5% polyethylene glycol and 12.5% PEG 300 inhibited precipitate formation. However, 25% propylene glycol was superior to the other formulations tested in inhibiting CNTF precipitation, with 10% glycerol yielding more protection than 2% glycerol.

In summary, the results obtained show that propylene glycol stabilizes CNTF against precipitation from agitation while having little effect on thermal stability.

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EXAMPLE 5. THE EFFECT OF HSA ON LOSS OF CNTF
THROUGH ADSORPTION.

One of the most critical problems for obtaining therapeutic delivery of CNTF is that of adsorption. CNTF is readily lost from solution by absorption to surface areas of the container in which the solution is stored and of the dispensing devices. The problem of adsorption was studied with delivery of CNTF solutions from catheter tubes. The assumption was that if CNTF adsorptive losses could be prevented from catheter tubes, loss from the pump could also be prevented.

The ability of HSA to protect CNTF from loss through absorption was studied. Table 12 shows the effect of HSA on the recovery of CNTF when a 2.0 mg/ml solution of CNTF was subjected to repeated cycles of freeze/thawing.

The formulations used in the experiments shown in Table 12 were composed of: 10 mM citrate acid (pH 6.0), 10% glycerol, 200 mM NaCl, 25% propylene glycol, and 1.5 mg/ml CNTF.

These results indicate that HSA does not significantly decrease the stability of CNTF in solution. The ability of HSA to decrease loss of CNTF through absorption to surface areas was studied by examining the effect of HSA on CNTF recovery after subjecting a 0.01 mg/ml solution of CNTF to one freeze/thaw cycle and dispersion from a syringe and needle. The results are shown in Table 13,

The formulation of CNTF used for the experiments shown in Table 13 was: 10 mM citrate acid (pH 6.0), 10% glycerol, 200 mM NaCl, 25% propylene glycol, and 0.01 mg/ml CNTF.

As can be seen, HSA is critical in protecting from loss of CNTF by absorption to the surface area of containers.

The applicants have discovered that the addition of the amino acids -- such as lysine, arginine and

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cysteine -- to solutions of CNTF provides superior protection from adsorptive losses (data not shown). Consequently, a preferred formulation of CNTF is comprised of 3-5% lysine. A formulation comprised of polyethylene glycol, glycerol and lysine has been found to be useful to deliver CNTF from an implantable pump for periods of time up to one week.

EXAMPLE 6. CNTF FORMULATIONS WITH ETHANOL.

Ethanol was found to stabilize solutions of CNTF from precipitation upon agitation. CNTF solutions of 1 mg/ml containing 25% glycerol were vortexed for up to 1 hour in the presence of 0, 1%, 3%, 5%, or 10% ethanol. The results show that 10% ethanol significantly inhibited CNTF precipitation (Table 14).

In the presence of 10%, 13%, 16%, or 20% ethanol, the greatest inhibition of precipitation was observed in the presence of 10% and 13% ethanol (Table 15).

Glycerol has been shown to be a key excipient for increasing the thermal stability of CNTF (see Example 2). The effect of adding ethanol to glycerol on thermal stability is shown in Table 16.

The presence of 10% ethanol stabilized a 0.1 mg/ml solution of CNTF containing either 10% or 20% glycerol when incubated at 37°C for up to 300 hours. Similar results were obtained with incubations of up to 700 hours (data not shown). The most improved thermal stability was obtained with a CNTF solution containing 7.5% ethanol and 20% glycerol.

EXAMPLE 7. INTRATHECAL FORMULATIONS OF CNTF.

Intrathecal CNTF formulations were tested for chemical and physical stability.

Two formulations were compared which contained 10 mM phosphate (pH 7.0), 150 mM NaCl, 10% ethanol, 5% lysine, and 0.5 mg/ml CNTF, with and without 20% glycerol. Samples of each formulation were frozen at -

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70°C and thawed at room temperature. As shown in Table 17, the intrathecal formulation of CNTF containing 10% glycerol did not precipitate out of solution after 1 freeze/thaw cycle.

5 The stability of the intrathecal formulation of CNTF was examined by subjecting different formulations of CNTF to vortexing for up to 1350 minutes. The results shown in Table 18 compares the physical stability of various intrathecal CNTF formulations to
10 agitation. Table 19 shows a comparison of the thermal stability of the same intrathecal CNTF formulations.

 CNTF formulations containing 4% PEG 8000, 10% glycerol, 5% lysine, and buffered by 10 mM citrate acid (pH 7.5) exhibited the best physical and thermal
15 stability.

 The effect of the molecular weight of PEG on the thermal stability of CNTF is shown in Table 20 and a comparison of the effects of PEG 300, PEG 1000, and PEG 8000 on physical stability is shown in Table 21.

20 The formulation containing 4% PEG 8000 exhibited the best overall thermal and physical stabilities.

 The thermal stability of the intrathecal formulation of CNTF was examined by incubation at room temperature (20°C, Table 22) or 37°C (Table 23).

25 As shown in Table 22, CNTF in the intrathecal formulation does not precipitate upon incubation at room temperature for up to 17 days. This intrathecal formulation of CNTF was less stable at 37°C. However, no significant amount of precipitation was detected
30 after one day of incubation at 37°C, and precipitation only became significant upon prolonged incubation.

EXAMPLE 9. EFFECT OF INCUBATION OF CNTF
FORMULATIONS ON LOSS FROM CATHETER TUBES
35 AND PUMP RESERVOIRS.

 Two experiments were performed to examine the adsorption of CNTF in an intrathecal formulation

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comprising 6 mg/ml CNTF, 10 mM citrate acid (pH 7.5), 150 mM NaCl, 10% glycerol, 5% lysine, and 4% PEG 8000. In the first experiment, the intrathecal formulation was incubated in a catheter tube 6 feet in length holding approximately 700 μ l for two days at 37°C. After two days, the catheter tube was emptied and the amount of CNTF remaining in solution determined. In the second experiment, the intrathecal formulation of CNTF was incubated in a Medication Cassette Reservoir for the Pharmacia Deltec external pump. The reservoir bag was incubated at 30°C, and samples removed as a function of time and analyzed for CNTF. The results are shown in Table 24.

As shown in Table 24, virtually all the CNTF was recovered from the intrathecal formulation after 1 day in the catheter tube or up to 3 days in the reservoir bag, regardless of the method of measurement.

Further studies of the recovery of CNTF from catheter tubing were performed, and the results are shown in Tables 25 and 26.

The intrathecal formulations of the present invention thus represents a major advance in solving the problem of CNTF adsorption out of solution.

25 EXAMPLE 10. LYOPHILIZED FORMULATIONS OF CNTF.

Lyophilization is a way of providing for the long term storage of biological proteins with minimal degradation, aggregation and/or nonspecific adsorption. Experiments were conducted to determine formulations of CNTF suitable for lyophilization.

Three CNTF formulations were analyzed under a variety of conditions by reverse phase HPLC (RP-HPLC), cation exchange chromatography (CEX), and size exclusion chromatography (SEC), as described in Example 1. Formulation I was comprised of 0.5 mg/ml CNTF, 85 mg/ml sucrose, 2.0 mg/ml polysorbate 80, 4.0 mg/ml cysteine, 0.1 mg/ml disodium EDTA, and 2.0 mg/ml citric

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acid (pH 5.0). Formulation II was comprised of 0.5 mg/ml CNTF, 85 mg/ml sucrose, 2.0 mg/ml polysorbate 80, 4.0 mg/ml cysteine, 0.1 mg/ml disodium EDTA, and 2.0 mg/ml tromethamine (pH 7.6). Formulation III was comprised of 0.5 mg/ml CNTF, 80 mg/ml polyethylene glycol 3350, 50 mg/ml lysine, 20 mg/ml arginine, 4.0 mg/ml cysteine, 0.58 mg/ml sodium chloride, and 1.6 mg/ml histidine (pH 7.5).

The three lyophilizable formulations of CNTF (I, II, III) were stored at 2-8°C, room temperature, 37°C and 45°C for 0, 1.4, 6, and 10 week periods. The stability of the formulations was determined by RP-HPLC, CEX, and SEC analysis. The results are shown in Table 27.

SDS-PAGE analysis indicates that in the absence of cysteine, CNTF dimers are formed during lyophilization and subsequent storage. These dimers are believed to be disulfide linked dimers since their presence was eliminated when placed on a reducing SDS-PAGE (data not shown). No difference in the molecular weight of CNTF was observed with mass spectrometry when non-lyophilized and lyophilized CNTF were compared, indicating that CNTF did not form cysteine adducts in the presence of cysteine.

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TABLE 1. EFFECT OF NaCl ON PRECIPITATE FORMATION WHEN A 1 MG/ML CNTF SOLUTION VORTEXED AT ROOM TEMPERATURE

hours	10 mM NaCl	50 mM NaCl	100 mM NaCl	200 mM NaCl	400 mM NaCl
0.3	0.000	0.000	0.000	0.000	0.000
0.6	0.000	0.000	0.000	0.000	0.000
1.0	0.000	0.000	0.000	0.000	0.000
1.5	0.000	0.000	0.001	0.000	0.000
2.0	0.000	0.000	0.000	0.000	0.000
3.0	0.000	0.000	0.005	0.000	0.000
4.0	0.001	0.000	0.005	0.000	0.001
5.0	0.002	0.000	0.005	0.004	0.002
7.0	0.001	0.000	0.005	0.004	0.001
10.0	0.001	0.000	0.003	0.002	0.000
14.0	0.003	0.001	0.007	0.004	0.002
22.0	0.006	0.003	0.003	0.006	0.002
30.0	0.006	0.004	0.008	0.008	0.002
42.0	0.028	0.004	0.006	0.003	0.001

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TABLE 2. EFFECT OF NaCl ON PRECIPITATE FORMATION WHEN A 1 MG/ML CNTF SOLUTION VORTEXED AT 37°C.

hours	10 mM NaCl	50 mM NaCl	100 mM NaCl	200 mM NaCl	400 mM NaCl
20	0.001	0.000	0.000	0.000	0.001
40	0.004	0.004	0.008	0.008	0.025
60	0.011	0.018	0.048	0.037	0.054
90	0.040	0.050	0.145	0.175	0.120
120	0.072	0.085	0.250	0.256	0.180
180	0.168	0.297	0.483	0.420	0.360
240	0.287	0.398	0.558	0.538	0.401

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TABLE 3. EFFECT OF TWEEN® 80 ON PRECIPITATE FORMATION WHEN CNTF IS VORTEXED AT ROOM TEMPERATURE

Time (min)	Tween® 80 Concentration			
	0.20%	0.40%	0.60%	0.75%
0.0	0.000	0.000	0.000	0.000
0.2	0.068	0.001	0.000	0.000
0.3	0.068	0.001	-0.001	0.000
0.7	0.068	0.001	-0.001	0.001
1.0	0.072	0.000	-0.001	0.000
2.0	0.087	0.002	-0.002	0.000
3.0	0.101	0.002	0.001	0.004
6.0	0.107	0.003	0.000	0.002
10.0	0.148	0.004	0.003	0.003
25.0	0.590	0.007	0.001	0.001
45.0	0.955	0.027	0.001	0.001
91.0	1.333	0.456	0.027	0.003
125.0	1.496	0.909	0.192	0.011
155.0	1.620	1.090	0.342	0.093
185.0	1.652	1.209	0.611	0.301

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TABLE 4. EFFECT OF TWEEN® 80 ON PRECIPITATE FORMATION AS A FUNCTION OF TIME WHEN CNTF IS INCUBATED AT ROOM TEMPERATURE

Time (min)	Tween® 80 Concentration			
	0.20%	0.40%	0.60%	0.75%
0.00	0.000	0.000	0.000	0.000
1.00	-0.001	0.001	0.000	0.000
3.00	-0.002	0.002	0.002	-0.001
4.67	0.000	0.000	0.001	-0.002
7.83	0.000	0.000	-0.001	-0.001
12.00	0.119	0.100	0.052	0.012
21.33	0.342	0.450	0.423	0.384

TABLE 5. EFFECT OF PH WITH AND WITHOUT(-) 25% PROPYLENE GLYCOL ON PRECIPITATE FORMATION WHEN CNTF IS VORTEXED AT ROOM TEMPERATURE

Time (min)	pH 6.0(-)	pH 6.0	pH 5.75	pH 5.5
10	0.511	0.000	0.000	0.001
20	0.491	0.000	0.000	0.001
40	0.483	0.000	0.000	0.009
60	0.467	0.000	0.001	0.021
90	0.513	0.000	0.007	0.077
180	0.399	0.000	0.017	0.083
240	0.455	0.008	0.048	0.166
300	0.384	0.003	0.094	0.263
420	-0.100	0.004	0.175	0.231

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TABLE 6. EFFECT OF PROPYLENE GLYCOL ON PRECIPITATE FORMATION WHEN CNTF IS VORTEXED AT ROOM TEMPERATURE

Time (min)	% Concentration of Propylene Glycol				
	0%	13%	16%	20%	25%
5	0.354	0.189	0.015	0.000	0.002
10	0.536	0.38	0.074	0.000	0.002
20	0.390	0.574	0.206	0.003	0.005
40		0.517	0.403	0.010	0.004
60		0.514	0.441	0.024	0.005
90		0.488	0.667	0.066	0.003
120		0.368	0.581	0.147	0.000
180		0.438	0.695	0.197	0.001
240		0.440	0.769	0.209	0.006
360				0.299	0.015
480				0.315	0.026

TABLE 7. EFFECT OF PROPYLENE GLYCOL ON PRECIPITATE FORMATION WHEN CNTF IS INCUBATED AT 37°C

Time (wks)	Concentration of Propylene Glycol					
	0%	13%	16%	20%	25%	30%
1	0.002	0.003	0.004	0.003	0.001	0.000
2	0.000	0.000	0.001	0.001	0.002	0.013
3	0.001	0.002	0.002	0.003	0.006	0.051
4	0.021	0.001	0.000	0.010	0.057	0.312

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TABLE 8. EFFECT OF CNTF CONCENTRATION ON PRECIPITATE FORMATION WHEN CNTF IS VORTEXED AT ROOM TEMPERATURE IN THE PRESENCE OR ABSENCE (-) OF 25% PROPYLENE GLYCOL.

Time (min)	Concentration of CNTF (mg/ml)				
	0.5 (-)	0.5	1.0	2.0	4.0
10	0.233	0.000	0.000	0.001	0.000
20	0.194	0.000	0.000	0.001	0.000
40	0.151	0.000	0.000	0.001	0.000
60	0.165	0.000	0.000	0.003	0.002
90	0.168	0.000	0.002	0.004	0.010
180		0.000	0.000	0.002	0.015
240		0.000	0.001	0.005	0.056
300		0.001	0.005	0.009	0.106
420		0.001	0.005	0.013	0.089

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TABLE 9. EFFECT OF PEG ON PRECIPITATE FORMATION WHEN CNTF IS VORTEXED AT ROOM TEMPERATURE WITH 10% (-) OR 20% GLYCEROL

Time (hrs)	No Additions	Propylene Glycol (-)	PEG 400	PEG 600	PEG 1000	Propylene Glycol
1	0.559	0.000	0.017	0.027	0.266	0.000
2	0.446	0.000	0.033	0.059	0.229	0.000
3	0.474	0.000	0.051	0.090	0.302	0.001

TABLE 10. EFFECT OF PROPYLENE GLYCOL (25%) OR PEG (25%) ON PRECIPITATE FORMATION WHEN CNTF IS INCUBATED AT 37°C WITH 10% OR 20% GLYCEROL.

Time (days)	No Additions	Propylene Glycol (10%)	PEG 400	PEG 600	PEG 1000	Propylene Glycol (20%)
6	0.000	0.012	0.022	0.054	0.286	0.009
13	0.000	0.009	0.133	0.223	0.354	0.027
30	0.003	0.095	0.039	0.135	0.255	0.165

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TABLE 11. EFFECT OF DIFFERENT FORMULATIONS OF CNTF ON PRECIPITATE FORMATION WHEN VORTEXED AT ROOM TEMPERATURE

Time (min)	10% Glycerol	25% Propylene Glycol + 10% Glycerol	25% PEG 300 + 10% Glycerol	25% PEG 300 + 12.5% Glycol	25% Glycol + 2% Glycerol
10	0.511	0.000	0.002	0.000	0.001
20	0.491	0.000	0.026	0.001	0.000
40	0.483	0.000	0.070	0.002	0.000
60	0.467	0.000	0.105	0.012	0.001
90	0.513	0.000	0.290	0.023	0.001
120	0.421	0.000	0.362	0.032	0.002
180	0.399	0.000	0.438	0.064	0.002
240	0.455	0.008	0.585	0.123	0.009
300	0.384	0.003	0.593	0.165	0.025
420		0.004		0.194	0.036

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TABLE 12. EFFECT OF PROPYLENE GLYCOL ON FREEZE/THAW RECOVERY OF CNTF

Freeze/Thaw Cycles	% CNTF Recovered No HSA	% CNTF Recovered With HSA
0	100	100
1	98	92
2	100	96
3	97	99
4	99	94
5	100	95

TABLE 13. RECOVERY OF 0.01 MG/ML CNTF AFTER ONE FREEZE/THAW CYCLE FOR ONE HOUR DISPENSED FROM SYRINGE AND NEEDLE.

	Without HSA	With HSA
Initial CNTF Sample	100	100
% Recovery After Treatment	89	105

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TABLE 14. EFFECT OF ETHANOL ON PRECIPITATE FORMATION WHEN 0.5 MG/ML CNTF (pH 7.5) IS VORTEXED AT ROOM TEMPERATURE

Formulation	Time (minutes)							
	0	15	30	60	120	180	240	300
0% Ethanol	0.0	0.290	0.299	0.312	0.331	0.324	0.341	0.337
7% Ethanol	0.0	0.019	0.040	0.087	0.235	0.248	0.267	0.287
8% Ethanol	0.0	0.003	0.003	0.004	0.007	0.006	0.010	0.015
9% Ethanol	0.0	0.001	0.002	0.004	0.005	0.005	0.005	0.007
10% Ethanol	0.0	0.000	0.000	0.003	0.004	0.004	0.006	0.006
13% Ethanol	0.0	0.003	0.002	0.006	0.007	0.004	0.007	0.005

TABLE 15. EFFECT OF ETHANOL ON PRECIPITATE FORMATION WHEN 1.0 MG/ML CNTF IS VORTEXED AT ROOM TEMPERATURE.

Formulation	Time (minutes)						
	0	5	10	20	40	60	
0% Ethanol	0.0	0.361	0.479	0.405	0.381	0.387	
10% Ethanol	0.0	0.001	0.002	0.005	0.010	0.011	
13% Ethanol	0.0	0.000	0.004	0.003	0.006	0.008	
16% Ethanol	0.0	0.001	0.001	0.005	0.010	0.014	
20% Ethanol	0.0	0.010	0.012	0.022	0.045	0.054	

TABLE 16. EFFECT OF ETHANOL ON PRECIPITATE FORMATION WHEN FORMULATED CNTF IS INCUBATED AT 37°C

Formulation	Time (minutes)							
	0	15	30	60	120	180	240	300
20% Glycerol 10% Ethanol 5% Lysine	0.00	0.001	0.001	0.002	0.003	0.004	0.003	0.003

TABLE 17. STABILITY OF INTRATHECAL CNTF FORMULATION TO 1 FREEZE/THAW CYCLE.

Formulation	0 Cycle	1 Cycle
10% ethanol + 5% lysine	0.041	0.147
20% glycerol + 10% ethanol + 5% lysine	0.042	0.043

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TABLE 18. PHYSICAL STABILITY OF INTRATHECAL CNTF FORMULATIONS UPON VORTEXING

Formulation	Buffer	pH	Vortex Time (minutes)					
			0	30	60	120	300	1350
25% glycol 10% glycerol 5% lysine	citrate	6.0	0.000	-0.001	0.000	0.002	0.002	0.018
8% ethanol 20% glycerol 5% lysine	phospha te	7.5	0.000	-0.001	0.000	0.010	0.002	0.008
4% PEG 8000 10% glycerol 5% lysine	phospha te	7.5	0.000	-0.001	-0.001	0.000	-0.001	0.004
4% PEG 8000 10% glycerol 5% lysine	carbona te	7.5	0.000	-0.001	0.000	0.003	-0.003	0.002
4% PEG 8000 10% glycerol 5% lysine	citrate	7.5	0.000	-0.008	-0.002	-0.003	-0.003	-0.005

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TABLE 19. THERMAL STABILITY OF INTRATHECAL CNTF FORMULATIONS WHEN INCUBATED AT 37°C.

Formulation	Buffer	pH	Incubation at 37°C (days)								Catheter 2 days
			0	2	3	4	5	6	11	16	
25% glycol 10% glycerol 5% lysine	citrate	6.0	0.000	0.006	0.007	0.011	0.014	0.010	0.023	0.008	0.002
8% ethanol 20% glycerol 5% lysine	phosphate	7.5	0.000	0.022	0.031	0.039	0.043	0.031	0.066	0.032	0.015
4% PEG 8000 10% glycerol 5% lysine	phosphate	7.5	0.000	0.011	0.014	0.013	0.016	0.014	0.026	0.018	0.010
4% PEG 8000 10% glycerol 5% lysine	carbonate	7.5	0.000	0.015	0.017	0.021	0.027	0.017	0.033	0.016	0.014
4% PEG 8000 10% glycerol 5% lysine	citrate	7.5	0.000	0.000	0.004	0.006	0.009	0.007	0.010	0.004	0.002

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TABLE 20. EFFECT OF MOLECULAR WEIGHT AND CONCENTRATION OF PEG ON THERMAL STABILITY OF CNF.

Formulation	Time (days)						
	0	1	2	3	4	5	6
0% PEG	0.000	0.013	0.018	0.014	0.023	0.066	0.066
2% PEG 8000	0.000	0.022	0.020	0.016	0.015	0.020	0.020
4% PEG 8000	0.000	0.020	0.014	0.011	0.012	0.022	0.022
6% PEG 8000	0.000	0.033	0.023	0.020	0.018	0.019	0.019
8% PEG 8000	0.000	0.055	0.026	0.024	0.026	0.025	0.025
10% PEG 8000	0.000	0.063	0.023	0.019	0.019	0.021	0.021
5% PEG 1000	0.000	0.071	0.052	0.042	0.066	0.045	0.045
7.5% PEG 1000	0.000	0.157	0.121	0.080	0.112	0.131	0.131
10% PEG 100	0.000	0.583	0.446	0.380	0.359	0.457	0.457
10% PEG 300	0.000	0.034	0.036	0.045	0.056	0.068	0.068
15% PEG 300	0.000	0.047	0.038	0.057	0.058	0.081	0.081
20% PEG 300	0.000	0.064	0.074	0.072	0.092	0.104	0.104
25% PEG 300	0.000	0.063	0.059	0.065	0.078	0.084	0.084

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TABLE 21. EFFECT OF MOLECULAR WEIGHT AND CONCENTRATION OF PEG ON THE PHYSICAL STABILITY OF CNIF.

Formulation	Time (minutes)									
	0	5	15	30	60	120	210	1080		
0% PEG	0.000	0.233	0.128	0.167						
2% PEG 8000	0.000	0.001	0.001	0.003	0.013	0.036	0.063	0.225		
4% PEG 8000	0.000	0.000	0.000	0.000	0.003	0.016		0.266		
6% PEG 8000	0.000	0.000	0.000	0.001	0.004	0.022		0.266		
8% PEG 8000	0.000	0.002	0.001	0.000	0.003	0.023		0.179		
10% PEG 8000	0.000	0.000	0.105	-0.001	0.001	0.014		0.127		
5% PEG 1000	0.000	0.026	0.090	0.210						
7.5% PEG 1000	0.000	0.022	0.000	0.188						
10% PEG 1000	0.000	-0.002	0.201	0.016	0.007	0.027	0.070	0.304		
10% PEG 300	0.000	0.042	0.135	0.323						
15% PEG 300	0.000	0.032	0.011	0.267						
20% PEG 300	0.000	0.005	0.000	0.043	0.111	0.245	0.319			
25% PEG 300	0.000	0.000	0.135	0.005	0.009	0.039	0.088			

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TABLE 22. EFFECT OF GLYCEROL AND ETHANOL ON CNTF THERMAL STABILITY: PRECIPITATION FORMED WHEN 0.1 MG/ML CNTF IS INCUBATED AT ROOM TEMPERATURE

Formulation	Time (days)							
	0	0.63	1.63	4.63	6.6	7.6	9.9	16.8
20% Glycerol 10% Ethanol 5% Lysine	0	0.002	0.001	0.002	0.002	0.003	0.005	0.007

TABLE 23. EFFECT OF GLYCEROL AND ETHANOL ON CNTF THERMAL STABILITY: PRECIPITATION FORMED WHEN 0.1 MG/ML CNTF IS INCUBATED AT 37°C.

Formulation	Time (days)						
	1	2	3	6	7	9	
20% Glycerol 10% Ethanol 5% Lysine	0.002	0.009	0.017	0.037	0.048	0.054	

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TABLE 24. ADSORPTIVE LOSSES OF CNTF FROM AN INTRATHECAL FORMULATION.

Assay	Catheter 2 day	Reservoir 1 day	Reservoir 2 days	Reservoir 3 days
OD ₂₈₀	103	100	95	
Protein Assay	93	118	104	
SDS-PAGE	89	105	117	
Cation Exchange HPLC	67	100	125	92
Size Exclusion HPLC	87	96	102	109
Bioassay			104	

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TABLE 25. RECOVERY OF CNTF IN INTRATHECAL FORMULATIONS FROM CATHETER TUBE

Formulation	Buffer	pH	t=0 mg/ml	Catheter mg/ml	% Recovery
25% glycol 10% glycerol 5% lysine	citrate	6.0	0.38	0.39	103
8% ethanol 20% glycerol 5% lysine	phosphate	7.5	0.41	0.44	107
4% PEG 8000 10% glycerol 5% lysine	phosphate	7.5	0.48	0.43	90
4% PEG 8000 10% glycerol 5% lysine	carbonate	7.5	0.42	0.42	100
4% PEG 8000 10% glycerol 5% lysine	citrate	7.5	0.41	0.46	112

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TABLE 26. RECOVERY OF CNTF FROM CATHETER TUBES

Formulation	Time (days)		% Recovery
	0	2	
4% PEG 8000	1.42	1.28	90
4% PEG 8000 10% Glycerol	1.42	0.93	65
4% PEG 8000 5% Lysine	1.56	1.35	87
4% PEG 8000 10% Glycerol 5% Lysine	1.65	1.67	101
4% PEG 8000 20% Glycerol 5% Lysine	1.60	1.69	106
10% Glycerol 5% Lysine	0.45	0.39	87
10% Glycerol 5% Lysine 2% Ethanol	0.58	0.53	91
10% Glycerol 5% Lysine 4% Ethanol	0.64	0.48	75
10% Glycerol 5% Lysine 6% Ethanol	0.65	0.47	72
10% Glycerol 6% Lysine 8% Ethanol	0.63	0.51	81

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TABLE 27. STABILITY OF LYOPHILIZABLE CNTF FORMULATIONS I, II, AND III.

FORMULATION	STORAGE TEMPERATURE	TIME (WEEKS)	% OXIDATION BY RP-HPLC	% DEAMINATION BY CEX	% AGGREGATION BY SEC
I	-	0	0	2.1	0
	2-8°C	6	0	2.2	< 1
	RT	6	0.9	2.1	0.7
II	-	0	0	2.1	0
	2.8°C	6	0	2.7	0
	RT	6	0	2.7	0
III	-	0	1.8	2.1	-
	8°C	28	1.3	2.2	-
	25°C	24	1.4	2.5	-
	37°C	20	2.1	4.2	-
	45°C	9	6.8	3.3	-

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CLAIMS

1. An aqueous pharmaceutical formulation comprising:
 (a) ciliary neurotrophic factor;
5 (b) stabilizing agent(s);
 (c) biologically acceptable salt in an amount
sufficient to maintain isotonicity;
 (d) a buffer in an amount sufficient to maintain
the pH of the formulation from about 6.0 to about 8.0;
10 and
 (e) water.
2. The aqueous pharmaceutical formulation of claim 1
wherein the stabilizing agent(s) include glycerol.
15
3. The aqueous formulation of claim 2 wherein the
stabilizing agent further includes a compound selected
from the group consisting of: a non-ionic surfactant,
propylene glycol, or ethanol.
20
4. The aqueous formulation of claim 3 wherein the non-
ionic surfactant is polysorbate.
5. The aqueous formulation of claim 3 wherein the
25 stabilizing agent is propylene glycol.
6. The aqueous formulation of claim 3 wherein the
stabilizing agent is ethanol.
- 30 7. The aqueous formulation of claim 3 further
comprising a biologically acceptable water soluble
carrier.
8. The aqueous formulation of claim 7 wherein the
35 carrier is human serum albumin.

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9. The aqueous formulation of claim 7 wherein:
- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
 - (b) the stabilizing agents comprise 0.1 - 1.0 mg/ml polysorbate-80 and 4-20% glycerol;
 - (c) the carrier comprises 0.1 - 5.0% human serum albumin;
 - (d) the salt comprises about 100 to 400 mM; and
 - (e) the buffer comprises about 1 - 50 mM sodium phosphate and 1 - 50 mM tris (hydroxymethyl) aminomethane.
10. The aqueous formulation of claim 7 wherein:
- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
 - (b) the stabilizing agents comprise 1.0 - 25% propylene glycol and 4-20% glycerol;
 - (c) the carrier comprises 0.1 - 5.0% human serum albumin;
 - (d) the salt comprises about 100 to 400 mM; and
 - (e) the buffer comprises about 1 - 50 mM sodium phosphate and 1 - 50 mM tris (hydroxymethyl) aminomethane.
11. The aqueous formulation of claim 7 wherein:
- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
 - (b) the stabilizing agents comprise 1.0 - 20% ethanol and 4-20% glycerol;
 - (c) the carrier comprises 0.1 - 5.0% human serum albumin;
 - (d) the salt comprises about 100 to 400 mM; and
 - (e) the buffer comprises about 1 - 50 mM sodium phosphate and 1 - 50 mM tris (hydroxymethyl) aminomethane.

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12. An aqueous pharmaceutical formulation suitable for intrathecal administration, comprising:

- (a) ciliary neurotrophic factor;
- 5 (b) two or more stabilizing agents;
- (c) biologically acceptable salt in an amount sufficient to maintain isotonicity;
- (d) a buffer in an amount sufficient to maintain the pH of the formulation at about 6.0; and
- 10 (e) water.

13. The aqueous pharmaceutical formulation of claim 12 wherein the stabilizing agents include glycerol, lysine and one or more of the compounds selected from the group consisting of: ethanol, propylene glycol, or polyethylene glycol.

14. The aqueous formulation of claim 13 wherein the stabilizing agent is ethanol.

15. The aqueous formulation of claim 13 wherein the stabilizing agent is propylene glycol.

16. The aqueous formulation of claim 13 wherein the stabilizing agent is polyethylene glycol.

17. The aqueous formulation of claim 13 wherein the stabilizing agents are polyethylene glycol and propylene glycol.

18. The aqueous formulation of claim 13 wherein:

- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
- (b) the stabilizing agents comprise 10 - 13% ethanol, 3 - 5% lysine, and 1.0 - 20% (w/v) glycerol;

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- (c) the salt comprises about 150 mM; and
- (e) the buffer comprises about 10 mM phosphate.

19. The aqueous formulation of claim 13 wherein:

- 5 (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
- (b) the stabilizing agents comprise 20 - 25% propylene glycol, 3 - 5% lysine, and 10.0% glycerol;
- (c) the salt comprises about 150 mM; and
- 10 (e) the buffer comprises about 10 mM phosphate.

20. The aqueous formulation of claim 13 wherein:

- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
- 15 (b) the stabilizing agents comprise 4% polyethylene glycol, 3 - 5% lysine, and 10.0% glycerol;
- (c) the salt comprises about 150 mM; and
- (e) the buffer comprises about 10 mM phosphate.

20 21. The aqueous formulation of claim 13 wherein:

- (a) The ciliary neurotrophic factor comprises about 0.01 to about 8.00 mg/ml;
- (b) the stabilizing agents comprise 2% polyethylene glycol, 10% propylene glycol, 3 - 5% lysine, and 10.0% glycerol;
- 25 (c) the salt comprises about 150 mM; and
- (e) the buffer comprises about 10 mM phosphate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02153

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 14/475; A61K 38/18

US CL :530/399; 514/12, 970

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399; 514/12, 970

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

search terms: CNTF, pharmaceutical, glycerol, polysorbate, ethanol, propylene glycol, albumin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,141,856 (COLLINS ET AL.) 25 August 1992, col. 1, lines 19-51.	1-21
Y	US, A, 5,272,135 (TAKRURI) 21 December 1993, col. 1, lines 35-46; col. 5, lines 4-54; col. 4, lines 46-51; col. 6, lines 5-8.	1-21
Y	US, A, 5,130,298 (CINI ET AL.) 14 July 1992, col. 6, lines 9-31.	2-21
Y	US, A, 4,061,736 (MORRIS ET AL.) 06 December 1977, see entire document.	13-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

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