

5 **CYANOCOBALAMIN LOW VISCOSITY AQUEOUS FORMULATIONS**
FOR INTRANASAL DELIVERY

BACKGROUND OF THE INVENTION

[001] Vitamin B12 is a dietary essential, a deficiency of which results in defective synthesis of DNA in any cell in which chromosomal replication and division
10 are taking place. Since tissues with the greatest rate of cell turnover show the most dramatic changes, the hematopoietic system is especially sensitive to vitamin B12 deficiencies. An early sign of B12 deficiency is a megaloblastic anemia. Dietary B12, in the presence of gastric acid and pancreatic proteases, is released from food and salivary binding protein and bound to gastric intrinsic factor. When the vitamin B12-
15 intrinsic factor complex reaches the ileum, it interacts with a receptor on the mucosal cell surface and is actively transported into circulation. Adequate intrinsic factor, bile and sodium bicarbonate (suitable pH) all are required for ileal transport of vitamin B12. Vitamin B12 deficiency in adults is rarely the result of a deficient diet; rather, it usually reflects a defect in one or another aspect of this complex sequence of absorption.
20 Achlorhydria and decreased secretion of intrinsic factor by parietal cells secondary to gastric atrophy or gastric surgery is a common cause of vitamin B12 deficiency in adults. Antibodies to parietal cells or intrinsic factor complex also can play a prominent role in producing deficiency. A number of intestinal diseases can interfere with absorption. Vitamin B12 malabsorption is seen with pancreatic disorders (loss of
25 pancreatic protease secretion), bacterial overgrowth, intestinal parasites, sprue, and localized damage to ileal mucosal cells by disease or as a result of surgery. The recommended daily intake of vitamin B12 in adults is 2.4 µg.

5 [002] There are four main forms of vitamin B₁₂: cyanocobalamin:
hydroxocobalamin, methylcobalamin and adenosylcobalamin. Methylcobalamin and
adenosylcobalamin are unstable and damaged by light. They are therefore unsuitable
for use in dietary supplements or pharmaceuticals and are not essential since they can
be formed from cyanocobalamin or hydroxocobalamin within the body. The main form
10 of vitamin B₁₂ found in food is hydroxocobalamin. The main form used therapeutically
and in nutritional supplements is cyanocobalamin, chosen because it is the most stable
form and therefore easiest to synthesize and formulate.

 [003] Because deficiencies of vitamin B₁₂ are generally caused by the inability
of the vitamin to be absorbed in the small intestine due to a breakdown in the vitamin
15 B₁₂-intrinsic factor complex transport mechanism, vitamin B₁₂ must therefore be
administered systemically. Currently, therapeutic amounts of cyanocobalamin are
administered by intramuscular or deep subcutaneous injection of cyanocobalamin.
However, patients must return to the physician's office periodically to receive
additional injections to maintain their levels of vitamin B₁₂. However, an intranasal
20 gel cyanocobalamin preparation, NASCOBAL® is currently being marketed in which
cyanocobalamin is administered intranasally as maintenance vitamin B₁₂ therapy.
However, many patients find the consistency of the intranasal gel unpleasant and
would prefer to have administered intranasally a low viscosity spray containing
cyanocobalamin.

25 [004] The prior art suggests that for vitamin B₁₂ to be absorbed intranasally in
therapeutically beneficial amounts, the concentration of the B₁₂ in solution must
either be greater than 1% by weight, see Merkus, U.S. Patent No. 5,801,161 (hereafter
"Merkus") or be administered intranasally in a viscous gel, Wenig, U.S. Patent No.
4,724,231 (hereafter "Wenig") so that the gel remains in the nostril for an extended
30 period of time. In fact Wenig states that B₁₂ administered intranasally in a low
viscosity solution is not in contact with the nasal mucosa long enough for a sufficient
period of time to permit useful absorption. Wenig claims that most of the B₁₂ is
wasted if the solution has a low viscosity. Merkus developed intranasal formulations
of hydroxocobalamin having a concentration of hydroxocobalamin greater than 1%,

5 however hydroxocobalamin is not very stable and thus has a short shelf-life.
Merkus chose hydroxocobalamin because cyanocobalamin is not soluble in an
aqueous solution at concentrations greater than 1%.

10 [005] The teachings of Wenig are clear in that the subject disclosure fails to
teach a cyanocobalamin formulation in an aqueous form, suitable and effective for
intranasal administration as described herein below. Wenig unambiguously teaches
away from any cyanocobalamin formulation having a viscosity below the expressly-
stated, critical range taught by Wenig of 2500-6000 cPs. In particular, Wenig teaches
that an intranasal cyanocobalamin formulation "will contain a sufficient amount of a
thickening agent so that the viscosity is from about 2500 to 6500 cps, although more
15 viscous compositions even up to 10,000 cps may be employed." (Col. 2, lines 37-39,
emphasis added). Nowhere does Wenig teach or suggest adjustment or selection to
lower viscosity of an intranasal cyanocobalamin formulation as disclosed by
Applicant. On the contrary, Wenig's disclosure teaches directly away from intranasal
cyanocobalamin formulations having a viscosity less than about 1000 cPs. The
20 cyanocobalamin gel compositions described by Wenig have a vastly higher viscosity
than this, in all embodiments contemplated. Thus, Wenig teaches that "[a] typical
composition of this invention" has a viscosity of "about 4500 cps" (Col. 3, lines 41-
51). Each of the reported working embodiments provided by Wenig (for which
viscosity values are provided, and which are reportedly validated in terms of
25 bioavailability by measurement of blood plasma cyanocobalamin levels), have
respective viscosities of 4000 cps, 3500-4000 cps, and 4000 cps (see, e.g., Example 1;
formulations A, B, and C). As noted above, Wenig further emphasizes that "more
viscous compositions even up to 10,000 cps may be employed."

30 [006] Accordingly, there is clearly no teaching nor suggestion provided by
Wenig to substitute an aqueous liquid (spray or drop) cyanocobalamin formulation for
the disclosed intranasal gel formulation of Wenig. In addition, Wenig expressly
teaches in the background section of the disclosure that non-gel (powder and aqueous)
formulations of cyanocobalamin are ineffective for intranasal administration to treat
vitamin B12 deficiency. With regard to aqueous formulations, Wenig cites, e.g., a

5 proposed aqueous isotonic sodium chloride solution of vitamin B12 reported by
Monto et al. (Am. J. Med. Sci. 223:113, 1953; Arch. Int. Med. 93:219, 1954). Wenig
states that this solution, along with powdered cyanocobalamin formulations, is
ineffective for intranasal use to treat vitamin B12 deficiency, because:

10 [M]ost of the B12 passes immediately into the throat. It is
not in contact with the nasal mucosa for a sufficient period
of time to permit useful and uniform absorption. Most of
the B12 so administered is, in fact wasted. (Col. 1, lines
63-68).

15 [007] Thus, Wenig not only teaches away from decreasing viscosity of an
intranasal cyanocobalamin formulation below a critical, minimum range of 2500-6500
cps, but also expressly negates the prospect of using an aqueous (spray or drops)
composition to substitute for the intranasal gel formulation described by Wenig. Wenig
provides clear evidence and reasoning that teaches directly away from such a proposed
modification. In particular, Wenig teaches that effective intranasal cyanocobalamin
20 formulations must have “a sufficient amount of a thickening agent so that the viscosity
is from about 2500 to 6500 cps” (supra)—which Wenig describes as a critical parameter
to render the formulations “sufficiently viscous to maintain themselves in the nasal
passages for a period of time which is long enough so that most of the B12 is
absorbed.” (Col. 2, lines 24-29). These properties are directly contrasted by Wenig to
25 the properties of non-gel (liquid and powder) cyanocobalamin compositions, which,
according to Wenig’s express teachings, fail to exhibit sufficient nasal mucosal
residence time to achieve effective intranasal delivery/bioavailability of
cyanocobalamin.

30 [008] In other attempts to develop useful cyanocobalamin formulations for nasal
administration, Garcia-Arieta et al., Biol. Pharm. Bull. 24:1411-1416, 2001 (hereafter,
“Garcia-Arieta et al.”) describes “Spray-Dried Powders as Nasal Absorption Enhancers
of Cyanocobalamin” (Title). The only disclosure by Garcia-Arieta et al. with regard to
any cyanocobalamin spray and drop formulation is derived from a comparative
experiment, from which the authors expressly report that liquid (spray and drop)
35 cyanocobalamin compositions are inoperable for intranasal use. More specifically,
Garcia-Arieta et al. tested bioavailability of three spray-dried, intranasal

5 cyanocobalamin formulations alongside two experimental cyanocobalamin nasal solutions (drops and spray containing 0.1% cyanocobalamin; no other formulation parameters specified) (p. 1412, right column). Garcia-Arieta et al. expressly report that the experimental nasal spray and drop formulations of cyanocobalamin yielded no detectable bioavailability whatsoever. As described at page 1415, left column, Garcia-
10 Arieta et al. found from their experiments that:

15 “[N]either the nasal solution in drops nor in spray were able to increase the basal level of serum cobalamin in rabbits to a statistically significant level. This means that either the cyanocobalamin is hardly absorbed by the nasal route when it is administered without absorption enhancers or that due to the lack of any viscosity-enhancing agent these formulations were not retained in the nasal cavity for long enough to allow their absorption. (emphasis supplied).”

[009] These reports expressly describe inoperability of an aqueous, low viscosity
20 cyanocobalamin formulation, which accords closely with the conclusions by Wenig noted above. Accordingly, both Wenig and Garcia-Arieta et al., teach that non-gel, liquid intranasal formulations of cyanocobalamin were understood to be ineffective to achieve useful therapeutic results. Wenig expressly describes a critical threshold viscosity above 2500 cps, and a much higher range of 3500-4500 cps for all
25 demonstrated working embodiments, of a useful intranasal gel formulation of cyanocobalamin. Garcia-Arieta et al. further provides direct experimental evidence that simple 1% aqueous cyanocobalamin spray and drop formulations yielded no detectable bioavailability.

[0010] In other attempts to provide useful intranasal formulations to treat vitamin
30 B12 deficiency, Slot et al., Gastroenterology 113:430-433, 1997 (hereafter “Slot et al.”), report an intranasal formulation of hydroxocobalamin. Notably, Slot et al. expressly teach that hydroxocobalamin is the preferred form of vitamin B12 for treating vitamin B12 deficiency, and that cyanocobalamin is not an effective or practical treatment agent for use in liquid, intranasal delivery formulations or methods. Like Wenig discussed
35 above, Slot et al. comment on a previously-described, isotonic saline solution of cyanocobalamin, stating that “[N]one of these proposals found a follow-up in clinical practice. Apparently the results were not very practical.” (page 432, right column).

5 Slot et al. further teach away from intranasal cyanocobalamin formulations and methods for treating vitamin B12 deficiency, by disclosing that:

10 “Hydroxocobalamin binds more extensively to plasma proteins and has a longer half time in the body than cyanocobalamin. As a result, hydroxocobalamin is better retained in the body and, therefore, requires less frequent dosing. Moreover, cyanocobalamin is contraindicated in patients with tropical amblyopia and simultaneous tobacco usage and in patients with pernicious anemia with optic neuropathy; hence, hydroxocobalamin is the drug of choice
15 in restoring vitamin B12 deficiencies. (page 432, right column).”

[0011] Another prior art reference that teaches away from the selection of cyanocobalamin as a useful form of cobalamin in intranasal formulations and methods is Merkus, USPN 5,801,161, discussed above. Similar to the teachings of Slot et al.,
20 Merkus expressly states that hydroxocobalamin is a preferred treatment agent for vitamin B12 deficiency in comparison to cyanocobalamin. In the specific context of nasal formulations, Merkus emphasizes that:

25 “[T]he most effective concentrations of vitamin B12 in the formulations for nasal administration are higher than 1%. The maximal concentration that can be reached with cyanocobalamin is about 1%. Concentrations above 1% can only be obtained with hydroxocobalamin, because its good solubility in water. The solubility of hydroxocobalamin substances can be as high as 10%,
30 which means that up to about 10 times more vitamin B12 per unit of volume can be administered and subsequently absorbed nasally, when hydroxocobalamin is used. (Col. 3, lines 43-53)”

[0012] Based on the foregoing teachings, persons of ordinary skill in the art
35 would not have found practical motivation to develop a low viscosity cyanocobalamin formulations and methods, particularly as an effective treatment tool for intranasal administration to treat vitamin B12 deficiency. Considering the prior art as a whole, there was simply no reasonable expectation that cyanocobalamin formulations and methods as described herein, below could be developed and employed to successfully
40 achieve therapeutically effective delivery/bioavailability of cyanocobalamin sufficient

5 to alleviate vitamin B12 deficiency, as presently disclosed. Both Wenig and Garcia-Arieta et al. teach directly away from low viscosity liquid cyanocobalamin formulations. Wenig expressly teaches a critical, minimum viscosity for an effective, intranasal cyanocobalamin between 2500-4000 cps. Both Wenig and Garcia-Arieta et al. teach that non-gel, liquid cyanocobalamin formulations would not be retained for a
10 sufficient time to allow for intranasal absorption. Garcia-Arieta et al. specifically report experimental results that no significant bioavailability was detected following nasal administration of a 1% simple aqueous cyanocobalamin formulation. Similarly with regard to the selection and concentration of cyanocobalamin as an active form of cobalamin for use in an intranasal liquid formulation, Slot et al. and Merkus
15 collectively teach that cyanocobalamin would be expected to be ineffective, or at best strongly disfavored in comparison to hydroxocobalamin, in an intranasal formulation for treating vitamin B12 deficiency (independent from viscosity considerations). Exemplifying these teachings, Merkus emphasizes that:

20 “A high and efficient intranasal absorption of vitamin B12 is advantageous in medical therapy and can be obtained only by using hydroxocobalamin, which shows a significant higher solubility in water than cyanocobalamin. Only with hydroxocobalamin a superior nasal composition
25 in an aqueous medium can be produced with by far the highest concentration of vitamin B12 and consequently a much more efficient nasal absorption of vitamin B12. Such a nasal formulation can be taken less frequently by patients, making the therapy much easier and less expensive. (Col. 2, lines 27-37).”

30 **[0013]** In other prior art pertaining to the general field of the invention, U.S. Patent No. 4,525,341, Deihl, discloses a method of administering vitamins intranasally but does not describe or enable a specific formulation containing only cyanocobalamin.

35 **[0014]** International Patent Application No. PCT/US86/00665, publication no. WO 86/05987, discloses nasal spray composition containing vitamin B₁₂ as cyanocobalamin. However, the specific spray formulations all contained a mercury compound as a preservative, however the disclosure did require the presence of mercury compounds. Other preservatives were also mentioned including

5 benzalkonium chloride and chlorobutanol. As was stated above, an intranasal gel
containing cyanocobalamin, NASCOBAL®, is currently being produced and
marketed by Natestch Pharmaceutical Company Inc. of Bothell, Washington. It is
very effective in maintaining levels of vitamin B12 for patients who have been
deficient in the past but have recovered their levels of B12 through intramuscular
10 injections. However, a number of patients find the consistency of the gel unpleasant
in their nose, and would prefer an intranasal formulation that has a lower viscosity
and is free of mercury compounds. Thus, there is a need to produce a
pharmaceutically stable aqueous solution of cyanocobalamin that has a low viscosity,
is optionally free of mercury compounds, and has sufficient bioavailability to be used
15 as a maintenance therapy for vitamin B12.

SUMMARY OF THE INVENTION

[0015] The present invention fills this need by providing a stable pharmaceutical
solution of cyanocobalamin suitable for intranasal administration, having a viscosity
20 less than about 1000 cPs, wherein said intranasal solution of cyanocobalamin has a
bioavailability of at least 5%, and in certain embodiments at least 6% or 7%, of the
bioavailability of an intramuscular injection of cyanocobalamin.

[0016] In alternate embodiments, a therapeutic or pharmaceutically effective
formulation of the invention may be comprised of cyanocobalamin, citric acid,
25 sodium citrate, and water wherein the viscosity is less than 1000 cPs, and wherein the
solution of cyanocobalamin has a bioavailability of at least about 5% of, and in
certain embodiments at least about 6% or 7% of, the bioavailability of an
intramuscular injection of cyanocobalamin. In certain embodiments the solution of
cyanocobalamin according to the invention has a bioavailability of at least about 8%,
30 9%, 10%, 11%, or 12% of, the bioavailability of an intramuscular injection of
cyanocobalamin. In certain embodiments, the solution is essentially free of mercury
and mercury-containing compounds.

5 **[0017]** Certain compositions within the scope of this invention will contain a humectant to inhibit drying of the mucous membranes and to prevent irritation. Any of a variety of humectants can be used including but not limited to sorbitol, propylene glycol or glycerol. An illustrative, useful humectant is glycerin.

10 **[0018]** A preservative is generally employed to increase the shelf life of the compositions. Examples of preservative include but are not limited to benzyl alcohol, chlorobutanol and benzalkonium chloride. In illustrative embodiments, the preservative is benzalkonium chloride. A suitable concentration of the preservative will be from 0.002% to 2.0% based upon the total weight, although there may be appreciable variation depending upon the agent selected.

15 **[0019]** An exemplary formulation has the concentration of cyanocobalamin at 0.5% (percent of total weight), citric acid 0.12%, sodium citrate 0.32%, glycerin 2.23%, benzalkonium chloride 0.02% and 96.79% water. In other detailed embodiments, a nasal spray solution of Cyanocobalamin is provided in a spray applicator containing 2.3 mL of a 500 mcg/0.1 mL solution of cyanocobalamin with
20 sodium citrate, citric acid, glycerin and benzalkonium chloride in purified water. An exemplary spray solution in this context has a pH between 4.5 and 5.5. After initial priming, an exemplary spray pump spray delivers an average of 500 mcg of cyanocobalamin and the 2.3 mL of spray solution contained in the bottle will deliver 8 doses of the nasal spray.

25 **[0020]** Another embodiment of the present invention is a method for administering cyanocobalamin comprised of infusing the nose with an aqueous solution of cyanocobalamin, wherein the solution of cyanocobalamin has a viscosity of less than 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of at least about 5% of, and in certain embodiments at least about 6%
30 or 7% of, the bioavailability of an intramuscular injection of cyanocobalamin. In certain embodiments the solution of cyanocobalamin according to the invention has a bioavailability of at least about 8%, 9%, 10%, 11%, or 12% of, the bioavailability of an intramuscular injection of cyanocobalamin. In more detailed embodiments, the cyanocobalamin solution administered according to the methods of the invention is

5 essentially free of mercury and mercury-containing compounds. In this context,
“essentially free” generally means a solution having less than 2% by weight of a
particular substance, while in other aspects the solution will have less than 1% by
weight, 0.05% by weight, 0.01% by weight, or be completely free of the substance so
that, e.g., a mercury or mercury-containing compound is not detectable in the solution
10 using conventional detection methods.

[0021] The present invention is further directed towards a method for elevating
the vitamin B12 levels in the cerebral spinal fluid (CSF) comprising intranasally
administering a solution of cyanocobalamin so as to increase the average ratio of
vitamin B12 in the CSF to that in the blood serum ($B12\text{ CSF}/B12\text{ Serum} \times 100$) to at
15 least about 1.1, wherein said solution of cyanocobalamin has a bioavailability of at
least 7% relative to an intramuscular injection of cyanocobalamin. In a more detailed
embodiment the B12 CSF levels are increased so that the ratio of B12 in the CSF to
the levels in the blood serum is at least 1.9.

[0022] The methods of present invention are further comprised of the
20 cyanocobalamin solutions being administered into a nose of an individual through an
actuator tip as a spray, wherein the spray in certain embodiments has one or more of
the following properties: a spray pattern ellipticity ratio of from about 1.0 to about 1.4
when measured at a height of 3.0 cm from the actuator tip; or the spray produces
droplets, less than about 5% of the droplets are less than 10 μm in size; the spray has a
25 spray pattern major axis of about 35.3 mm and a minor axis of about 30.8 mm; 50%
of the droplets produced by the spray are 26.9 μm or less in size; 90% of the droplets
produced by the spray are 55.3 μm or less in size; or 10% of the droplets produced by
the spray are 12.5 μm or less in size.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows a nasal spray pump kit containing the cyanocobalamin solution of the present invention having an actuator that is not engaged.

FIG. 1B shows the nasal spray pump kit containing the cyanocobalamin solution of the present invention having an actuator that is engaged and expelling a spray plume of the cyanocobalamin solution of the present invention.

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FIG. 2 shows the spray pattern produced by the actuator of the spray pump kit.

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DETAILED DESCRIPTION OF THE INVENTION

[0023] The following definitions may aid in the understanding of the present invention.

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[0024] "About": is taken to be a relative term denoting an approximation of plus or minus 20% of the nominal value it refers to. For the field of pharmacology and clinical medicine and analogous arts that are the subject of this disclosure, this level of approximation is appropriate unless the value is specifically stated to be critical or to require a tighter range.

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[0025] "Nasal mucosa": the nasal mucosa is taken to be the lining of the vestibule of the nose, where vascularized, and extending interiorly to the boundaries of the oropharynx and sinuses.

[0026] "Aqueous": refers to a solution formed in water, but may contain lesser amounts of other co-solvents.

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[0027] "Bioavailability" is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action, [21 CFR §320.1(a)].

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[0028] "Bioavailability of the intranasal spray relative to an intramuscular injection of cyanocobalamin" means the percent amount a dose of the intranasal taken up by the systemic vascular system in comparison to the same amount of cyanocobalamin injected. For example, assuming an intramuscular injection of a solution of cyanocobalamin containing 100 µg of cyanocobalamin would have a 100% bioavailability, if an intranasal dose of cyanocobalamin contains 100 µg and has at least 5% of, and in certain embodiments at least about 6% or 7% of, the bioavailability of an intramuscular injection of cyanocobalamin, at least 5 µg, 6 µg, or 7 µg of cyanocobalamin would be taken up into the blood vasculature. Likewise, if the intranasal dose of cyanocobalamin contained 500 µg, at least 35 µg of cyanocobalamin would be taken up into the blood vasculature, if the intranasal formulation had a bioavailability of at least 7%.

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5 [0029] “Stability”: during storage, any compositional change measured in a parameter, examples of which include but are not limited to concentration, degradation, viscosity, pH, or particle size, that is considered to significantly affect the quality attributes of the product over time, denotes instability. In a similar vein, changes that are not considered to significantly affect the quality attributes of the product connote stability. The time period over which stability is measured is relative depending on the intended utility of the composition. Accelerated stability at higher temperature is sometimes taken as a more speedy way of extrapolating stability over longer periods of time than are actually measured.

15 [0030] “Pharmaceutically acceptable”: refers to a composition which when administered to a human or a mammal by the indicated route of administration, provokes no adverse reaction which is disproportionate to the benefit gained by administration of said compound.

20 [0031] “Mammal” shall include any of a class of warm-blooded higher vertebrates that nourish their young with milk secreted by mammary glands and have skin usually more or less covered with hair, and non-exclusively includes humans and non-human primates, their children, including neonates and adolescents, both male and female, livestock species, such as horses, cattle, sheep, and goats, and research and domestic species, including dogs, cats, mice, rats, guinea pigs, and rabbits. “Patient” or “subject” is used herein interchangeably with “mammal.”

25 [0032] “Intranasal delivery” shall mean delivery of a drug primarily via the mucosa of the nasal cavity. This includes the superior, middle and inferior nasal turbinates and the nasal pharynx. Note that the olfactory region is concentrated in the superior (upper 1/3) of the nasal turbinates. Cilial action pushes material back toward the oropharynx, so material deposited in the nasal vestibule encounters the nasal mucosa before entering the throat.

30 [0033] “Substantially free” refers to the level of a particular active ingredient in the compositions of the invention, wherein the particular active ingredient constitutes less than 20%, preferably less than 10%, more preferably less than 5%, and most

5 preferably less than 1%, by weight based on the total weight of active ingredients in the composition.

[0034] Delivery vehicles herein found useful include actuator dispensers commonly used for nasal solutions and gels. Embodiments of this technology include multiple, single-dose, metered dose, child resistant, and disposable dispensers, and
10 their kits.

[0035] As used herein “peak concentration ($C_{\max}$) of cyanocobalamin in a blood plasma”, “area under concentration vs. time curve (AUC) of cyanocobalamin in a blood plasma”, “time to maximal plasma concentration ($t_{\max}$) of vitamin in a blood plasma” are pharmacokinetic parameters known to one skilled in the art. [Laursen *et al.*, *Eur. J. Endocrinology*, 135: 309-315, (1996)]. The “concentration vs. time curve”
15 measures the concentration of cyanocobalamin in a blood serum of a subject vs. time after administration of a dosage of cyanocobalamin to the subject either by intranasal, subcutaneous, or other parenteral route of administration. “ $C_{\max}$ ” is the maximum concentration of cyanocobalamin in the blood serum of a subject following a single
20 dosage of cyanocobalamin to the subject. The term “ $t_{\max}$ ” is the time to reach maximum concentration of cyanocobalamin in a blood serum of a subject following administration of a single dosage of cyanocobalamin to the subject.

[0036] As used herein, “area under concentration vs. time curve (AUC) of cyanocobalamin in a blood plasma” is calculated according to the linear trapezoidal
25 rule and with addition of the residual areas. A decrease of 23% or an increase of 30% between two dosages would be detected with a probability of 90% (type II error $\beta = 10\%$). The “delivery rate” or “rate of absorption” is estimated by comparison of the time ($t_{\max}$) to reach the maximum concentration ($C_{\max}$). Both $C_{\max}$ and $t_{\max}$ are analyzed using non-parametric methods. Comparisons of the pharmacokinetics of
30 subcutaneous, intravenous and intranasal cyanocobalamin administrations were performed by analysis of variance (ANOVA). For pair wise comparisons a Bonferroni-Holmes sequential procedure was used to evaluate significance. The dose-response relationship between the three nasal doses was estimated by regression

5 analysis. $P < 0.05$ was considered significant. Results are given as mean values $\pm$ SEM. (Laursen et al., 1996.)

[0037] The above-described cyanocobalamin solutions are designed to be administered to the nasal mucosa either in drop or in spray form. However, the preferred mode of administration is in spray form, *i.e.*, in the form of finely divided
10 droplets. An example of a suitable spray pump is the Pfeiffer Spray Pump Model # 63385 produced by Pfeiffer GmbH, Radolfzell, Germany.

Nasal Administration of Cyanocobalamin

[0038] Cyanocobalamin is administered intranasally using a nasal spray according
15 to the present invention. In this area the following definitions are useful.

[0039] Aerosol – A product that is packaged under pressure and contains therapeutically active ingredients that are released upon activation of an appropriate valve system.

[0040] Metered aerosol – A pressurized dosage form comprised of metered dose
20 valves, which allow for the delivery of a uniform quantity of spray upon each activation.

[0041] Powder aerosol – A product that is packaged under pressure and contains therapeutically active ingredients in the form of a powder, which are released upon activation of an appropriate valve system.

25 [0042] Spray aerosol – An aerosol product that utilizes a compressed gas as the propellant to provide the force necessary to expel the product as a wet spray; it generally applicable to solutions of medicinal agents in aqueous solvents.

[0043] Spray – A liquid minutely divided as by a jet of air or steam.

[0044] Metered spray – A non-pressurized dosage form consisting of valves that
30 allow the dispensing of a specified quantity of spray upon each activation.

[0045] Suspension spray – A liquid preparation containing solid particles dispersed in a liquid vehicle and in the form of coarse droplets or as finely divided solids.

5 **[0046]** The fluid dynamic characterization of the aerosol spray emitted by metered nasal spray pumps as a drug delivery device ("DDD"). Spray characterization is an integral part of the regulatory submissions necessary for Food and Drug Administration ("FDA") approval of research and development, quality assurance and stability testing procedures for new and existing nasal spray pumps.

10 **[0047]** Thorough characterization of the spray's geometry has been found to be the best indicator of the overall performance of nasal spray pumps. In particular, measurements of the spray's divergence angle (plume geometry) as it exits the device; the spray's cross-sectional ellipticity, uniformity and particle/droplet distribution (spray pattern); and the time evolution of the developing spray have been found to be
15 the most representative performance quantities in the characterization of a nasal spray pump. During quality assurance and stability testing, plume geometry and spray pattern measurements are key identifiers for verifying consistency and conformity with the approved data criteria for the nasal spray pumps.

Definitions

20 **[0048]** Plume Height – the measurement from the actuator tip to the point at which the plume angle becomes non-linear because of the breakdown of linear flow. Based on a visual examination of digital images, and to establish a measurement point for width that is consistent with the farthest measurement point of spray pattern, a height of 30 mm is defined for this study

25 **[0049]** Major Axis – the largest chord that can be drawn within the fitted spray pattern that crosses the COMw in base units (mm)

[0050] Minor Axis – the smallest chord that can be drawn within the fitted spray pattern that crosses the COMw in base units (mm)

[0051] Ellipticity Ratio – the ratio of the major axis to the minor axis

30 **[0052]** D_{10} – the diameter of droplet for which 10% of the total liquid volume of sample consists of droplets of a smaller diameter (μm)

[0053] D_{50} – the diameter of droplet for which 50% of the total liquid volume of sample consists of droplets of a smaller diameter (μm), also known as the mass median diameter

5 [0054] D_{90} – the diameter of droplet for which 90% of the total liquid volume of sample consists of droplets of a smaller diameter (μm)

 [0055] Span – measurement of the width of the distribution, The smaller the value, the narrower the distribution. Span is calculated as $\frac{(D_{90} - D_{10})}{D_{50}}$.

10 [0056] % RSD – percent relative standard deviation, the standard deviation divided by the mean of the series and multiplied by 100, also known as % CV.

 [0057] **Cyanocobalamin nasal spray kit.**

 [0058] The present invention is further comprised of a cyanocobalamin nasal spray kit and method of administering the cyanocobalamin solution using the nasal spray kit.

15 [0059] The nasal spray kit is exemplified by Figures 1A, 1B and Figure 2. Figure 1A and 1B show a nasal spray device 10 before engagement (FIG. 1A) and after engagement (FIG. 1B). The cyanocobalamin nasal spray kit, 10, is comprised of a container, in this case a bottle 12 into which the cyanocobalamin formulation is placed, and an actuator 14, attached to bottle 12 and in fluid connection with the
20 solution of cyanocobalamin in bottle 12. When the actuator, 14 is actuated or engaged, it forces a spray plume, 16 of cyanocobalamin through tip 15 of the actuator. The spray plume is comprised of droplets of the solution of cyanocobalamin. A spray pattern is determined by taking a photograph of a cross-section of the spray plume 16 above a predetermined height, of the plume. The spray plume also has angle of
25 ejection, 20, as it leaves actuator, 14. A spray pattern of spray plume 16 is shown on FIG. 2. The Spray pattern of Figure 2, is elliptical and has a major axis, 24, and a minor axis 26.

 [0060] In one exemplary embodiment, the actuator produces spray of the cyanocobalamin solution having a spray pattern ellipticity ratio of from about 1.0 to
30 about 1.4 when measured at a height of 3.0 cm from the actuator tip. In certain embodiments less than 5% of the droplets of the cyanocobalamin solution are less than 10 μm in size, the spray pattern has a major axis and minor axis of 25 and 40 mm, respectively, 50% of the droplets are 26.9 μm or less in size, 90% of the droplets are 55.3 μm or less in size, and 10% of the droplets are 12.5 μm or less in size.

5 [0061] As noted above, the present invention provides improved methods and compositions for intranasal delivery cyanocobalamin to mammalian subjects for treatment or prevention of a variety of diseases, disorders and conditions. Examples of appropriate mammalian subjects for treatment and prophylaxis according to the methods of the invention include, but are not restricted to, humans and non-human
10 primates, livestock species, such as horses, cattle, sheep, and goats, and research and domestic species, including dogs, cats, mice, rats, guinea pigs, and rabbits.

 [0062] In exemplary therapy methods, the cyanocobalamin nasal spray of the present invention may be directed towards maintenance of the hematological status of patients who are in remission following intramuscular vitamin B12 therapy. For
15 example, an initial therapy regimen may involve the patient receiving daily intramuscular injections of 100 µg of cyanocobalamin for about 1 to 2 weeks, together with 1 to 5 mg of folic acid. Intramuscular injections of cyanocobalamin should not be greater than 100 µg as doses in excess of 100 µg are rapidly cleared from the plasma into the urine, and administration of larger amounts of vitamin B12
20 will not result in greater retention of larger amounts of the vitamin. Treatment using the methods and compositions of the invention may be enlisted following such a course of initial therapy, as follows.

 [0063] Instead of a once a month injection of 100 µg of cyanocobalamin, using the cyanocobalamin spray described herein the patient self-administers a dose of the
25 nasal spray, e.g., containing 500 µg of cyanocobalamin, once or twice a week. The maintenance therapy of the intranasal cyanocobalamin is for any patient that had been diagnosed with a vitamin B12 deficiency, but especially for those treated for pernicious anemia and dietary deficiency of vitamin B12 occurring in strict vegetarians, the so-called vegans who eat no animal products. Maintenance
30 cyanocobalamin therapy using the cyanocobalamin solution of the present invention is also indicated for those afflicted with malabsorption of vitamin B12 resulting from structural or functional damage to the stomach, where intrinsic factor is secreted or to the ileum, where intrinsic factor facilitates B12 absorption. These conditions include

5 tropical sprue and nontropical sprue (Idiopathic steatorrhea, gluten-induced enteropathy).

[0064] Maintenance cyanocobalamin therapy using the cyanocobalamin solution of the present invention is also indicated for those afflicted with malabsorption of vitamin B12 resulting from inadequate secretion of intrinsic factor, resulting from
10 lesion that destroys the gastric mucosa (ingestion of corrosives, extensive neoplasia), and a number of conditions associated with a variable degree of gastric atrophy (such as multiple sclerosis, human immunodeficiency viral (HIV) infection certain endocrine disorders, iron deficiency, and subtotal gastrectomy). Structural lesions that lead to B12 deficiency include ileitis, ileal resections, Crohn's disease and
15 malignancies. Vitamin B12 deficiencies may also be the result of competition by intestinal parasites, and inadequate utilization of vitamin B12 occurring if antimetabolites for the vitamin are employed in the treatment of neoplasia.

[0065] The intranasal cyanocobalamin solution of the present invention can also be used for individuals who require above normal levels of vitamin B12, due to, for
20 example, pregnancy, thyrotoxicosis, hemolytic anemia, hemorrhage, malignancy, hepatic and renal disease.

[0066] As noted above, the present invention provides for a stable pharmaceutical solution of cyanocobalamin suitable for intranasal administration, having a viscosity less than about 1000 cPs, wherein said intranasal solution of cyanocobalamin has
25 when administered intranasally a bioavailability of at least 5%, 6%, or 7% of the bioavailability of an intramuscular injection of cyanocobalamin. The intranasal formulation will often be comprised of, in addition to water and cyanocobalamin, a buffering agent to maintain the pH between 4 and 6 preferably about 5, an optional humectant to inhibit drying of the mucous membranes, and an optional preservative.

[0067] In certain exemplary embodiments, a composition according to the
30 invention is comprised of cyanocobalamin, citric acid, sodium citrate, and water wherein the viscosity is less than 1000 cPs, and wherein the solution of cyanocobalamin has a bioavailability of at least 5%, 6%, or 7%, and in certain

5 embodiments at least about 8%, 9%, 10%, 11%, or 12% or more, of the
bioavailability of an intramuscular injection of cyanocobalamin.

 [0068] In certain embodiments, compositions according to the invention will
contain a humectant to inhibit drying of the mucous membranes and to prevent
irritation. Any of a variety of humectants can be used including, for example sorbitol,
10 propylene glycol or glycerol. An exemplary humectant is glycerin.

 [0069] A preservative is generally employed to increase the shelf life of the
compositions. Examples of preservative include benzyl alcohol, parabens thimerosal,
chlorobutanol, benzethonium chloride and benzalkonium chloride. An exemplary
preservative useful within the formulations and methods of the invention is
15 benzalkonium chloride. A suitable concentration of the preservative will be from
0.002% to 2% based upon the total weight, although there may be appreciable
variation depending upon the agent selected.

 [0070] In certain exemplary embodiments, a formulation according to the
invention has the concentration of cyanocobalamin at 0.5% (percent of total weight),
20 citric acid 0.12%, sodium citrate 0.32%, glycerin 2.23%, benzalkonium chloride
solution 0.02% and 96.79% water.

 [0071] Other buffering agent combinations useful within the invention include but
are not limited to: Monopotassium phosphate and disodium phosphate; Potassium
biphthalate and sodium hydroxide; and Sodium acetate and acetic acid.

25 [0072] Another embodiment of the present invention is a method for
administering cyanocobalamin comprised of infusing the nose with an aqueous
solution of cyanocobalamin, wherein the solution of cyanocobalamin has a viscosity
of less than 1000 cPs, and wherein said solution of cyanocobalamin has a
bioavailability of at least about 5%, 6%, or 7% relative to an intramuscular injection
30 of cyanocobalamin. In certain embodiments, the bioavailability of the
cyanocobalamin solution of the invention is at least about 8%, 9%, 10%, 11%, and up
to 12% or greater compared to bioavailability of an intramuscular injection of
cyanocobalamin.

5 [0073] The present invention is further directed towards a method for elevating the vitamin B12 levels in the cerebral spinal fluid (CSF) comprising intranasally administering a solution of cyanocobalamin so as to increase the average ratio of vitamin B12 in the CSF to that in the blood serum ($B12\text{ CSF}/B12\text{ Serum} \times 100$) to at least about 1.1, wherein said solution of cyanocobalamin has a bioavailability of at least 7% relative to an intramuscular injection of a cyanocobalamin. In certain
10 embodiments the B12 CSF levels are increased so that the ratio of B12 in the CSF to the levels in the blood serum is at least 1:9.

 [0074] This is a significant embodiment of the present invention because vitamin B12 deficiency can result in irreversible damage to the nervous system. Progressive
15 swelling of myelinated neurons, demyelination, and neuronal cell death are seen in the spinal column and cerebral cortex. This causes a wide range of neurological signs and symptoms, including paresthesias of the hands and feet, diminution of vibration and position senses with resultant unsteadiness, decreased deep tendon reflexes, and, in the later stages, confusion, moodiness, loss of memory, and even a loss of central
20 vision. The patient may exhibit delusions, hallucinations, or even an overt psychosis. Since the neurological damage can be dissociated from the changes in the hematopoietic, vitamin B12 deficiency must be considered as a possibility in elderly patients with dementia and psychiatric disorders, even if they are not anemic. Thus, the embodiment of the present invention directed towards increasing the level of
25 vitamin B12 in the CSF can have tremendous benefit for neurological patients. Thus, intranasal administration of vitamin B12 according to the invention can be used to treat such diseases as Alzheimer's disease, dementia, and multiple sclerosis.

- 5 [0075] Exemplary formulations of the invention include the following:

Cyanocobalmin Nasal Spray 500 mcg/0.1 mL

Formulation:

Component	Nasal Solution
	Quantity (% w/w)
Cyanocobalamin, USP	0.50
Citric acid anhydrous, USP	0.12
Sodium citrate dihydrate, USP	0.32
Glycerin, USP	2.23
Benzalkonium chloride (50%), NF	0.04
Purified water q.s.	100.0

- 10 [0076] Alternative buffer systems and amounts that can be used for
Cyanocobalamin Nasal Spray

	Quantity (% w/w)
1) Citric Acid-Phosphate buffer	
Citric Acid anhydrous, USP	0.240
Dibasic Sodium Phosphate anhydrous	0.357
2) Acetate buffer	
Sodium Acetate anhydrous, USP	0.220
Acetic Acid, glacial, USP	0.064
3) Phosphate buffer	
Monobasic Potassium Phosphate anhydrous, NF	0.483
Dibasic Sodium Phosphate anhydrous	0.004

5 [0077] The intranasal formulations of the present invention can be administered using any spray bottle or syringe. A preferred nasal spray bottle is the, "Nasal Spray Pump w/ Safety Clip, Pfeiffer SAP # 60548, which delivers a dose of 0.1mL per
1 squirt and has a diptube length of 36.05 mm. It can be purchased from Pfeiffer of America of Princeton, NJ.

10 [0078] The following examples are provided by way of illustration, not limitation.

EXAMPLE 1

Comparison of Intranasal Cyanocobalamin Solution of the Present Invention with NASCOBAL® and Intramuscular Injections of Cyanocobalamin

Introduction

15 [0079] Nascobal® (Cyanocobalamin, USP) is a synthetic form of vitamin B₁₂ with equivalent vitamin B₁₂ activity. The chemical name is 5,6-dimethyl-benzimidazolyl cyanocobamide. Currently, Nascobal® (Cyanocobalamin, USP) is marketed as a self-administered nasal gel. The recommended dose of Nascobal® (Cyanocobalamin, USP) in subjects with vitamin B₁₂ malabsorption who are in remission following
20 inject able vitamin B₁₂ therapy is 500-µg administered intranasally once weekly.

 [0080] Vitamin B₁₂ deficiency has a number of causes, including malabsorption of vitamin B₁₂ resulting from structural or functional damage to the gastrointestinal system and dietary deficiency of vitamin B₁₂.

25 [0081] The purposes of this study are to compare the bioequivalence of vitamin B₁₂ nasal gel versus the nasal spray, and to evaluate the relative bioavailability of three preparations of vitamin B₁₂ in a fasted state in normal healthy male and female subjects.

30 [0082] Intranasal cyanocobalamin gel is approved for a dose of 500 µg. The current study also utilizes a cyanocobalamin nasal spray at the same 500 µg dose and an intramuscular dose of 100 µg.

5 Study Objectives

[0083] To compare the pharmacokinetic profile of a single intranasally-administered spray, single intranasally-administered gel (Nascobal[®]), and single intramuscular-administered vitamin B₁₂ in a fasted state in normal healthy male and female subjects.

10

5 Investigational plan

Overall Study Design and Plan

[0084] This study was a single-site, open-label, 3-way (3-treatment, 6-sequence) crossover, pharmacokinetic study of vitamin B₁₂ administered via intranasal (IN) spray (500-μg), IN gel (Nascobal[®]) (500-μg), and intramuscular (IM) injection (100-μg) in fasted normal healthy male and female subjects, as follows:

[0085] Treatment A: One IN spray administration of 500-μg vitamin B₁₂. The intranasal formulation was comprised of an exemplary embodiment of the present invention and contained cyanocobalamin at a concentration of 0.5% (percent of total weight), citric acid 0.12%, sodium citrate 0.32%, glycerin 2.23%, 50% benzalkonium chloride solution 0.04% and 96.79% water.

[0086] Treatment B: One IN gel administration of 500-μg vitamin B₁₂ (Nascobal[®]).

[0087] Treatment C: One IM administration of 100-μg vitamin B₁₂.

[0088] Subjects were on a Vitamin B₁₂-free diet throughout each confinement period. Subsequent treatments will be dosed no sooner than 14 days following the preceding treatment dose administration.

Treatments

Treatments Administered

[0089] On Day 1 of Periods I, II, and III after an 8 hour fast, subjects received a single IN spray of 500-μg vitamin B₁₂ (Treatment A), a single IN gel of 500-μg vitamin B₁₂ (Nascobal[®]) (Treatment B), or a single IM administration of 100-μg vitamin B₁₂ (Treatment C) based upon a randomization generated by the PPD Development Biostatistician in one of six sequences. Following all periods, all subjects were to have received each treatment in a crossover manner. A washout period of 14 days separated the three dosing periods.

[0090] On the morning of Day 1, subjects assigned to Treatment A received a single IN spray administration of 500 μg of vitamin B₁₂. Subjects assigned to

5 Treatment B received a single IN gel administration of 500 µg of vitamin B₁₂ (Nascobal®). Subjects assigned to Treatment C received a single IM administration of 100 µg of vitamin B₁₂. Doses were preceded by an overnight fast (i.e., at least 8 hours) from food (not including water) and were followed by a fast from food (not including water) for at least 4 hours post-dose.

10 [0091] While confined at the clinical site, subjects received a standardized vitamin B₁₂-deficient diet at scheduled times which did not conflict with other study-related activities. A registered dietician set up the diet, and the food staff maintained a diet diary. No dietary supplements were permitted during the study. Subjects abstained from consuming alcohol-containing, grapefruit-containing, or caffeine-containing foods
15 or beverages for 72 hours prior to Check-in.

Study Variables

[0092] For each subject, the following pharmacokinetic parameters were calculated whenever possible, based on the serum concentrations of vitamin B₁₂ from Treatments A, B, and C according to the model independent approach: C_{max}, T_{max}, and
20 AUC_{0-t}

Pharmacokinetic Measurements

[0093] Blood samples for PK analysis of vitamin B₁₂ levels were collected via an indwelling catheter and/or via direct venipuncture using 5-mL yellow-top Vacutainer® Hemogard™ evacuated serum separator collection tube. Blood samples for PK analysis
25 of vitamin B₁₂ levels were collected on Day -1 at 0, 6, and 12 hours and Day 1 at 0 hour (i.e., pre-dose); 30 minutes; 1, 1.5, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84 and 96 hours post-dose during each period.

Appropriateness of Measurements

[0094] The pharmacokinetic parameters used in this study were those typically
30 used to assess bioequivalence. All assessments of bioequivalence were based on comparisons of AUC_{0-t}, T_{max}, and C_{max} (test versus reference treatments).

[0095] AUC is a measure of the extent of drug bioavailability and reflects the total amount of drug that reaches the systemic circulation.

5 [0096] $C_{\max}$ represents the maximum serum concentration obtained after drug administration and provides an indication that sufficient drug has reached the systemic circulation to provide a therapeutic response. In addition, $C_{\max}$ provides warning of possible toxic drug levels.

 [0097] $T_{\max}$ was calculated and presented as median $\pm$ range.

10 Pharmacokinetic Variables

 [0098] For each subject, the following pharmacokinetic parameters were calculated, whenever possible, based on the serum concentrations of vitamin B₁₂ from Treatments A, B, and C, according to the model independent approach (Ref. 1):

 [0099] $C_{\max}$ Maximum observed concentration.

15 [00100] $t_{\max}$ Time to maximum concentration.

 [00101] AUC_{0-t} Area under the concentration-time curve from time 0 to the time of last measurable concentration, calculated by the linear trapezoidal rule.

 [00102] Pharmacokinetic calculations were performed, using SAS (SAS Inst., Version 8.02).

20 **Statistical Methods Planned in the Protocol and Determination of Sample Size**
 Statistical and Analytical Plans/Pharmacokinetic Analysis

 [00103] Levels of vitamin B₁₂ in serum samples were measured as pg/mL. Serum concentration values below the quantifiable limits of detection were treated as zero. Actual sampling times, rather than scheduled sampling times, were used in all
25 computations of the pharmacokinetic parameters. For ease of presentation, however, scheduled sampling times were used to present results in tables, listings, and figures.

 [00104] From the concentration data, non-compartmental pharmacokinetic parameters (AUC_{0-t} , $C_{\max}$, $T_{\max}$,) were calculated as described in Section 8.4.3.

Statistical Analysis

30 [00105] All statistical tests were conducted at the 0.05 significance level, unless otherwise specifically identified. Summary statistics of continuous parameters consisted of number (N), mean, median, SD, and range.

5 **[00106]** Descriptive statistics were obtained and tabulated by treatment for levels of vitamin B₁₂ at each time point and for the pharmacokinetic parameters calculated.

[00107] Bioequivalence was evaluated for the test (Treatment A - Nasal Spray) versus the reference (Treatment B - Gel). An analysis of variance (ANOVA, Ref. 2) was performed and the 90% confidence intervals were generated for the ratio of
10 test/reference. C_{max} and AUC_{0-t} were natural log (log_e) transformed prior to analysis. The corresponding 90% confidence intervals for the geometric mean ratio were obtained by taking the antilog of the 90% confidence intervals for the difference between the means on the log scale.

[00108] It was assumed that the test (Treatment A) is non-inferior (with respect to
15 the reference (Treatment B) if the lower bound of the 90% confidence intervals from log_e-transformed C_{max} and AUC_{0-t} were greater than or equal to 80%. If the lower bound of the 90% confidence intervals from log_e-transformed C_{max} and AUC_{0-t} were less than 80%, it was assumed that non-inferiority could not be established.

[00109] The sequence effect was tested using the mean square error (MSE) for
20 subject within sequence as the error term. All other main effects were tested against the MSE from the ANOVA model.

[00110] Bioavailability was evaluated for the test (Treatments A and B - Nasal Spray and Gel, respectively) and the reference (Treatment C – IM) groups. Relative bioavailability was assessed by examining the 90% confidence intervals for the ratio of
25 the test (Treatments A and B) group means relative to the reference (Treatment C) group mean.

[00111] For T_{max}, the analyses were run using Wilcoxon's matched pairs method to determine if differences exist between the test group and each reference group.

SUMMARY – CONCLUSIONS

PHARMACOKINETIC RESULTS:

The relative bioavailability for the two IN formulations was 0.9715. Bioavailability when comparing Treatment A (spray) versus Treatment C (IM) was 0.6105, and 0.6284 when comparing Treatment B (gel) versus Treatment C (IM).

The pharmacokinetic profiles of the spray formulation and the gel formulation were similar for C_{max} (1480 pg/mL, 1670 pg/mL, respectively) and AUC_{0-t} (92000 pg*hr/mL, 97000 pg*hr/mL, respectively). Additionally, the median difference for T_{max} between the spray and gel IN formulation was less than 15 minutes (-0.24). The C_{max} value for the IM formulation was significantly higher than the C_{max} values for the two IN formulations ($p < 0.0001$).

Bioequivalence was established for the Vitamin B₁₂ IN spray with regard to the gel data based on C_{max} and AUC_{0-t} . The 90% confidence intervals for the $\log_e$ -transformed C_{max} and AUC_{0-t} for the spray and gel formulations fell within the range of 80% to 125%. Additionally, non-inferiority can be assumed when comparing the two IN formulations because the lower bounds of the confidence intervals are greater than 80% for both AUC_{0-t} and C_{max} .

CONCLUSIONS:

- The relative bioavailability for the two IN formulations was 0.9715. Bioavailability for Treatment A (spray) versus Treatment C (IM) was 0.6105, and 0.6284 when comparing Treatment B (gel) versus Treatment C (IM).
- The pharmacokinetic profiles of the spray formulation and the gel formulation are similar for C_{max} (1480 pg/mL, 1670 pg/mL, respectively) and AUC_{0-t} (92000 pg*hr/mL, 97000 pg*hr/mL, respectively). Additionally, the median difference for T_{max} between the spray and gel IN formulation was less than 15 minutes (-0.24). The C_{max} value for the IM formulation was significantly higher than the C_{max} values for the two IN formulations ($p < 0.0001$).
- Bioequivalence between the Vitamin B₁₂ spray formulation and the Vitamin B₁₂ gel formulation was established using $\log_e$ -transformed 90% confidence intervals for AUC_{0-t} and C_{max} . The 90% confidence intervals for the $\log_e$ -transformed C_{max} and AUC_{0-t} for the spray and gel formulations fell within the range of 0.80 to 1.25. Noninferiority can be assumed for the two IN formulations (Treatment A versus Treatment B).

All Vitamin B₁₂ formulations were safe and well tolerated by healthy male and female volunteers.

5 **[00112]** As noted above, in certain embodiments of the invention a minimum relative or comparative bioavailability (e.g., in side-by-side test subjects administered comparable doses of intramuscular cyanocobalamin, or intranasal cyanocobalamin, tested for plasma or CSF concentration of cyanocobalamin against suitable control subjects administered sham or placebo preparations) of the intranasal cyanocobalamin
10 formulations of the invention will be at least 5%, 6%, or 7% of the bioavailability achieved by intramuscular injection, in some cases at least 8%, 9%, 10%, 11%, or 12% or greater.

[00113] The present disclosure provides detailed comparative bioavailability studies and results to evince these unexpected performance characteristics of the
15 methods and compositions of the invention. As described above, the relative bioavailability for two exemplary intranasal (IN) formulations was 0.9715. Relative bioavailability when comparing treatment A (Spray) versus treatment C (intramuscular = IM) was 0.6105, and 0.6284 when comparing Treatment B (gel) versus Treatment C (IM). The pharmacokinetic profiles of the compared
20 cyanocobalamin spray formulation and gel formulation were similar for C_{max} (1480 pg/mL, 1670 pg/mL, respectively) and AUC_{0-t} (9200 pg*hr/mL, 9700 pg*hr/mL, respectively). Additionally, the median difference for T_{max} between the spray and gel IN formulation was less than 15 minutes (-0.24). The C_{max} value for the IM formulation was significantly higher than the C_{max} values for the two IN
25 formulations ($p < 0.0001$).

[00114] While these data are not expressed directly in the form of comparative AUC values for IN versus IM bioavailability of cyanocobalamin, the relative AUC values are readily and accurately derivable from the data presented above. In particular, the comparative bioavailability study results presented above demonstrate
30 that the “relative bioavailability” ratio of an exemplary spray cyanocobalmin formulation compared to IM cyanocobalamin bioavailability, and of an exemplary gel formulation compared to IM bioavailability, is 0.6105, and 0.6284, respectively. These values represent ratios of the natural log of geometric means of the AUC based

5 on nominal doses. These data were dosed normalized according to conventional practice to the appropriate dose multiple based on a dose of 500 µg given intranasal and 100 µg given by IM. The skilled artisan will readily comprehend these data and fully appreciate that the dose normalized data yield a ratio of bioavailability between an IN cyanocobalamin solution of the invention and IM-administration that
10 reasonably corresponds to the disclosed relative minimum relative bioavailability of at least about 5%, 6%, or 7%. This determination can be made by a standard mathematical operation to derive the dose normalized relative AUC values for an IN spray and IM injection. In the example provided above, this standard operation/result is $0.6105 \times 100 \mu\text{g}/500\mu\text{g} \times 100 = 12\%$; or a ratio of the AUC between the IN spray and IM injection of 0.12. In addition, the actual arithmetic AUC are provided above
15 for an exemplary IN cyanocobalamin spray and gel, as 92000 and 97000 pg*hr/mL, respectively. These data likewise evince the corresponding AUC for the IM injected study comparator, according to the instant disclosure. For example, the arithmetic mean of the AUC for IM is calculated as 147155 pg*hr/mL (as readily derived by
20 reverse mathematical operation from the ratios given above--for example for the spray $92000/147155 = 0.62$ ratio). When dose normalized these data correspond directly to an exemplary relative bioavailability value within the presently-described range of at least 7%, and in other embodiments at least 9%, 10%, 11% or 12% or greater for an IN cyanocobalamin formulation of the invention compared to IM cyanocobalamin
25 bioavailability.

EXAMPLE 2

[00115] Also provided by the instant disclosure are results of a non-blinded, single dose, parallel group study to compare the uptake of Vitamin B₁₂ into the cerebrospinal fluid (CSF) after intranasal and intramuscular administration in healthy male and non-
30 pregnant female volunteers. This study compared CSF levels to plasma levels produced by both formulations.

[00116] Thirty-six healthy male and non-pregnant female subjects, age 18 and over, were enrolled in the study. Eighteen subjects received a single

5 intranasal dose of 500 mcg delivered as a 0.1 mL spray and eighteen subjects received a single intramuscular dose of 100 mcg delivered intramuscularly. Each subject visited the clinical site three times in a one-month period. These visits consisted of a screening visit, one dosing visit and a final visit.

10 [00117] After each dosing, each subject underwent lumbar puncture only once, with the retrieval of a total 4.0 mL of CSF (4 tubes, 1.0 mL per tube). One third of the subjects had a CSF sample collected at 60 minutes post dosing, one third of subjects had a CSF sample collected at 90 minutes post dosing, and one third of subjects had a CSF sample collected at 120 minutes post dosing

15 [00118] In addition to the above, on the day of dosing 7 mL blood samples were drawn before dosing and post dosing at 5, 10, 15 and 20 minutes, and at 0.5, 1, 1 ½, 2, 3, 4, 6, and 8 hours post-dose (prior to discharge).

[00119] The cerebrospinal fluid was evaluated for total Vitamin B₁₂ content. It was the objective of the study described herein to measure the amount of Vitamin B₁₂ present in the blood and CSF following intramuscular (IM) and nasal administration.

20 **Reference and Test Products**

[00120] Reference Product: Cyanocobalamin 100mcg intramuscular injection.

[00121] Cyanocobalamin Injection, USP is a sterile solution of cyanocobalamin (Vitamin B₁₂) for intramuscular or subcutaneous injection. Each mL contains 1,000 mcg cyanocobalamin.

25 [00122] Test Product: Vitamin B₁₂ Nasal Spray = 500 mcg/0.1 mL spray. The cyanocobalamin intranasal aqueous solution in this study contained cyanocobalamin at a concentration of 0.5% (percent of total weight), citric acid 0.12%, sodium citrate 0.32%, glycerin 2.23%, 50% benzalkonium chloride solution 0.04% and 96.79% water.

30 [00123] Vitamin B₁₂ Nasal Spray was supplied as a 2.3 mL bottle to deliver one dose: 500 mcg/0.1 mL per dose.

[00124] Before intranasal dosing, all subjects were given an orientation of the proper dosing technique and general conduct of the study.

5 **[00125]** The subjects were instructed to gently blow his/her nose. The subjects remained in a seated position, and the primed IN applicator was inserted into the nostril by the subject, under the direction of the study staff. During dosing, the contralateral nostril was closed with the forefinger. Subjects were instructed to tilt their heads slightly back for dosing and to return their heads to an upright position while sniffing in
10 gently immediately following dosing. According to this protocol, a 0.1 mL dose of vitamin B₁₂ spray was released into the nasal cavity of each subject (a dose is a single application to one nostril). Subjects were instructed to refrain from blowing their nose for 1 hour following IN treatment.

15 **[00126]** After dosing, each subject underwent lumbar puncture, involving the retrieval of 4.0 mL of CSF (4 tubes, 1.0 mL per tube). One third of subjects from each group had a CSF sample collected at 60 minutes post dosing, one third of subjects had a CSF sample collected at 90 minutes post dosing, and one third of subjects had a CSF sample collected at 120 minutes post dosing. At the appropriate time after dosing, the investigator positioned the patient appropriately for lumbar puncture. The lumbar area
20 was prepared and draped in the usual aseptic fashion. Local anesthesia was utilized (1% xylocaine, 1-5 mL). Upon reaching a state of adequate anesthesia, a spinal needle (20 or 22G) was introduced into the spinal canal, at the level deemed appropriate by the Investigator. The CSF samples were collected
25 60, 90 or 120 minutes after administration. A total of 4.0 mL of CSF were collected from each patient, and distributed into 4 separate collection tubes. The tubes were appropriately labeled with a patient identifier and submitted for bioanalytical analysis. Upon completion of CSF collection, the spinal needle was removed.

30 **[00127]** The levels of vitamin B₁₂ were determined in both the CSF and blood serum using Vitamin B₁₂ concentrations in the CSF will be analyzed for determination of Vitamin B₁₂ using a validated TOSOH Nex. 1A procedure.

5 **Results and Conclusion**

 [00128] The data showed that the ratio of vitamin B12 to serum was higher in those individuals receiving intranasal administration of vitamin B12 than those receiving intramuscular injections of vitamin B12.

10 [00129] The average ratio (B12 CSF/B12 Serum x 100) ranged from 1.1 to 1.9 for those individuals receiving intranasal administration of vitamin B12 while those who received intramuscular injections of vitamin B12 had an average ratio ranging from 0.17 to 0.24. This is a surprising result in that intranasal administration only has about a 7-12% bioavailability in the blood serum relative to intramuscular injection of vitamin B12. This indicates that intranasal administration of vitamin B12 reaches the
15 CSF much more effectively than by intramuscular injection.

5

EXAMPLE 3**Production of a Cyanocobalamin Solution**

[00130] A 4000 g batch of a cyanocobalamin solution of the present invention was prepared, which had a concentration of 500 mcg/0.1g of solution.

Starting Materials

10 I. Formula Record

Ingredient Name	Theoretical Weight (Grams)
Cyanocobalamin, USP	20.0
Citric Acid, USP (Anhydrous)	4.8
Sodium Citrate, USP (Dihydrate)	12.8
Glycerin, USP	89.2
Benzalkonium Chloride Solution, NF (50%)	1.6
Purified Water, USP	3871.6*

[00131] The 3871.6 grams of water was placed in a stainless steel container, which had been placed on a hot plate. The water was heated to about 30° C and stirred. Into the heated water was added 12.8 g of sodium citrate while the water was being stirred at 300 rpm for 5 minutes. The 4.8 g of citric acid was then added and stirred for 10 minutes. Into this mixture was added 20.0 g of cyanocobalamin and stirred for 30 minutes at 30° C at 300 rpm. The hot plate was then turned off. The 89.2 g of glycerin was added and stirred for 5 minutes at 300 rpm. Into the cyanocobalamin solution was then added 1.6 g of an aqueous solution containing 50% by weight of Benzalkonium Chloride was added to the solution and stirred for 5 minutes at 300 rpm. The pH was then measured and adjusted if the pH was not with the 4.5-5.5 range. Additional water was added to bring the weight of the solution to 4000 g.

5

EXAMPLE 4

[00132] This example describes an exemplary pharmaceutical composition of the invention comprising an aqueous solution of salmon cyanocobalamin at a concentration sufficient to produce therapeutically effective plasma concentrations, delivered via an actuator to produce an aerosol of said solution, wherein the spray pattern ellipticity ratio of said aerosol is between 1.00 and 1.40 when measured at a height of 30 cm distance from the actuator tip.

[00133] The volume of the aerosol can be between about 5 microliters and 1.0 ml, preferably between 20 and 200 microliters.

[00134] This test method describes the procedure for characterizing plume geometry of the cyanocobalamin nasal solution formulations using the SprayView NSP system. The plume geometry is characterized using a SprayView High Speed Optical Spray Characterization System (SprayView NSP) with Integrated SprayView NSx actuation station (Image Therm Engineering, Inc., Sudbury, MA) according to the methods described in U.S. Patent No. 6,665,421 and U.S. Patent Application Publication No. 20030018416 published January 23, 2003.

[00135] Using the formulation of table 1 the spray characterization and droplet size of the formulation in both a 3 mL bottle both having a nasal Spray Pump w/ Safety Clip, Pfeiffer SAP # 65550, which delivers a dose of 0.1mL per squirt and has a diptube length of 36.05 mm.

[00136] The droplet size data are shown in the following table.

Droplet Size for Nasal Spray Bottle and Pfeiffer SAP # 60548

		D ₁₀	D ₅₀	D ₉₀	Span	% < 10 micromet
1mL	Salmon cyanocobalamin	12.5	26.9	55.3	1.6	5.1

- 5 Below are listed the spray pattern results:

Spray Pattern		MajorAxis	MinorAxis	EllipticityRatio
Active	3mL	35.3mm	30.8 mm	1.14

5 WHAT IS CLAIMED IS:

1. A stable pharmaceutical aqueous solution of cyanocobalamin comprised of cyanocobalamin and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered
10 intranasally of at least about 5% relative to an intramuscular injection of cyanocobalamin.
2. The aqueous solution of cyanocobalamin of claim 1, wherein the solution is comprised of citric acid, and sodium citrate and water and has a pH of about 4-6.
3. The aqueous solution of claim 2 wherein the pH of the solution is about 5.
4. The aqueous solution of claim 2 further comprised of a humectant.
- 15 5. The aqueous solution of claim 4 wherein the humectant is selected from the group consisting of sorbitol, propylene glycol, and glycerin.
6. The aqueous solution of claim 5 wherein the humectant is glycerin.
7. The aqueous solution of claim 6 wherein the glycerin is present at a concentration of about 2.23%.
- 20 8. The aqueous solution of claim 2 wherein the solution is further comprised of a preservative.
9. The aqueous solution of claim 8 wherein the preservative is selected from the group consisting of benzyl alcohol, chlorobutanol and benzalkonium chloride.
10. The aqueous solution of claim 9 wherein the preservative is benzalkonium
25 chloride.
11. The aqueous solution of claim 10 wherein the benzalkonium chloride is present in solution at a concentration of about 0.02%.
12. The aqueous solution of claim 2 wherein cyanocobalamin is present at about concentration 0.5% percent of total weight, citric acid is present at a concentration of
30 about 0.12%, sodium citrate is present at a concentration of about 0.32%.
13. The aqueous solution of claim 12 wherein the pH of the solution.
14. The aqueous solution of claim 12 further comprised of a humectant.

- 5 15. The aqueous solution of claim 14 wherein the humectant is selected from the group consisting of sorbitol, propylene glycol, and glycerin.
16. The aqueous solution of claim 15 wherein the humectant is glycerin.
17. The aqueous solution of claim 16 wherein glycerin is present in solution at a concentration of about 2.23%.
- 10 18. The aqueous solution of claim 12 further comprised of a preservative.
19. The aqueous solution of claim 18 wherein the preservative is selected from the group consisting of benzyl alcohol, chlorobutanol and benzalkonium chloride.
20. The aqueous solution of claim 19 wherein the preservative is benzalkonium chloride.
- 15 21. The aqueous solution of claim 20 wherein the benzalkonium chloride is present in solution at a concentration of about 0.02%.
22. The aqueous solution of claim 1 wherein the solution of cyanocobalamin has at least about 7% bioavailability relative to an intramuscular injection of cyanocobalamin.
23. A stable pharmaceutical aqueous solution of cyanocobalamin comprised of
- 20 cyanocobalamin at a concentration of about 0.5% of total weight of solution, citric acid at a concentration of about 0.12%, sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about 2.23%, benzalkonium chloride at concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of
- 25 cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an intramuscular injection of cyanocobalamin.
24. A method for administering cyanocobalamin comprised of infusing the nose with an aqueous solution of cyanocobalamin, wherein the solution of cyanocobalamin has a viscosity of less than 1000 cPs, and wherein said solution of cyanocobalamin has a
- 30 bioavailability of cyanocobalamin of about 5% relative to an intramuscular injection of cyanocobalamin.
25. The method of claim 24 wherein the solution of cyanocobalamin is further comprised of citric acid and sodium citrate wherein the solution has a pH of from about 4-6.

- 5 26. The method of claim 25 wherein the pH of the solution is about 5.
27. The method of claim 25 wherein cyanocobalamin is present in solution at a concentration of between 0.5-1% by weight.
28. The method of claim 27 wherein the concentration of cyanocobalamin in solution is about 0.5%.
- 10 29. The method of claim 28 wherein the citric acid is present in solution at a concentration of about 0.12%, and the sodium citrate is present in solution at a concentration of about 0.32%, in water.
30. A method for administering cyanocobalamin comprised of infusing the nose with an aqueous solution of cyanocobalamin wherein said aqueous solution of cyanocobalamin
- 15 is comprised of cyanocobalamin at a concentration of about 0.5% of total weight of solution, citric acid at a concentration of about 0.12%, sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about 2.23%, benzalkonium chloride at concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and
- 20 wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an intramuscular injection of cyanocobalamin.
31. A method for elevating the vitamin B12 levels in the cerebral spinal fluid (CSF) comprising administering intranasally a sufficient amount of a solution of
- 25 cyanocobalamin so that the average ratio of vitamin B12 in the CSF to that in the blood serum ($B12\text{ CSF}/B12\text{ Serum} \times 100$) is increased to at least about 1.1, wherein said aqueous solution of cyanocobalamin is comprised of cyanocobalamin at a concentration of about 0.5% of total weight of solution, citric acid at a concentration of about 0.12%, sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about
- 30 2.23%, benzalkonium chloride at concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an intramuscular injection of cyanocobalamin.

- 5 32. A pharmaceutical kit for nasal drug delivery comprising:
an aqueous solution of cyanocobalamin and excipients in a container and;
a droplet-generating actuator attached to said container and fluidly connected to
the cyanocobalamin solution in the container; wherein said actuator produces a spray of
the cyanocobalamin solution through a tip of the actuator when said actuator is engaged,
10 wherein said spray of cyanocobalamin solution has a spray pattern ellipticity ratio of from
about 1.0 to about 1.4 when measured at a height of 3.0 cm from the actuator tip.
33. The kit of claim 32 wherein said spray comprises droplets wherein less than 5% of
said droplets are less than 10 μm in size.
34. The kit of claim 32 wherein the aqueous solution of cyanocobalamin has a
15 viscosity of less than 1000 cPs, and wherein said solution of cyanocobalamin has a
bioavailability of cyanocobalamin of about 5% relative to an intramuscular injection of
cyanocobalamin.
35. The kit of claim 34 wherein the spray is comprised of droplets of the
cyanocobalamin solution wherein less than 5% of the droplets are less than 10 μm in size.
- 20 36. The kit of claim 3 wherein the spray has a spray pattern major axis and minor axis
of between 25 and 40 mm each.
37. The kit of claim 34 wherein the solution of cyanocobalamin is further comprised
of citric acid and sodium citrate wherein the solution has a pH of from about 4-6.
38. The kit of claim 37 wherein the pH of the solution is about 5.
- 25 39. The kit of claim 34 wherein cyanocobalamin is present in solution at a
concentration of between 0.5-1 % by weight.
40. The kit of claim 39 wherein the concentration of cyanocobalamin in solution is
about 0.5% by weight.
41. The kit of claim 37 wherein the citric acid is present in solution at a concentration
30 of about 0.12%, and the sodium citrate is present in solution at a concentration of about
0.32%, in water.
42. The kit of claim 34 wherein the cyanocobalamin spray is comprised of droplets of
the cyanocobalamin solution wherein 50% of the droplets are 26.9 μm or less in size.

- 5 43. The kit of claim 34 wherein the cyanocobalamin spray is comprised of droplets of the cyanocobalamin solution, wherein 90% of the droplets are 55.3 μm or less in size.
44. The kit of claim 34 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 10% of the droplets are 12.5 μm or less in size.
45. A kit for administering intranasally a cyanocobalamin solution comprised of a
10 container, a solution of cyanocobalamin in the container, and an actuator attached to said container, wherein a spray of cyanocobalamin solution is expelled through a tip of said actuator when said actuator is engaged wherein said aqueous solution of cyanocobalamin is comprised of cyanocobalamin at a concentration of about 0.5% of total weight of
15 solution, citric acid at a concentration of about 0.12%, sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about 2.23%, benzalkonium chloride at concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an intramuscular injection of
20 cyanocobalamin, and wherein the spray has a spray pattern ellipticity ratio of from about 1.0 to about 1.4 when measured at a height of 3.0 cm from the actuator tip.
46. The kit of claim 45 wherein the cyanocobalamin spray is comprised of droplets of the cyanocobalamin solution, wherein less than 5% of the droplets of the cyanocobalamin spray are less than 10 μm in size.
- 25 47. The kit of claim 45 wherein the cyanocobalamin spray is comprised of droplets of the cyanocobalamin solution, and wherein 50% of the droplets of the cyanocobalamin spray are 26.9 μm or less in size.
48. The kit of claim 45 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 90% of the droplets are 55.3 μm or less in size.
- 30 49. The kit of claim 45 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 10% of the droplets are 12.5 μm or less in size.
50. The kit of claim 45 wherein the spray has a spray pattern major axis of about 35.3 mm and a minor axis of about 30.8 mm.

- 5 51. A method for administering cyanocobalamin intranasally comprised of providing an aqueous solution of cyanocobalamin, wherein the solution of cyanocobalamin has a viscosity of less than 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin of about 5% relative to an intramuscular injection of cyanocobalamin, wherein the cyanocobalamin formulation is administered into a nose of
10 an individual through an actuator tip as a spray, wherein the spray has a spray pattern ellipticity ratio of from about 1.0 to about 1.4 when measured at a height of 3.0 cm from the actuator tip.
52. The method of claim 51 wherein the spray produces droplets, wherein less than 5% of the droplets are less than 10 μm in size.
- 15 53. The method of claim 51 wherein the spray has a spray pattern major axis and minor axis of between 25 and 40 mm each.
54. The method of claim 51 wherein the solution of cyanocobalamin is further comprised of citric acid and sodium citrate wherein the solution has a pH of from about 4-6.
- 20 55. The method of claim 51 wherein the pH of the solution is about 5.
56. The method of claim 51 wherein cyanocobalamin is present in solution at a concentration of between 0.5-1 % by weight.
57. The method of claim 56 wherein the concentration of cyanocobalamin in solution is about 0.5%.
- 25 58. The method of claim 51 wherein the citric acid is present in solution at a concentration of about 0.12%, and the sodium citrate is present in solution at a concentration of about 0.32%, in water.
59. The method of claim 51 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 50% of the droplets are 26.9 μm or less in size.
- 30 60. The method of claim 51 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 90% of the droplets are 55.3 μm or less in size.
61. The method of claim 51 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 10% of the droplets are 12.5 μm or less in size.

- 5 62. A method for administering cyanocobalamin comprised of providing an aqueous solution of cyanocobalamin wherein said aqueous solution of cyanocobalamin is comprised of cyanocobalamin at a concentration of about 0.5% of total weight of solution, citric acid at a concentration of about 0.12%, sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about 2.23%, benzalkonium chloride at
10 concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an intramuscular injection of cyanocobalamin, and wherein the cyanocobalamin solution is administered into a nose of
15 an individual through an actuator tip as a spray, wherein the spray has a spray pattern ellipticity ratio of from about 1.0 to about 1.4 when measured at a height of 3.0 cm from the actuator tip.
63. The method of claim 62 wherein the cyanocobalamin spray produces droplets of the solution, wherein less than 5% of the droplets are less than 10 μm in size.
- 20 64. The method of claim 62 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 50% of the droplets are 26.9 μm or less in size.
65. The method of claim 62 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 90% of the droplets are 55.3 μm or less in size.
66. The method of claim 62 wherein the cyanocobalamin spray produces droplets of
25 the solution, and wherein 10% of the droplets are 12.5 μm or less in size.
67. The method of claim 62 wherein the spray has a spray pattern major axis and a minor axis of about 25-40 mm each.
68. A method for elevating the vitamin B12 levels in the cerebral spinal fluid (CSF) comprising administering intranasally a sufficient amount of a solution of
30 cyanocobalamin so that the average ratio of vitamin B 12 in the CSF to that in the blood serum ($\text{E12 CSF/B12 Serum} \times 100$) is increased to at least about 1.1, wherein said aqueous solution of cyanocobalamin is comprised of cyanocobalamin at a concentration of about 0.5% of total weight of solution, citric acid at a concentration of about 0.12%,

5 sodium citrate at a concentration of about 0.32%, glycerin at a concentration of about 2.23%, benzalkonium chloride at concentration of about 0.02% and water wherein said solution of cyanocobalamin is suitable for intranasal administration, has a viscosity less than about 1000 cPs, and wherein said solution of cyanocobalamin has a bioavailability of cyanocobalamin when administered intranasally of at least about 5% relative to an
10 intramuscular injection of cyanocobalamin, and wherein the cyanocobalamin solution is administered into a nose of an individual through an actuator tip as a spray, wherein the spray has a spray pattern ellipticity ratio of from about 1.0 to about 1.4 when measured at a height of 3.0 cm from the actuator tip.

69. The method of claim 68 wherein the cyanocobalamin spray produces droplets of
15 the solution, wherein less than 5 % of the droplets are less than 10 μm in size.

70. The method of claim 68 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 50% of the droplets are 26.9 μm or less in size.

71. The method of claim 68 herein the cyanocobalamin spray produces droplets of the solution, and wherein 90% of the droplets are 55.3 μm or less in size.

20 72. The method of claim 68 wherein the cyanocobalamin spray produces droplets of the solution, and wherein 10% of the droplets are 12.5 μm or less in size.

73. The method of claim 68 wherein the spray has a spray pattern major axis and a minor axis of between 25-40 mm each.