LINACLOTIDE-CONTAINING FORMULATIONS FOR ORAL ADMINISTRATION

FIELD

This disclosure concerns solid formulations of a guanylate cyclase-C receptor agonist polypeptide suitable for oral administration and methods for preparing such formulations.

PRIORITY CLAIM

This application claims priortiy to United States Application Serial No. 61/089,422, filed August 15, 2008 and to the United States Provisional Application filed August 03, 2009 and entitled, "Stable Solid Formulation of a GC-C Receptor Agonist Polypeptide Suitable for Oral Administration". The entire contents of the aforementioned applications are incorporated herein by reference.

BACKGROUND

Many therapeutic polypeptides are formulated in aqueous solution because they are most active in this form. However, most polypeptides are not particularly stable in aqueous solution, such that the formulations often have a short half-life and require refrigeration. Although aqueous solutions of polypeptides can be dried by freeze-drying, spray-drying or other methods, such dried formulations may also be unstable and have reduced activity relative to an aqueous solution of the polypeptide. Typical break-down mechanisms that occur both in aqueous solution and in dried formulations include aggregation and oxidative or hydrolytic degradation. Thus, the majority of therapeutic polypeptides, whether in aqueous solution or dried, are stored under refrigerated conditions due to their limited stability.

Linaclotide is a peptide having the amino acid sequence Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr that activates the guanylate cyclase-C (GC-C) receptor. Linaclotide, which may be administered orally, is useful for the treatment of gastrointestinal disorders and conditions, including irritable bowel syndrome (IBS) and chronic constipation (CC). Formulations comprising linaclotide have needed to be refrigerated in order to avoid degradation over time. However, refrigeration is inconvenient both for commercial distribution of the drug and for storage by patients. Thus, there is a need to have a solid linaclotide formulation that is stable at room temperature for at least 12 months.

SUMMARY

In one aspect of the present disclosure, there is provided a solid pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide, a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+}, and a sterically hindered primary amine.

In another aspect of the present disclosure, there is provided a solid pharmaceutical composition comprising:

linaclotide;

Ca^{2+};

leucine; and

hydroxypropyl methylcellulose,

wherein the molar ratio of Ca^{2+}:leucine:linaclotide is between 5-100:5-50:1.

In yet another aspect of the present disclosure, there is provided a solid pharmaceutical composition as defined herein for use in treating a gastrointestinal disorder selected from impaired intestinal motility, irritable bowel syndrome, constipation, pain associated with constipation, dyspepsia, gastroparesis, chronic intestinal pseudo obstruction, Crohn's disease, ulcerative colitis, or inflammatory bowel disease.

In a further aspect of the present disclosure, there is provided a use of linaclotide or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a gastrointestinal disorder selected from impaired intestinal motility, irritable bowel syndrome, constipation, pain associated with constipation, dyspepsia, gastroparesis, chronic intestinal pseudo obstruction, Crohn's disease, ulcerative colitis, or inflammatory bowel disease, said medicament comprising a solid pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide, a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+}, and a sterically hindered primary amine.

In another aspect of the present disclosure, there is provided a unit dosage form comprising a solid pharmaceutical composition as defined herein.

In yet another aspect of the present disclosure, there is provided a sealed container comprising a plurality of unit dosage forms as defined herein.

In a further aspect of the present disclosure, there is provided a method for preparing a solid pharmaceutical composition comprising linaclotide or a salt thereof, the method comprising:

(a) providing an aqueous solution comprising:

(i) linaclotide or a pharmaceutically acceptable salt thereof

(ii) one or more of a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} or a sterically hindered primary amine; and,

(iii) optionally, a pharmaceutically acceptable binder; and

(b) applying the aqueous solution to a pharmaceutically acceptable filler to generate linaclotide-coated filler.

Solid, stable formulations of linaclotide suitable for oral administration are described herein as are methods for preparing such formulations. The formulations described herein contain a polypeptide consisting of the amino acid sequence Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr ("linaclotide") or a pharmaceutically acceptable salt thereof.

The linaclotide formulations described herein are stable and have a sufficient shelf life for manufacturing, storing and distributing the drug. For example, formulations described herein are expected to have a shelf life of at least 12 months at room temperature storage conditions (e.g., 25°C/60% relative humidity (RH)). In further embodiments, the formulations described herein are expected to have a shelf life of at least 18 months or at least 24 months at room temperature storage conditions (e.g., 25°C/60% RH).

In some embodiments, formulations are described wherein ≥ 95% of the original amount of linaclotide in the composition remains after three months when packaged samples are stored at accelerated conditions (40°C/75% RH) when assessed in an assay on a weight/weight basis as determined by high pressure liquid chromatography (HPLC) against a linaclotide reference standard. In further embodiments, ≥ 90% of the original amount of linaclotide in the composition remains after at least 6 months when packaged samples are stored at accelerated conditions (40°C/75% RH). In other embodiments, formulations are described wherein chromatographic purity of the linaclotide as determined as area percent by HPLC remains at ≥ 95% over the course of at least three months when packaged samples are stored at accelerated conditions (40°C/75% RH). In further embodiments, the chromatographic purity of the linaclotide as determined by area percent by HPLC remains at ≥ 90% over the course of at least 6 months when packaged samples are stored at accelerated conditions (40 °C/75% RH). Thus, for example, no more than about 10% of the linaclotide undergoes degradation to other products such as an oxidation product of linaclotide, a hydrolysis product of linaclotide or a formaldehyde-mediated imine product of linaclotide ("formaldehyde imine product").

In one embodiment, the present disclosure comprises a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by

less than 10% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24 months of storage of the pharmaceutical

composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the present disclosure comprises a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by less than 10% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the present disclosure comprises a unit dosage form of a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by less than 10% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the present disclosure comprises a unit dosage form of a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by less than 10% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the present disclosure comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by less than 10% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In another embodiment, the present disclosure comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising linaclotide, wherein the chromatographic purity of the linaclotide decreases by less than 10% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity. In a further

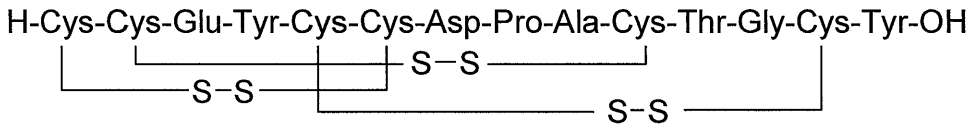
embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

In one embodiment, the present disclosure comprises a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for linaclotide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the present disclosure comprises a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the linaclotide decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the present disclosure comprises a unit dosage form of a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for linaclotide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the present disclosure comprises a unit dosage form of a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for linaclotide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

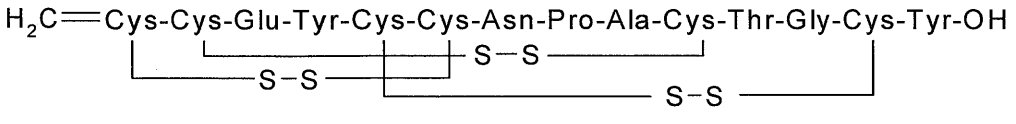
In one embodiment, the present disclosure comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the sealed container at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for linaclotide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In another embodiment, the present disclosure comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising linaclotide, wherein the assay value for linaclotide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity. In a further embodiment, the assay value for linaclotide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

In some embodiments, there is provided a pharmaceutical composition comprising linaclotide and a hydrolysis product comprising:



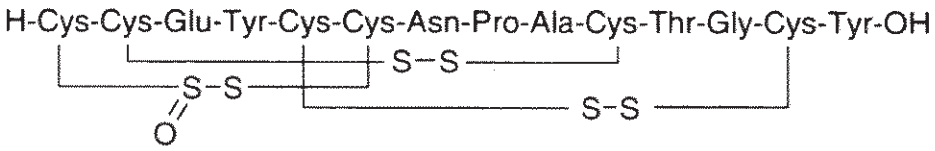
In some embodiments, the hydrolysis product comprises less than about 15% by weight of the composition, less than about 10% by weight of the composition, less than about 7% by weight of the composition or less than about 5% by weight of the composition. In other embodiments, the hydrolysis product comprises from about 0.01% to about 15% by weight of the composition, about 0.05% to about 10% by weight of the composition, about 0.05% to about 7% by weight of the composition or about 0.05% to about 5% by weight of the composition. In further embodiments, there is provided a method of treating a gastrointestinal disorder in a patient in need thereof comprising administering a pharmaceutical composition comprising linaclotide and a hydrolysis product.

In some embodiments, there is provided a pharmaceutical composition comprising linaclotide and a formaldehyde imine product comprising:



In some embodiments, the formaldehyde imine product comprises less than about 15% by weight of the composition, less than about 10% by weight of the composition, less than about 7% by weight of the composition or less than about 5% by weight of the composition. In other exemplary embodiments, the formaldehyde imine product comprises from about 0.01% to about 15% by weight of the composition, about 0.05% to about 10% by weight of the composition, about 0.05% to about 7% by weight of the composition or about 0.05% to about 5% by weight of the composition. In further embodiments, there is provided a method of treating a gastrointestinal disorder in a patient in need thereof comprising administering a pharmaceutical composition comprising linaclotide and a formaldehyde imine product.

In some embodiments, there is provided a pharmaceutical composition comprising linaclotide and a linaclotide oxidation product. In one embodiment, the linaclotide oxidation product has a molecular weight of 1542.8, which most likely forms as the addition of a single oxygen atom to one of the six cysteinyl sulfurs in linaclotide. One potential structure of the product is depicted below, although one of skill in the art will recognize that the oxygen atom may be attached to any of the other five sulfurs:



In another embodiment, there may be an addition of more than one oxygen atom to linaclotide, which would increase its molecular weight by 16 AU per added oxygen atom.

In some embodiments, the linaclotide oxidation product comprises less than about 15% by weight of the composition, less than about 10% by weight of the composition, less than about 7% by weight of the composition or less than about 5% by weight of the composition. In other exemplary embodiments, the linaclotide oxidation product comprises from about 0.01% to about 15% by weight of the composition, about 0.05% to about 10% by weight of the composition, about 0.05% to about 7% by weight of the composition or about 0.05% to about 5% by weight of the composition. In further embodiments, there is provided a method of treating a gastrointestinal disorder in a patient in need thereof comprising administering a pharmaceutical composition comprising linaclotide and a linaclotide oxidation product.

The assay value on a weight/weight basis ("weight/weight assay") may be determined by comparing, e.g., by HPLC, the amount of linaclotide in a sample, to a linaclotide reference

standard. As used herein, the weight of linaclotide in a composition after storage at room temperature or accelerated conditions at a specified time point (e.g., three or six months of storage under accelerated conditions [40°C/75% RH] or 12, 18 or 24 months of storage under room temperature conditions [25 °C/60% RH]) is compared to the weight of linaclotide in a composition at an initial time (e.g., the time when the pharmaceutical composition is released for clinical or patient use ("the release date")) to provide the weight/weight assay value. For example, the weight of linaclotide in a composition is measured after storage for a specified time at accelerated conditions (40°C/75% RH) and compared to the weight of linaclotide that was present in the sample at the release date. In another example, the weight of linaclotide in a composition is measured after storage for a specified time at room temperature conditions (25°C/60% RH) and compared to the weight of linaclotide that was present in the sample at the release date. Thus, the phrase "≥ 90% of the original amount of linaclotide in the composition remains after at least 6 months when packaged samples are stored at accelerated conditions (40°C/75% RH)" means the weight of linaclotide in the composition measured in an assay on a weight/weight basis as determined by HPLC after at least 6 months storage at accelerated conditions is ≥ 90% of the amount of linaclotide in the composition present at the initial time (e.g., the release date of the linaclotide composition).

Chromatographic purity of linaclotide may be assessed by performing HPLC under the conditions described herein. The area under the linaclotide peak is measured and compared to the total area under all peaks excluding the solvent peak and any non-polypeptide related peaks (i.e., peaks associated with excipients that may be observed in a placebo). As used herein, the chromatographic purity of linaclotide in a composition after storage at room temperature or accelerated conditions at a specified time point (e.g., three or six months of storage under accelerated conditions [40°C/75% RH] or 12, 18 or 24 months of storage under room temperature conditions [25 °C/60% RH]) is compared to the chromatographic purity of linaclotide in a composition at an initial time (e.g., the time when the pharmaceutical composition is released for clinical or patient use ("the release date")) to provide the chromatographic purity value. For example, the chromatographic purity of linaclotide in a composition is measured after storage for a specified time at accelerated conditions (40°C/75% RH) and compared to the chromatographic purity of linaclotide in the composition at the release date. In another example, the chromatographic purity of linaclotide in a composition is measured after storage for a specified time at room

temperature conditions (25°C/60% RH) and compared to the chromatographic purity of linaclotide in the composition at the release date.

This disclosure features a method for preparing a pharmaceutical composition comprising linaclotide or a pharmaceutically acceptable salt thereof, the method comprising: (a) providing a solution, e.g., an aqueous solution ("the coating solution"), comprising: (i) linaclotide or a pharmaceutically acceptable salt thereof; (ii) a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} and/or a sterically hindered primary amine (e.g., leucine) and, optionally, (iii) a pharmaceutically acceptable binder; and (b) applying the coating solution to a pharmaceutically acceptable filler to generate polypeptide-coated filler (e.g., by spraying, mixing or coating the pharmaceutically acceptable filler with the coating solution). The method can optionally include one or more of: (i) blending the polypeptide-coated filler with a pharmaceutically acceptable glidant, a pharmaceutically acceptable lubricant or a pharmaceutically acceptable additive that acts as both a glidant and lubricant; (ii) blending the polypeptide-coated filler with filler that is not polypeptide-coated, (iii) blending the polypeptide-coated filler with other additives; (iii) applying a pharmaceutically acceptable coating additive to the polypeptide-coated filler. The final pharmaceutical composition can be placed into capsules (e.g., gelatin capsule) or used to form tablets.

It has been found that a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or $Al^{3+}$is useful for suppressing the formation of an oxidation product of linaclotide during storage. It has also been found that a sterically hindered primary amine, e.g., leucine, is useful for suppressing the formation of a formaldehyde imine adduct of linaclotide ("formaldehyde imine product") during storage. Thus, a linaclotide formulation comprising a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} (e.g., a divalent cation selected from Zn^{2+}, Mg^{2+} or Ca^{2+}) and/or a sterically hindered primary amine, such as an amino acid, has a sufficient shelf life (as measured by chromatographic purity and/or by a weight/weight assay) for manufacturing, storing and distributing the drug. Further, while the presence of a sterically hindered amine alone can increase the formation of a hydrolysis product of linaclotide during storage, the combination of a sterically hindered primary amine and a cation, e.g., the combination of leucine and Ca^{2+}, suppresses the formation of the hydrolysis product of linaclotide as well as the oxidation product of linaclotide during storage, leading to an even greater overall stability as determined by a weight/weight assay and/or by chromatographic purity.

In some embodiments, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide and one or more agents selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} and a sterically hindered primary amine, wherein the agent improves at least one attribute of the composition, relative to a pharmaceutical composition without said agent. In further embodiments, the agent is Mg^{2+}, Ca^{2+} or Zn^{2+}. In a further embodiment, the agent is Ca^{2+}. In some embodiments, the cation is provided as, without limitation, magnesium acetate, magnesium chloride, magnesium phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate, manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum phosphate or aluminum sulfate. In further embodiments, the cation is provided as magnesium chloride, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, manganese chloride, potassium chloride, sodium chloride or aluminum chloride. In other embodiments, the cation is provided as calcium chloride, magnesium chloride or zinc acetate.

In another embodiment, the agent is a sterically hindered primary amine. In a further embodiment, the sterically hindered primary amine is an amino acid. In yet a further embodiment, the amino acid is a naturally-occurring amino acid. In a still further embodiment, the naturally-occurring amino acid is selected from the group consisting of: histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine and valine; yet further, the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine; in another embodiment, the naturally-occurring amino acid is leucine or methionine; still further, the naturally-occurring amino acid is leucine. In another embodiment, the sterically hindered primary amine is a non-naturally occurring amino acid or amino acid derivative (e.g., 1-aminocyclohexane carboxylic acid, lanthionine or theanine). In a further embodiment, the sterically hindered primary amine is cyclohexylamine, 2-methylbutylamine or chitosan.

In other embodiments, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide, a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} (e.g., a divalent cation selected from Zn^{2+}, Mg^{2+} or Ca^{2+}) and a sterically hindered primary amine. In one embodiment, the cation is Ca^{2+}. In another embodiment, the cation is a mixture of two or three of Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or

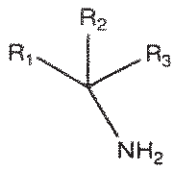
Al^{3+} (e.g., a mixture of two or three of Zn^{2+}, Mg^{2+} or Ca^{2+}). In a further embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable binder and/or a pharmaceutically acceptable glidant, lubricant or additive that acts as both a glidant and lubricant and/or an antioxidant. In a further embodiment, the sterically hindered primary amine is an amino acid. In yet a further embodiment, the amino acid is a naturally-occurring amino acid. In a still further embodiment, the naturally-occurring amino acid is selected from the group consisting of: histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine and valine; yet further, the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine; in another embodiment, the naturally-occurring amino acid is leucine or methionine; still further, the naturally-occurring amino acid is leucine. In another embodiment, the sterically hindered primary amine can be a mixture of more than one sterically hindered primary amines. For example, the sterically hindered primary amine may be a mixture of two or more sterically hindered primary amines, e.g., a mixture of two or more amino acids.

In some cases the molar ratio of cation:sterically hindered primary amine:linaclotide (e.g., Ca^{2+}:leucine:linaclotide) in the aqueous solution applied to the carrier is 5-100:5-50:1. It can be desirable for the molar ratio of cation:sterically hindered primary amine (e.g., Ca^{2+}:leucine) to be equal to or greater than 2:1 (e.g., between 5:1 and 2:1). Thus, in some cases the molar ratio of cation:sterically hindered primary amine:linaclotide (e.g., Ca^{2+}:leucine:linaclotide) applied to the carrier is 100:50:1, 100:30:1, 80:40:1, 80:30:1, 80:20:1, 60:30:1, 60:20:1, 50:30:1, 50:20:1, 40:20:1, 20:20:1, 10:10:1, 10:5:1 or 5:10:1. When binder, e.g., methylcellulose, is present in the linaclotide solution applied to the carrier it can be present at 0.5% - 2.5% by weight (e.g., 0.7%-1.7% or 0.7% - 1% or 1.5% or 0.7%).

The weight of linaclotide applied to a given weight of filler (e.g., microcrystalline cellulose) can vary from about 0.02:100 to about 2.67:100. Thus, about 0.05 mg to about 6.0 mg of linaclotide can be applied to 225 mg of filler. In a further embodiment, the weight of linaclotide applied to a given weight of filler is about 0.05 mg to about 2.0 mg of linaclotide (e.g., 0.1, 0.2, 0.3. 0.4, 0.5, 0.6, 0.7 mg peptide for 225 mg of filler).

In various embodiments: the sterically hindered primary amine is an amino acid (e.g., a naturally-occurring amino acid or a naturally-occurring amino acid selected from histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, methionine, asparagine, tyrosine, threonine, leucine, isoleucine, tryptophan, or valine). In other cases the sterically

hindered primary amine is a non-naturally occurring amino acid or amino acid derivative (e.g., lanthionine, theanine or 1-amino cyclohexane). In other cases, the sterically hindered primary amine is an amino sugar (e.g., chitosan or glucosamine).



In some cases, the sterically hindered primary amine has the formula: ,

wherein R_1, R_2 and R_3 are independently selected from: H; –C(O)OH; C1-C6 alkyl, optionally substituted by $–CO_2H$, $–CONH_2$, or a 5-10 membered aryl or heteroaryl; C1-C6 alkoxyalkyl; or C1-C6 thioalkoxyalkyl , , wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or $–NH_2$, and provided that no more than two of R_1, R_2 and R_3 are H. In a further embodiment, no more than one of R_1, R_2 and R_3 is H.

The term "alkyl", as used herein, refers to a saturated linear or branched-chain monovalent hydrocarbon radical. Unless otherwise specified, an alkyl group contains 1-20 carbon atoms (e.g., 1-20 carbon atoms, 1-10 carbon atoms, 1-8 carbon atoms, 1-6 carbon atoms, 1-4 carbon atoms or 1-3 carbon atoms). Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, t-butyl, pentyl, hexyl, heptyl, octyl and the like.

The terms C_{n-m} "alkoxyalkyl" and C_{n-m} "thioalkoxyalkyl" mean alkyl, substituted with one or more alkoxy or thioalkoxy groups, as the case may be, wherein the combined total number of carbons of the alkyl and alkoxy groups, or alkyl and thioalkoxy groups, combined, as the case may be, is between the values of n and m. For example, a C_{4-6} alkoxyalkyl has a total of 4-6 carbons divided between the alkyl and alkoxy portion; e.g. it can be $–CH_2OCH_2CH_2CH_3$, $–CH_2CH_2OCH_2CH_3$ or $–CH_2CH_2CH_2OCH_3$.

As used herein, the term "aryl" (as in "aryl ring" or "aryl group"), used alone or as part of a larger moiety, refers to a carbocyclic ring system wherein at least one ring in the system is aromatic and has a single point of attachment to the rest of the molecule. Unless otherwise specified, an aryl group may be monocyclic, bicyclic or tricyclic and contain 6-18 ring members. Examples of aryl rings include, but are not limited to, phenyl, naphthyl, indanyl, indenyl, tetralin, fluorenyl, and anthracenyl.

The term "heteroaryl" (or "heteroaromatic" or "heteroaryl group" or "aromatic heterocycle") used alone or as part of a larger moiety as in "heteroaralkyl" or

"heteroarylalkoxy" refers to a ring system wherein at least one ring in the system is aromatic and contains one or more heteroatoms, wherein each ring in the system contains 3 to 7 ring members and which has a single point of attachment to the rest of the molecule. Unless otherwise specified, a heteroaryl ring system may be monocyclic, bicyclic or tricyclic and have a total of five to fourteen ring members. In one embodiment, all rings in a heteroaryl system are aromatic. Also included in this definition are heteroaryl radicals where the heteroaryl ring is fused with one or more aromatic or non-aromatic carbocyclic or heterocyclic rings, or combinations thereof, as long as the radical or point of attachment is in the heteroaryl ring. Bicyclic 6,5 heteroaromatic system, as used herein, for example, is a six membered heteroaromatic ring fused to a second five membered ring wherein the radical or point of attachment is on the six membered ring.

Heteroaryl rings include, but are not limited to the following monocycles: 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, N-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, pyridazinyl (e.g., 3-pyridazinyl), 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, tetrazolyl (e.g., 5-tetrazolyl), triazolyl (e.g., 2-triazolyl and 5-triazolyl), 2-thienyl, 3-thienyl, pyrazolyl (e.g., 2-pyrazolyl), isothiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,3-triazolyl, 1,2,3-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, pyrazinyl, 1,3,5-triazinyl, and the following bicycles: benzimidazolyl, benzofuryl, benzothiophenyl, benzopyrazinyl, benzopyranonyl, indolyl (e.g., 2-indolyl), purinyl, quinolinyl (e.g., 2-quinolinyl, 3-quinolinyl, 4-quinolinyl), and isoquinolinyl (e.g., 1-isoquinolinyl, 3-isoquinolinyl, or 4-isoquinolinyl).

In various cases: the antioxidant is selected from BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), vitamin E, propyl gallate, ascorbic acid and salts or esters thereof, tocopherol and esters thereof, alpha-lipoic acid, beta-carotene; the pharmaceutically acceptable binder is polyvinyl alcohol or polyvinyl pyrrolidone; the pharmaceutically acceptable binder is selected from: a starch (e.g., corn starch, pre-gelatinized potato starch, rice starch, wheat starch, and sodium starch glycollate), maltodextrin or a cellulose ether (e.g., methylcellulose, ethylcellulose, carboxymethylcellulose, hydroxyethyl cellulose, hydroxyethyl methylcellulose, hydroxypropyl cellulose and hydroxypropyl methylcellulose); the pharmaceutically acceptable filler is cellulose (e.g., microfine cellulose or microcrystalline cellulose such as Celphere CP-305 or Avicel); the pharmaceutically acceptable filler is a sugar or a sugar alcohol (e.g., mannitol, isomalt, sorbitol, dextrose,

xylitol, sucrose and lactose); the filler comprises particles having an average diameter between 50 μm and 1000 μm; the lubricant and/or glidant is selected from: talc, leucine, magnesium stearate, stearic acid and polyvinyl alcohol; and the lubricant and/or glidant is selected from: calcium stearate, mineral oil, vegetable oil, polyethylene glycol (PEG ; e.g., PEG that is liquid or solid at room temperature), sodium benzoate, and sodium lauryl sulfate.

In some cases, the linaclotide solution used in a method for preparing the formulation has a pH below 7 (e.g., a pH between 1 and 3 or a pH between about 1.5 and about 2.5). The pH can be adjusted with, e.g., phosphoric acid. In some cases, the solution is buffered. Various pharmaceutically acceptable buffers can be used (e.g., phosphate buffer).

In some cases, the linaclotide solution used in a method for preparing the formulation comprises both a cation (e.g., $CaCl_2$) and a sterically hindered primary amine (e.g., leucine).

In some cases the linaclotide solution comprises $CaCl_2$ and leucine; the binder is methylcellulose; the filler is microcrystalline cellulose; the glidant and/or lubricant comprises talc or leucine.

Also provided is a pharmaceutical composition prepared by any of the methods described herein.

In another embodiment, a pharmaceutical composition is disclosed that comprises a pharmaceutically acceptable carrier, linaclotide and one or more agents selected from (i) a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^+, Na^+ or Al^{3+}, or (ii) a sterically hindered primary amine. In some embodiments, the pharmaceutical composition comprises at least one cation and at least one sterically hindered primary amine.

Methods of using the pharmaceutical compositions to treat a variety of gastrointestinal disorders are also described.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 demonstrates an example of an analysis of linaclotide by HPLC, wherein "Oxidation" refers to the linaclotide oxidation product, "Formaldehyde Imine" refers to the linaclotide formaldehyde imine product and "Hydrolysis" refers to the linaclotide hydrolysis product.

This figure is provided by way of example and is not intended to limit the scope of the present invention.

DETAILED DESCRIPTION

Oral compositions containing linaclotide can be used to treat a variety of gastrointestinal disorders. In various embodiments, the patient is suffering from a gastrointestinal disorder; the patient is suffering from a disorder selected from the group consisting of: gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus, ulcerative colitis, chronic constipation, constipation, pain associated with constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders described herein); the patient is suffering from a gastrointestinal motility disorder, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, inflammatory bowel disease, irritable bowel syndrome (e.g. diarrhea-predominant irritable bowel syndrome (d-IBS), constipation-predominant irritable bowel syndrome (c-IBS) and/or alternating irritable bowel syndrome (a-IBS)), post-operative ileus, ulcerative colitis, chronic constipation, constipation, pain associated with constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders described herein); the patient has been diagnosed with a functional gastrointestinal disorder according to the Rome Criteria (e.g. Rome II), the patient has been diagnosed with irritable bowel syndrome (e.g. (e.g. diarrhea predominant-IBS, constipation predominant-IBS, and/or alternating-IBS), according to the Rome Criteria (e.g. Rome II).

The dose range of linaclotide for adult humans is generally from 25 μg to 6 mg per day orally. In a further embodiment, the dose range is 25 μg to 2 mg per day orally. In some embodiments, the dose range for adult humans is 50 μg to 1 mg per day orally (e.g., 50 μg, 67.5 μg, 100 μg, 133 μg, 150 μg, 200 μg, 250 μg, 266 μg, 300 μg, 350 μg, 400 μg, 450 μg, 500 μg, 550 μg, 600 μg, 650 μg, 700 μg, 750 μg, 800 μg, 850 μg, 900 μg, 950 μg or 1 mg). In further embodiments, the dose range is 100 μg to 600 μg per day orally. In other embodiments, the dose is 50 μg, 67.5 μg, 100 μg, 133 μg, 150 μg, 200 μg, 266 μg, 300 μg, 400 μg, 500 μg or 600 μg linaclotide per day orally. In one embodiment, the linaclotide

composition is provided in a discrete unit, a unit dosage form, (e.g., a tablet, a capsule, a sachet) that is effective at such dosage or as a multiple of the same. In certain embodiments, the unit dosage form and daily dose are equivalent. In various embodiments, the unit dosage form is administered with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast). In various embodiments, the unit dosage form is administered once a day, twice a day or three times a day. The unit dosage form can optionally comprise other additives. In some embodiments, one, two or three unit dosage forms will contain the daily oral dose of linaclotide. The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity.

In one embodiment, there is provided a method for treating irritable bowel syndrome with constipation (IBS-c) in an adult patient in need thereof, comprising administering to the patient once daily an effective amount of a pharmaceutical composition described herein. In various embodiments, the pharmaceutical composition comprises 133 μg or 266 μg linaclotide per unit dose per day. In other embodiments, the pharmaceutical composition is administered for a period of at least one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks or longer. In some embodiments, treatment with the linaclotide composition improves at least one symptom selected from reduced abdominal pain, an increase in the number of complete spontaneous bowel movements (CSBM) in a week, an increase in the number of spontaneous bowel movements (SBM) in a week, improved stool consistency, reduced straining, reduced abdominal discomfort, reduced bloating or reduced IBS-c symptom severity.

In one embodiment, there is provided a method for treating chronic constipation in an adult patient in need thereof, comprising administering to the patient once daily an effective amount of a pharmaceutical composition described herein. In various embodiments, the pharmaceutical composition comprises 133 μg or 266 μg linaclotide per unit dose per day. In other embodiments, the pharmaceutical composition is administered for a period of at least one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks or longer. In some embodiments, treatment with the linaclotide composition improves at least one symptom selected from an increase in the number of complete spontaneous bowel movements (CSBM) in a week, an increase in the number of spontaneous bowel movements (SBM) in a week, improved stool consistency, reduced

straining, reduced abdominal discomfort, reduced bloating or reduced severity of constipation.

Stool consistency of each BM may be monitored by the 7-point Bristol Stool Form Scale (BSFS) (1 = hard lumps, 2 = lumpy sausage, 3 = cracked sausage, 4 = smooth sausage, 5 = soft lumps, 6 = mushy, 7 = watery). Straining may be monitored by the 7-point Ease of Passage Scale (1 = manual disimpaction/enema needed, 2 = severe straining, 3 = moderate straining, 4 = mild straining, 5 = no straining, 6 = urgency, 7 = incontinent). CSBM may be measured by the sensation of complete emptying after an SBM (yes/no). Abdominal discomfort, bloating and severity of constipation may be measured using, e.g., a 5-point ordinal scale (1 = none, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe).

A cation of the invention may be provided as a pharmaceutically acceptable salt i.e., a cation with an appropriate counterion. Examples of pharmaceutically acceptable salts that may be used in the invention include, without limitation, magnesium acetate, magnesium chloride, magnesium phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate, manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum phosphate or aluminum sulfate. In some embodiments, the pharmaceutically acceptable salts include calcium chloride, calcium carbonate, calcium acetate, magnesium chloride, magnesium acetate, zinc acetate and zinc chloride. In further embodiments, a pharmaceutically acceptable salt that may be used is calcium chloride, magnesium chloride and zinc acetate.

As used herein, the term "binder" refers to any pharmaceutically acceptable binder that may be used in the practice of the invention. Examples of pharmaceutically acceptable binders include, without limitation, a starch (e.g., corn starch, potato starch and pre-gelatinized starch (e.g., STARCH 1500® and STARCH 1500 LM®, sold by Colorcon, Ltd.) and other starches), maltodextrin, gelatin, natural and synthetic gums such as acacia, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., methylcellulose, hydroxyethyl cellulose, hydroxyethyl methylcellulose, hydroxypropyl cellulose and hydroxypropyl methylcellulose (hypromellose), ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose, carboxymethylcellulose, microcrystalline cellulose (e.g. AVICEL™, such as, AVICEL-PH-101™, -103™ and -105™,

sold by FMC Corporation, Marcus Hook, PA, USA)), polyvinyl alcohol, polyvinyl pyrrolidone (e.g., polyvinyl pyrrolidone K30), and mixtures thereof.

As used herein, the term "filler" refers to any pharmaceutically acceptable filler that may be used in the practice of the invention. Examples of pharmaceutically acceptable fillers include, without limitation, talc, calcium carbonate (e.g., granules or powder), dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate (e.g., granules or powder), microcrystalline cellulose (e.g., Avicel PH101 or Celphere CP-305), powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch (e.g., Starch 1500), pre-gelatinized starch, lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, isomalt, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, xylitol, myoinositol, and mixtures thereof.

Examples of pharmaceutically acceptable fillers that may be particularly used for coating with linaclotide include, without limitation, talc, microcrystalline cellulose (e.g., Avicel PH101 or Celphere CP-305), powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, isomalt, dibasic calcium phosphate, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, xylitol, mannitol, myoinositol, and mixtures thereof.

As used herein, the term "additives" refers to any pharmaceutically acceptable additive. Pharmaceutically acceptable additives include, without limitation, disintegrants, dispersing additives, lubricants, glidants, antioxidants, coating additives, diluents, surfactants, flavoring additives, humectants, absorption promoting additives, controlled release additives, anti-caking additives, anti-microbial agents (e.g., preservatives), colorants, desiccants, plasticizers and dyes.

As used herein, an "excipient" is any pharmaceutically acceptable additive, filler, binder or agent.

As used herein, "purified linaclotide" is linaclotide or a pharmaceutically acceptable salt thereof that is greater than or equal to 90 percent pure or greater than or equal to 95 percent pure. In some embodiments, linaclotide as used in the methods and compositions described herein is purified. Linaclotide purity can be measured, for example, by chromatographic purity of linaclotide using reversed phase HPLC as described in Example 21. Linaclotide Assay [w/w] can be determined, for example, by using reversed phase HPLC with quantitation via external calibration with a reference standard as described in Example 21.

In one aspect, the pharmaceutical composition may be prepared by spraying a solution comprising linaclotide or a pharmaceutically acceptable salt thereof, on a pharmaceutically acceptable filler to generate linaclotide-coated filler. In one embodiment, the method comprises: (a) providing a solution, e.g., an aqueous solution ("the coating solution"), comprising: (i) linaclotide or a pharmaceutically acceptable salt thereof; (ii) a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} and/or a sterically hindered primary amine (e.g., leucine) and, optionally, (iii) a pharmaceutically acceptable binder; and (b) applying the coating solution to a pharmaceutically acceptable filler to generate polypeptide-coated filler (e.g., by spraying, mixing or coating the pharmaceutically acceptable filler with the coating solution). The method can optionally include one or more of: (i) blending the polypeptide-coated filler with a pharmaceutically acceptable glidant, a pharmaceutically acceptable lubricant or a pharmaceutically acceptable additive that acts as both a glidant and lubricant; (ii) blending the polypeptide-coated filler with filler that is not polypeptide-coated, (iii) blending the polypeptide-coated filler with other additives; and (iv) applying a pharmaceutically acceptable coating additive to the polypeptide-coated filler. The final pharmaceutical composition can be placed into capsules (e.g., gelatin capsule) or used to form tablets.

In another embodiment, the pharmaceutical composition is prepared by spray drying, which is a technique used to prepare microparticles (e.g., microcapsules or microspheres) of drugs. Spray-dried peptides generally retain their biological activity upon dissolution and may have useful physical characteristics, including a uniform particle size and a spherical shape. In addition, the microparticles prepared by spray drying are often free flowing, which is helpful for pharmaceutical manufacturing processes such as forming tablets and filling capsules. Spray drying processes are also useful because they may be readily scaled up for clinical and commercial manufacturing.

Thus, this disclosure features a method for preparing a pharmaceutical composition comprising linaclotide or a pharmaceutically acceptable salt thereof, the method comprising: (a) providing a solution, e.g., an aqueous or organic solution, comprising: (i) linaclotide or a pharmaceutically acceptable salt thereof; and (ii) a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} and/or a sterically hindered primary amine (e.g., leucine) and (b) spray drying the linaclotide-containing solution to produce microparticles. The linaclotide-containing solution can optionally include a polymer, such as one or more of the binders described herein, a lipid or phospholipid, and/or a filler, such as mannitol. The method can

optionally include one or more additional steps of: (i) blending the linaclotide microparticles with a pharmaceutically acceptable glidant, a pharmaceutically acceptable lubricant or a pharmaceutically acceptable additive that acts as both a glidant and lubricant; (ii) blending the microparticles with a filler, and/or (iii) blending the microparticles with other additives. The final pharmaceutical composition can be placed into capsules (e.g., gelatin capsule) or used to form tablets.

In other embodiments, the pharmaceutical composition is prepared by spray freeze drying, supercritical fluid processing or lyophilization of a solution, e.g., an aqueous or organic solution, comprising: (i) linaclotide or a pharmaceutically acceptable salt thereof; and (ii) a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} and/or a sterically hindered primary amine (e.g., leucine).

In some embodiments, the linaclotide composition is provided in a solid form for oral administration. Examples of such forms include, without limitation, a tablet, a sachet, a pellet, a capsule or a powder. In some embodiments, the compositions can be used to create unit dosages forms, e.g., tablets, capsules, sachets or pellets. Orally administered compositions can include, for example, binders, lubricants, inert diluents, lubricating, surface active or dispersing additives, flavoring additives, and humectants. Orally administered formulations such as tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the linaclotide therein. The linaclotide can be co-administered or co-formulated with other medications. In one embodiment, the linaclotide composition can be co-administered with other medications used to treat gastrointestinal disorders. The linaclotide composition can also be used for treatment of disorders outside the gastrointestinal tract such as congestive heart failure and benign prostatic hypertrophy.

The compositions can include, for example, various additional solvents, dispersants, coatings, absorption promoting additives, controlled release additives, and one or more inert additives (which include, for example, starches, polyols, granulating additives, microcrystalline cellulose, diluents, lubricants, binders, disintegrating additives, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or non-aqueous techniques. Compositions can also include, for example, anti-caking additives, preservatives, sweetening additives, colorants, flavors, desiccants, plasticizers, dyes, and the like.

Suitable disintegrants include, for example, agar-agar, calcium carbonate,

microcrystalline cellulose, croscarmellose sodium, crospovidone, povidone, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums, and mixtures thereof.

Suitable lubricants include, for example, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil), zinc stearate, ethyl oleate, ethyl laurate, agar, syloid silica gel (AEROSIL 200, W.R. Grace Co., Baltimore, MD USA), a coagulated aerosol of synthetic silica (Evonik Degussa Co., Plano, TX USA), a pyrogenic silicon dioxide (CAB-O-SIL, Cabot Co., Boston, MA USA), and mixtures thereof.

Suitable glidants include, for example, leucine, colloidal silicon dioxide, magnesium trisilicate, powdered cellulose, starch, talc, and tribasic calcium phosphate.

Suitable anti-caking additives include, for example, calcium silicate, magnesium silicate, silicon dioxide, colloidal silicon dioxide, talc, and mixtures thereof.

Suitable anti-microbial additives that may be used, e.g., as a preservative for the linaclotide compositions, include, for example, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, butyl paraben, cetylpyridinium chloride, cresol, chlorobutanol, dehydroacetic acid, ethylparaben, methylparaben, phenol, phenylethyl alcohol, phenoxyethanol, phenylmercuric acetate, phenylmercuric nitrate, potassium sorbate, propylparaben, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimersol, thymo, and mixtures thereof.

Suitable coating additives include, for example, sodium carboxymethyl cellulose, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methyl cellulose phthalate, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, and mixtures thereof. Suitable protective coatings include Aquacoat (e.g. Aquacoat Ethylcellulose Aquaeous Dispersion, 15% w/w, FMC Biopolymer, ECD-30), Eudragit (e.g. Eudragit E PO PE-EL, Roehm Pharma Polymers) and Opadry (e.g Opadry AMB dispersion, 20% w/w, Colorcon).

In certain embodiments, suitable additives for the linaclotide composition include one or more of sucrose, talc, magnesium stearate, crospovidone or BHA.

In certain embodiments, the term "95%" may be 95.0%, the term "90%" may be 90.0%, the term "10%" may be 10.0%, the term "9%" may be 9.0%, the term "8%" may be

8.0%, the term "7%" may be 7.0%, the term "6%" may be 6.0%, the term "5%" may be 5.0%, the term "4%" may be 4.0%, the term "3%" may be 3.0%, the term "2%" may be 2.0%, and the term "1%" may be 1.0%.

In certain embodiments, the linaclotide composition is provided in a unit dosage form. In some embodiments, the unit dosage form is a capsule, a tablet, a sachet, a pellet or a powder. In one such embodiment, the unit dosage form is a capsule or tablet. Such unit dosage forms may be contained in a container such as, without limitation, a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. It is feasible that more than one container can be used together in a single package to provide a single dosage form. For example, tablets or capsules may be contained in a bottle which is in turn contained within a box. In some embodiments, the unit dosage forms are provided in a container further comprising a desiccant. In a further embodiment, the unit dosage forms, e.g., a quantity of tablets or capsules, are provided in a container, e.g., a bottle, jar or re-sealable bag, containing a desiccant. In a further embodiment, the container containing the unit dosage forms is packaged with administration or dosage instructions. In certain embodiments, the linaclotide composition is provided in a kit. The linaclotide composition described herein and combination therapy agents can be packaged as a kit that includes single or multiple doses of two or more agents, each packaged or formulated individually, or single or multiple doses of two or more agents packaged or formulated in combination. Thus, the linaclotide composition can be present in first container, and the kit can optionally include one or more agents in a second container. The container or containers are placed within a package, and the package can optionally include administration or dosage instructions.

EXAMPLES

The following examples are merely illustrative of the present invention and should not be construed as limiting the scope of the invention in any way as many variations and equivalents that are encompassed by the present invention will become apparent to those skilled in the art upon reading the present disclosure.

Linaclotide or a pharmaceutically acceptable salt thereof may be produced and purified using standard techniques known in the art, e.g., chemical synthesis or recombinant expression followed by and purification using standard techniques.

Formulation Scheme A

Preparation of the Coating Solution: Approximately 32 g to 42 g of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The cation, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. The sterically hindered primary amine, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. Other additives, such as antioxidants, are then added, if desired. The pH of the solution is tested, and hydrochloric acid is added, if necessary, to produce a solution having a pH between 1.5 and 2.0. The binder is then added to the solution and the mixture is then stirred for sufficient time to achieve a clear solution. The desired amount of linaclotide is added to the solution and mixed for 30-100 minutes to provide the coating solution.

Preparation of the Active Beads: Approximately 30-36 g of dried microcrystalline cellulose beads are added to a Mini Column Fluid Bed Coater. The microcrystalline cellulose beads are fluidized and heated prior to layering. Next, the coating solution is layered to the beads. The spraying temperature is controlled between 24°C and 55°C by controlling inlet temperature, spray rate, atomization pressure, and air volume. After the entire coating solution is layered to the beads, the beads are dried. The product of this process is referred to as active beads.

Preparation of Active Beads with Protective Coating: Approximately 35 g of Active Beads are added to a Mini Column Fluid Bed Coater. The Active Beads are fluidized and heated prior to coating with Aquacoat (e.g. Aquacoat Ethylcellulose Aquaeous Dispersion, 15% w/w, FMC Biopolymer, ECD-30), Eudragit (e.g. Eudragit E PO PE-EL, Roehm Pharma Polymers) or Opadry (e.g Opadry AMB dispersion, 20% w/w, Colorcon). Next, the coating solution is layered to the beads. The spraying temperature is controlled between 24°C and 55°C by controlling inlet temperature, spray rate, atomization pressure, and air volume. After the entire coating solution is layered to the beads, the beads are dried.

Formulation Scheme B

Preparation of the Coating Solution: Approximately 8.3 kg of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The cation, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. The sterically hindered primary amine, if used, is added to the solution in a quantity to provide the desired

concentration, and the solution is mixed for sufficient time to produce a clear solution. Other additives, such as antioxidants, are then added, if desired. The binder is then added to the solution and the solution is mixed for sufficient time to achieve a clear solution. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is Solution 1. Approximately 8.3 kg of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The desired amount of linaclotide is added to the solution and mixed for 10 to 30 minutes. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is Solution 2. Solution 1 and Solution 2 are then mixed together. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is the coating solution.

Preparation of the Active Beads: Approximately 24.19 kg of microcrystalline cellulose beads are added to a Wurster Column of a Glatt GPCG-30 Fluid Bed. The microcrystalline cellulose beads are fluidized and heated to product temperature of 45-47°C. Next, the coating solution is layered to the beads. The product spraying temperature is controlled between 37°C and 47°C by controlling inlet temperature, spray rate, atomization pressure, and air volume. After the entire coating solution is layered to the beads, the beads are dried with a product drying temperature of 37°C to 47°C. The product of this process is referred to as active beads.

Examples 1-15: Preparation of Linaclotide Formulations

The linaclotide formulations of Examples 1-15 were produced essentially as described in Formulation Scheme A wherein Table 1 provides the amounts of cation, sterically hindered primary amine, binder, linaclotide and beads, while Table 2 provides the conditions under which the beads were coated:

Table 1

Example	Cation Amount [ ]*	Amine Amount [ ]	Binder Amount	Amount of Linaclotide **	Beads Amount
1	$CaCl_2.2H_2O$ 0.6740g [60]	Leucine 0.2005g [20]	Hypromellose 1.019g	0.1282g	Celphere CP-305 33.38g
2	$CaCl_2.2H_2O$ 0.6740g [60]	Leucine 0.3007g [30]	Hypromellose 0.3063g	0.1329g	Celphere CP-305 33.87g

Example	Cation Amount [ ]*	Amine Amount [ ]	Binder Amount	Amount of Linaclotide **	Beads Amount
3	$CaCl_2.2H_2O$ 0.2247g [20]	Leucine 1.002g [100]	Hypromellose 0.0656g	0.1282g	Celphere CP-305 33.86g
4	$CaCl_2.2H_2O$ 1.123g [100]	Leucine 0.2005g [20]	Hypromellose 1.969g	0.1282g	Celphere CP-305 32.36g
5	$CaCl_2.2H_2O$ 0.4493g [40]	Leucine 0.4009g [40]	Hypromellose 0.5425g	0.1282g	Celphere CP-305 33.78g
6	$MgCl_2.6H_2O$ 0.2590g [10]	Leucine 0.3341g [20]	Hypromellose 0.6636g	0.2100g	Celphere CP-305 33.83g
7	$ZnAc.2H_2O$ 0.2796g [10]	Leucine 0.3341g [20]	Hypromellose 0.6636g	0.2100g	Celphere CP-305 33.82g
8	N/A	Leucine 0.8944g [27]	Hypromellose 0.6636g	0.4387g	Celphere CP-305 33.40g
9	$CaCl_2.2H_2O$ 0.3745g [10]	N/A	Hypromellose 0.6636g	0.4227g	Celphere CP-305 33.83g
10	N/A	N/A	Hypromellose 0.6811g	0.2114g	Celphere CP-305 34.28g
11	N/A	N/A	Hypromellose 0.6636g	0.4227g	Celphere CP-305 34.13g
12	$CuCl_2.2H_2O$ 0.4342g [10]	N/A	Hypromellose 0.6636g	0.4227g	Celphere CP-305 33.79g
13	$ZnAc.2H_2O$ 0.5590g [10]	N/A	Hypromellose 0.6636g	0.4227g	Celphere CP-305 33.68g
14	$MgCl_2.6H_2O$ 0.5178g [10]	N/A	Hypromellose 0.6636g	0.4227g	Celphere CP-305 33.72g
15	N/A	Methionine 0.0380g [1]	Hypromellose 0.6636g	0.4387g	Celphere CP-305 34.08g

* "Cation" refers to the divalent cation contained in the salt used in the example, "Amine" refers to the sterically hindered primary amine, [ ] refers to the molar ratio of the cation and/or amine to linaclotide.

** The Amount of linaclotide in this and all following examples is determined based on peptide content and chromatographic purity as listed on the Certificate of Analysis provided for each manufactured lot of linaclotide Active Pharmaceutical Ingredient (API).

Table 2

Example	Product Spraying Temp (°C)	Inlet Temp (°C)	Spray rate (mL/min)	Atomization Pressure (psig)	Air Flow
1	34.0 – 37.0	55.7 – 57.7	0.33 – 0.40	20	Low
2	27.4 – 32.3	37.01 – 42.1	0.40	22	Low
3	32.6 – 34.7	60.0 – 60.1	0.33 – 0.40	20	Low
4	35.3 – 39.3	58.9 – 59.2	0.40	18	Low
5	27.8 – 27.9	58.7 – 59.8	0.35 – 0.33	20	Low
6	32.1 – 38.3	42.0 – 53.4	0.39 – 0.75	22	Low
7	31.7 – 39.3	50.0 – 52.5	0.27 – 0.57	22	Low
8	33.3 – 41.3	50.5 – 57.0	0.57 – 0.65	22	Low
9	33.2 – 40.0	49.5 – 58.7	0.82 – 1.00	20	Low
10	42.5	59.5	0.49	22	Low
11	39.7	52.0	0.66	22	Low
12	36.6 – 40.0	47.2 – 54.8	0.65 – 0.75	20-22	Low
13	32.4	57.4	0.65	22	Low
14	34.0	49.0	0.75	20	Low
15	24.1 – 39.9	48.5 – 55.9	0.39 - 0.65	22-23	Low

Example 16: Preparation of Linaclotide Formulation

The linaclotide formulation of Example 16 was produced essentially as described in Formulation Scheme B wherein Table 3 provides the amounts of cation, sterically hindered primary amine, binder, linaclotide and beads, while Table 4 provides the conditions under which the beads were coated:

Table 3

Example	Cation Amount [ ]	Amine Amount [ ]	Binder Amount	Amount of Linaclotide	Beads Amount
16	$CaCl_2.2H_2O$ 385.1 g [60]	Leucine 171.8 g [30]	Hypromellose 175.0 g	73.5 g	Celphere CP-305 24.19 kg

Table 4

Example	Product Spraying Temp (°C)	Inlet Temp (°C)	Spray rate (g/min)	Atomization Pressure (bar)	Process Air Volume (cfm)	Product Drying Temp (°C)
16	64.9 – 65.1	80	150	2.0	515-564	54.9 – 55.0

Example 17: Preparation of Linaclotide Formulation

The linaclotide formulation of Example 17 was produced essentially as described in Formulation Scheme A except that the formulation contained 22.96mg butylated

hydroxyanisole (BHA), wherein Table 5 provides the amounts of cation, sterically hindered primary amine, binder, linaclotide and beads, while Table 6 provides the conditions under which the beads were coated.

Table 5

Example	Cation Amount [ ]	Amine Amount [ ]	Binder Amount	Amount of Linaclotide	Beads Amount
17	$CaCl_2.2H_2O$ 0.3745g [20]	N/A	Hypromellose 0.6636g	0.2100g	Celphere CP-305 33.99g

Table 6

Example	Product Spraying Temp (°C)	Inlet Temp (°C)	Spray rate (mL/min)	Atomization Pressure (psig)	Air Flow
17	33.5 – 34.8	47.7 – 48.6	0.56 – 0.74	26	Low

Example 18: Preparation of Capsules Containing Linaclotide Formulation

The linaclotide content on active beads may be measured as described in Example 21 or by other equivalent methods.

To form capsules suitable for oral administration, an appropriate amount of active beads is used to fill gelatin capsules (e.g., Size 2 gelatin capsules). An appropriate amount of active beads may contain 50 μg to 2 mg linaclotide per capsule with a range of ± 5%. In some embodiments, the appropriate amount of linaclotide on active beads may be 50 μg, 67.5 μg, 100 μg, 133 μg, 150 μg, 200 μg, 266 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1 mg, 2 mg, 4 mg or 6 mg. In a particular embodiment, the appropriate amount of linaclotide on active beads is 67.5 μg, 100 μg, 133 μg, 150 μg, 200 μg, 266 μg, 300 μg, 400 μg, 500 μg, 600 μg. In a more particular embodiment, the appropriate amount of linaclotide on active beads is 67.5 μg, 133 μg, 150 μg, 266 μg or 300 μg per capsule.

In another embodiment, an appropriate amount of active beads to fill a desired number of gelatin capsules is placed in a container. One or more pharmaceutically acceptable fillers or other pharmaceutically acceptable additives may be added, if desired, to the container. In some embodiments, a filler or additive is talc, leucine, microcrystalline cellulose or mannitol. The contents of the container are blended and the mixture is used to fill gelatin capsules with an appropriate amount of active beads containing linaclotide (e.g., 50 μg to 2 mg linaclotide per capsule with a range of ± 5%).

In an alternative embodiment, an appropriate amount of active beads is used to fill gelatin capsules and one or more pharmaceutically acceptable fillers or other pharmaceutically acceptable additives are added to the gelatin capsules.

Example 19: Preparation of Capsules Containing Linaclotide Formulation

Preparation of the Coating Solution: First, 41.98 g of purified water was mixed with 1.13 g of hydrochloric acid in order to create a solution with a pH between 1.5 and 2.0. Next, 7.49 g of calcium chloride dihydrate and 6.68 g of leucine were added to the solution, which was then mixed for 30 minutes in order to produce a clear solution. The pH was tested, and 1.70 g of hydrochloric acid was added to produce a solution having a pH between 1.5 and 2.0. Next, 13.27 g of hypromellose (hydroxypropyl methylcellulose; Dow Chemical Company; Midland, MI) was added to the solution and the mixture was stirred for 60 minutes to achieve a clear solution. Next, 4.39 g of a linaclotide was added to the solution and mixed for 90 minutes. The pH of the solution was 1.73. This was the coating solution.

Preparation of the Active Beads: 674.5 g of microcrystalline cellulose beads (Celphere CP-305; Ashai Kasei Corporation (Tokyo; Japan) were added to a Wurster Column of a Glatt GPCG-2 Fluid Bed. The microcrystalline cellulose beads were fluidized and heated for 30 minutes at a product temperature of 60°C. Next, the coating solution was layered to the beads. The product temperature was controlled between 45°C and 49°C by an inlet temperature of 80°C, spray rate of 5.0-11 g/min, an atomization pressure of 2.0 bar, and air volume of 40 to 50 m^3h. After the entire coating solution was layered to the beads, the beads were dried for 10 minutes with a product temperature of 46.9°C to 50.9°C. The product of this process was referred to as active beads.

Reverse phase liquid chromatography of linaclotide extracted from a formulation prepared as described above demonstrated that the extracted linaclotide and a linaclotide reference standard exhibited the same retention time and that there was no significant change in purity as a result of the formulation process.

To form capsules, 49.50 g of active beads were added to a clear bag. Next, 0.25 g of leucine, screened through a 60 mesh screen, was added to the bag. The bag was tied and mixed for 125 turns in order to blend all of the materials. Next, 0.25 g of talc, screened through a 60 mesh screen, was added to the bag. The bag was tied and mixed for 125 turns to blend all of the materials. Once all of the materials were blended, the mixture was used to fill Size 2 gelatin capsules at target weight of 227 mg/capsule with a range of ± 5%.

Example 20: Preparation of Capsules Containing Linaclotide Formulation

Active beads were prepared according to Example 16. The active beads were tested for linaclotide content. Based on the assay of the active beads, an appropriate amount of active beads (96mg – 123mg) were filled into size 2 hard gelatin capsules using an MG2 Futura encapsulation machine, to achieve a linaclotide concentration of 300 μg.

Active beads were prepared according to Example 15. The active beads were tested for linaclotide content. Based on the assay of the active beads, an appropriate amount of active beads (48mg – 62mg) were filled into size 2 hard gelatin capsules using an MG2 Futura encapsulation machine, to achieve a linaclotide concentration of 150 μg.

Example 21: Measurement of Linaclotide Content and Purity

Linaclotide content and purity, as well as measurement of linaclotide-related substances may be determined by reverse phase gradient liquid chromatography using an Agilent Series 1100 LC System with Chemstation Rev A.09.03 software or equivalent. A YMC Pro™ C18 column (dimensions: 3.0 x 150 mm, 3.5 um, 120 Å; Waters Corp., Milford, MA) or equivalent is used and is maintained at 40°C. Mobile phase A (MPA) consists of water with 0.1% trifluoroacetic acid while mobile phase B (MPB) consists of 95% acetonitrile:5% water with 0.1% trifluoroacetic acid. Elution of linaclotide and its related substances is accomplished with a gradient from 0% to 47% MPB in 28 minutes followed by a ramp to 100% MPB in 4 minutes with a 5 minute hold at 100% MPB to wash the column. Re-equilibration of the column is performed by returning to 0% MPB in 1 minute followed by a 10 minute hold at 100% MPA. The flow rate is 0.6 mL/min and detection is accomplished by UV at 220 nm.

Samples for analysis are prepared by addition of the contents of linaclotide capsules to 0.1 N HCl to obtain a target concentration of 20 μg linaclotide/mL. 100 μL of this solution is injected onto the column.

Linaclotide content is measured by determining the linaclotide concentration in the prepared sample against a similarly prepared external linaclotide standard.

An example of an analysis of linaclotide by HPLC is shown in Figure 1, wherein "Oxidation" refers to the linaclotide oxidation product, "Formaldehyde Imine" refers to the linaclotide formaldehyde imine product and "Hydrolysis" refers to the linaclotide hydrolysis product.

Example 22: Linaclotide Formulation Stability Testing

For the formulations of Examples 1-15 and 17, gelatin capsules were filled with approximately 225 mg of active beads. Five filled capsules were placed in plastic bottles. The bottles contained 1 to 2g of desiccant and were induction sealed. The bottles were stored at 40°C/75 % RH for six months.

Linaclotide content and purity as well as the amount of linaclotide-related substances were measured essentially as described in Example 21 or by an equivalent method. Results are provided in Table 7.

Table 7

Example	Assay [w/w] % of Initial	Area % by HPLC			
		Linaclotide (% of Initial)	Oxidation	Hydrolysis	Formaldehyde Imine
1	107.56	96.88 (99.13)	0.11	0.24	0.19
3	98.87	97.36 (99.42)	0.07	0.52	0.15
4	95.67	95.61 (97.83)	0.10	0.16	0.24
5	103.41	95.87 (98.68)	0.07	0.25	0.24
6	99.46	93.64 (95.51)	0.14	0.70	0.55
7	98.64	93.44 (95.36)	0.45	1.45	0.63
8	92.81	88.20 (94.90)	0.37	1.85	0.49
9	93.53	93.81 (96.55)	0.2	0.41	1.06
10	77.12	84.85 (87.77)	0.37	0.29	4.45
11	85.73	89.09 (91.63)	1.18	0.49	1.38
12	33.60	41.98 (43.15)	ND	ND	ND
13	87.69	91.91 (94.01)	1.98	0.74	0.86
14	86.94	90.59 (92.70)	0.25	0.54	1.23
15	87.71	87.54 (93.24)	0.24	0.66	1.67
17	98.94	93.65 (95.16)	ND	0.32	0.73

For the formulation of Example 16, gelatin capsules were filled with approximately 113 mg of total beads. 35 filled capsules were placed in plastic bottles. The bottles contained 2g of desiccant and were induction sealed. The bottles were stored at 40°C/75 % RH for one month.

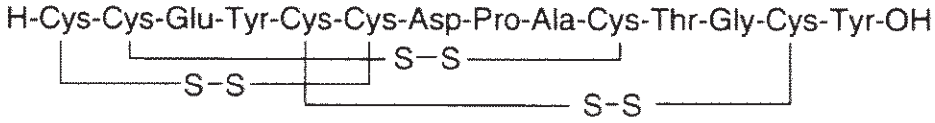
Linaclotide content and purity as well as the amount of linaclotide-related substances may be measured essentially as described in Example 21 or by an equivalent method. Results are shown in Table 8.

Table 8

Example	Assay [w/w] % of Initial	Area % by HPLC			
		Linaclotide (% of Initial)	Oxidation	Hydrolysis	Formaldehyde Imine
16	97.01	97.12 (99.79)	<0.1	<0.1	0.34

Example 23: Isolation and Preparation of Linaclotide Hydrolysis Product

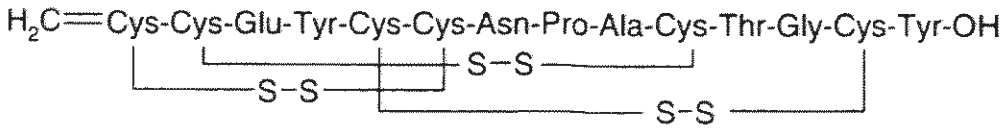
The linaclotide hydrolysis product occurs as a transformation of Asn in the 7 position to Asp (the numbering of linaclotide starts with 1 at the N-terminal Cys). Its structure is depicted below:



The linaclotide hydrolysis product has been independently synthesized for confirmation of identity using standard solid phase peptide synthesis techniques. The linaclotide hydrolysis product may also be prepared by other methods known in the art, e.g., by isolation from linaclotide preparations using chromatographic techniques or by recombinant expression of a nucleic acid encoding the linaclotide hydrolysis product (Cys Cys Glu Tyr Cys Cys Asp Pro Ala Cys Thr Gly Cys Tyr), optionally followed by oxidation of the cysteine residues to form the disulfide linkages.

Example 24: Isolation and Preparation of Linaclotide Formaldehyde Imine Product

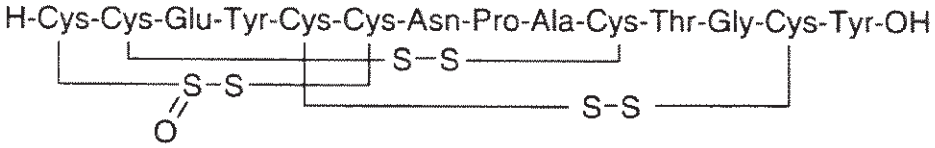
The formaldehyde imine product occurs as the addition of an imine to the N-terminal Cys (Cys1) via a formaldehyde-mediated reaction. A proposed structure of the product is depicted below:



The linaclotide formaldehyde imine product has been independently synthesized for confirmation of identity by reacting linaclotide with formaldehyde (1:5 molar ratio) in absolute ethanol at room temperature for 4 days. The formaldehyde imine product may also be prepared by other methods known in the art, e.g., by isolation from linaclotide preparations using chromatographic techniques or by chemical peptide synthesis or recombinant expression of a nucleic acid encoding linaclotide followed by formylation as described herein or by other methods known in the art, optionally followed by oxidation of the cysteine residues to form the disulfide linkages.

Example 25: Isolation and Preparation of Linaclotide Oxidation Product

The linaclotide oxidation product has a molecular weight of 1542.8. The oxidation product most likely forms as the addition of a single oxygen atom to one of the six cysteinyl sulfurs in linaclotide. One potential structure of the product is depicted below, although one of skill in the art will recognize that the oxygen atom may be attached to any of the other five sulfurs:



To support this identification, the linaclotide oxidation product has been produced by reacting linaclotide with hydrogen peroxide (3% aqueous) at room temperature or 40°C for up to 24 hours. The resulting product is enriched in the oxidation product by 1-10%. The linaclotide oxidation product may also be prepared by other methods known in the art, e.g., by isolation from linaclotide preparations using chromatographic techniques or by chemical peptide synthesis or recombinant expression of a nucleic acid encoding linaclotide followed by oxidation of the cysteine residues to form the disulfide linkages followed by reacting linaclotide with hydrogen peroxide or similar oxidizing reagent to form the linaclotide oxidation product.

Example 26: Linaclotide Tablet Formation

Fluid Bed Granulation

Linaclotide, $CaCl_2$, leucine and polyvinyl pyrrolidone (PVP) K30 were dissolved in 0.0001N HCl to form the coating solution (see Table 9). Isomalt was charged to the bowl of the fluid bed. With fluidizing the isomalt powder, the drug solution was top-sprayed at a speed of ~10g/min, with product temperature of ~40°C to coat the powder with the coating solution. Upon finishing spraying, the linaclotide granules were dried for 30 minutes and the product was discharged.

Table 9

Example	Cation Amount [ ]	Amine Amount [ ]	Binder Amount	Amount of Linaclotide	Filler Amount
26A	$CaCl_2.2H_2O$ 15.4 g [60]	Leucine 6.9 g [30]	PVP K30 40 g	3.08 g	Isomalt 935 g

Dicalcium phosphate or Avicel were also used as filler for fluid bed granulation.

Wet Granulation

Linaclotide was weighed and dissolved under agitation in 250 g of 0.1 N HCl (pH 1.7) to form Solution 1 (see Table 10). $CaCl_2$ and leucine were weighed and dissolved under agitation in 100 g 0.1 N HCl to form Solution 2. Solution 1 and Solution 2 were mixed together under agitation to form the coating solution. Avicel was added to the bowl of a high shear granulator. With mixing at 500 rpm, the coating solution was added into the Avicel. Upon finishing adding the solution, the granules were mixed and chopped for 1 minute. The wet granules obtained were charged into the bowl of a fluid bed, and dried for 15 minute and then the linaclotide granules were discharged.

Table 10

Example	Cation Amount [ ]	Amine Amount [ ]	Binder Amount	Amount of Linaclotide	Filler Amount
26B	$CaCl_2.2H_2O$ 7.68 g [60]	Leucine 3.42 g [30]	N/A	1.54g	Isomalt 488 g

In the wet granulation formula, the molar ratio of $CaCl_2$ and leucine to linaclotide was adjusted in the range of 60 to 100 and 30 to 50, respectively. Also, sucrose was added in one example. See Table 11.

Table 11

Example	Strength	Filler	$CaCl_2$:Leu:Linaclotide	Sucrose	HCl

	(Linaclotide/Filler)				
26C	600 μg/225 mg	Avicel	60:30:1	No	0.1N
26D	600 μg/225 mg	Avicel	80:40:1	No	0.1N
26E	600 μg/225 mg	Avicel	100:50:1	No	0.1N
26F	600 μg/225 mg	Avicel	60:30:1	5%	0.1N

Tablet Formulation

The linaclotide granules were blended with the following excipients (see Table 12) and compressed into tablets with a hardness of ~4kp.

Table 12

Ingredient Function	**Weight in 200 mg tablet with 150 μg Linaclotide**	**Weight in 400 mg tablet with 300 μg Linaclotide**	**Weight in 800 mg tablet with 600 μg Linaclotide**	**Weight in 1600 mg tablet with 1200 μg Linaclotide**
Linaclotide granules API	53.4 mg	106.8 mg	213.6 mg	427.2 mg
Isomalt Tablet filler	134.1 mg	268.2 mg	536.4 mg	1072.8 mg
Crospovidone Disintegrant	10 mg	20 mg	40 mg	80 mg
Magnesium stearate Lubricant	1.5 mg	3 mg	6 mg	12 mg
Talc Glidant	1 mg	2 mg	4 mg	8 mg
Total of dry material	200 mg	400 mg	800 mg	1600 mg

Isomalt, starch 1500 or dicalcium phosphate were also used as the tablet filler based on the above formula (see Table 13).

Table 13

Granulation	**Filler**	**$CaCl_2$:leucine:Linaclotide**	**Tablet Filler**
Fluid bed	isomalt	60:30:1	isomalt
			starch 1500
			dicalcium phosphate
Fluid bed	Avicel	60:30:1	starch 1500
Wet granulation	Avicel	100:50:1	starch 1500
Wet granulation	Avicel	60:30:1 + 5% sucrose	starch 1500

After two weeks storage at 40°C and 75% relative humidity, all tablets described in Table 13 exhibited assay values of linaclotide is greater than 90%.

Examples 27-53: Preparation of Linaclotide Formulations

The linaclotide formulations of Examples 27-53 were produced essentially as described in Formulation Scheme A and Examples 1-15. The linaclotide coating solution contained 0.7% binder (w/v) and the coating solution was sprayed on Celphere CP-305 beads as described in Examples 1-15. Table 14 provides the type of cation, amine and/or other excipient along with their molar ratios relative to linaclotide, as well as the type of binder used, while Table 15 provides the conditions under which the beads were coated:

Table 14

Example	Cation	Amine	Molar Ratio	Binder	Additive
27	$CaCl_2 \cdot 2H_2O$	--	20:0:1	Hypromellose	--
28	$MnCl_2 \cdot 4H_2O$	--	20:0:1	Hypromellose	--
29	KCl	--	20:0:1	Hypromellose	--
30	$AlCl_3 \cdot 6H_2O$	--	20:0:1	Hypromellose	--
31	$CaCl_2 \cdot 2H_2O$	Leucine	60:30:1	Hypromellose	--
32	Ca Alginate	Leucine	60:30:1	Hypromellose	--
33	$CaHPO_4$	Leucine	60:30:1	Hypromellose	--
34	Ca Stearate	Leucine	60:30:1	Hypromellose	--
35	$CaSO_4 \cdot 2H_2O$	Leucine	60:30:1	Hypromellose	--
36	$Zn(OAc)_2$	Leucine	60:30:1	Hypromellose	--
37	$CaCl_2 \cdot 2H_2O$	Isoleucine	60:30:1	Hypromellose	--
38	$CaCl_2 \cdot 2H_2O$	Valine	60:30:1	Hypromellose	--
39	$CaCl_2 \cdot 2H_2O$	Methionine	60:30:1	Hypromellose	--
40	$CaCl_2 \cdot 2H_2O$	Phenylalanine	60:30:1	Hypromellose	--
41	--	Histidine	0:20:1	Hypromellose	--
42	--	Tryptophan	0:20:1	Hypromellose	--
43	$CaCl_2 \cdot 2H_2O$	--	0:20:1:20 (Vit. E)	Hypromellose	Vitamin E
44	--	1-aminocyclohexane carboxylic acid	0:20:1	Hypromellose	--

Example	Cation	Amine	Molar Ratio	Binder	Additive
45	--	cyclohexylamine	0:20:1	Hypromellose	--
46	--	2-methylbutylamine	0:20:1	Hypromellose	--
47	--	chitosan	0:20:1	Hypromellose	--
48	$CaCl_2 \cdot 2H_2O$	Leucine	60:30:1	Polyvinyl pyrrolidone	--
49	$CaCl_2 \cdot 2H_2O$	Leucine	60:30:1	Methyl cellulose (Methocel A15)	--
50	$CaCl_2 \cdot 2H_2O$	Leucine	60:30:1	Hydroxypropyl cellulose	--
51	NaCl	--	20:0:1	Hypromellose	--
52	$CaCl_2 \cdot 2H_2O$	Leucine	60:30:1	Gelatin	--
53	$CaCl_2 \cdot 2H_2O$	Glycine	60:30:1	Hypromellose	--

* "Cation" refers to the cation contained in the salt used in the example, "Amine" refers to the sterically hindered primary amine, "Molar Ratio" refers to the molar ratio of the cation:amine:linaclotide:Additive (if applicable).

Table 15

Example	Product Spraying Temp (°C)	Inlet Temp (°C)	Spray rate (g/min)	Atomization Pressure (psig)	Air Flow
27	25.1-35.1	37.0-50.1	0.44-0.62	20	Low
28	24.1-35.8	37.3-50.9	0.30-0.61	18-20	Low
29	28.1-34.7	37.6-47.8	0.50-0.63	18	Low
30	29.8-35.0	33.9-50.2	0.32-0.47	20	Low
31	25.5-35.1	34.6-50.4	0.40-0.61	20	Low
33	30.4-35.2	38.7-51.0	0.48-0.52	20	Low
35	29.9-34.9	37.8-50.4	0.37-0.76	20	Low
36	29.9-35.4	38.0-50.1	0.38-0.50	21	Low
37	27.3-34.9	36.2-50.1	0.45-0.54	20	Low
38	27.6-36.2	36.9-47.3	0.43-0.66	20	Low
39	30.1-35.8	40.6-47.1	0.30-0.48	20	Low
40	31.7-37.5	41.3-51.0	0.40-0.67	18	Low
41	29.4-36.2	41.7-49.5	0.48-0.53	20	Low
42	31.0-38.6	42.4-51.2	0.52-0.64	20	Low
44	31.0-37.6	39.5-48.8	0.40-0.46	18	Low
45	28.7-36.5	37.1-49.2	0.49-0.61	18	Low
46	28.6-35.2	37.1-47.2	0.39-0.53	18	Low
47	33.4-38.7	40.6-48.5	0.48-0.47	18-26	Low
48	31.6-36.1	41.6-46.7	0.36-0.72	18	Low
49	28.5-36.5	36.8-48.1	0.45-0.51	18	Low
50	27.9-36.4	37.1-48.6	0.35-0.60	18	Low

Example	Product Spraying Temp (°C)	Inlet Temp (°C)	Spray rate (g/min)	Atomization Pressure (psig)	Air Flow
51	29.3-37.9	36.7-49.2	0.42-0.55	18	Low
52	29.8-36.3	36.1-49.1	0.44-0.54	18	Low
53	28.9-35.8	36.5-47.7	0.45-0.52	18	Low

Processing issues were experienced during spraying on the beads for examples 32 (Calcium Alginate), 34 (Calcium Stearate) and 43 ($CaCl_2$:Vitamin E). Thus, the coating solution was mixed with the Celphere beads and the beads were dried on a tray.

Example 54: Linaclotide Formulation Stability Testing

For the formulations of Examples 27-53, gelatin capsules were filled with approximately 225 mg of active beads (600 µg linaclotide/capsule). Five filled capsules were placed in plastic bottles. The bottles contained 1g of desiccant and were induction sealed. The bottles were stored at 40°C/75 % RH for three months or six months.

Linaclotide content (µg/mg) and percent chromatographic purity (%CP) were measured essentially as described in Example 21 or by an equivalent method. Results are provided in Table 16A (three months stability) or Table 16B (six month stability).

Table 16A

Example	Assay [w/w] % of Initial *	% CP	% CP [% of Initial]
27	96.30	93.98 %	98.07
28	96.82	93.59 %	96.07
29	101.56	92.71 %	95.40
30	109.06	93.07 %	95.76
31	103.59	95.98 %	99.12
32	66.53	82.66 %	85.27
33	96.81	91.94 %	93.55
34	30.75	55.47 %	56.88
35	101.37	93.07 %	95.02
36	105.27	91.49 %	93.45
37	109.22	95.73 %	97.99
38	99.24	95.79 %	97.59
39	95.22	95.76 %	97.82
40	102.98	95.68 %	97.60
41	110.92	94.03 %	96.30
42	120.05	88.57 %	91.65
43	58.51	70.99 %	74.06
44	98.83	93.84 %	96.88
45	91.72	90.07 %	93.71
46	90.17	89.45 %	91.67

Example	Assay [w/w] % of Initial *	% CP	% CP [% of Initial]
47	105.70	88.59 %	91.31
48	106.92	95.11 %	97.62
49	96.48	94.62 %	96.60
50	112.30	95.86 %	98.98
51	102.92	91.80 %	99.79
52	108.12	83.10 %	86.80
53	104.22	95.25 %	97.95

* Variability in the values for Assay [w/w % of Initial] reflects the imperfect control over content uniformity for these capsule lots, which manufactured at small scale.

It is believed that the difficulties encountered during processing and the resulting modified processing procedure for Examples 32, 34 and 43 (see above) could explain the lower stability observed in these samples.

Table 16B

Example	Assay [w/w] % of Initial	Area % by HPLC			
		Linaclotide (% of Initial)	Oxidation	Hydrolysis	Formaldehyde Imine
27	91.58	89.68 (93.58)	0.09	0.60	1.59
28	93.36	88.44 (90.78)	0.24	0.41	1.55
29	93.73	87.79 (90.34)	0.18	0.53	1.82
30	108.63	93.93 (96.65)	0.39	1.11	0.44
31	94.53	86.83 (89.67)	-	0.41	0.98
32	69.28	73.15 (75.46)	0.97	1.93	1.69
33	88.91	85.96 (87.46)	0.97	3.86	0.17
34	77.37	70.42 (72.21)	0.67	0.99	1.78
35	95.34	88.85 (90.71)	0.39	1.80	0.33
36	102.83	87.27 (89.14)	3.31	1.86	0.21
37	99.33	87.23 (89.29)	-	0.59	0.25
38	93.97	86.27 (87.89)	-	0.42	0.45
39	87.78	85.23 (87.07)	-	0.40	0.31

Example	Assay [w/w] % of Initial	Area % by HPLC			
		Linaclotide (% of Initial)	Oxidation	Hydrolysis	Formaldehyde Imine
40	94.36	86.28 (88.01)	-	0.46	0.41
41	104.28	90.04 (92.22)	0.33	1.61	0.52
42	117.92	76.85 (79.52)	0.14	1.21	0.10
43	54.21	59.54 (62.12)	5.92	4.44	1.83
44	92.56	90.24 (93.17)	0.16	1.47	0.54
45	76.23	79.57 (82.78)	0.17	0.87	1.22
46	73.07	78.92 (80.88)	0.51	0.66	0.65
47	97.65	82.73 (85.27)	0.92	0.60	2.68
48	93.94	85.24 (87.49)	0.05	0.69	0.20
49	51.65	63.46 (64.79)	0.96	0.58	2.24
50	104.75	92.61 (95.62)	-	0.38	0.48
51	94.15	88.19 (92.01)	-	0.58	1.35
52	100.06	72.81 (75.62)	0.06	0.49	0.41
53	95.74	89.80 (92.35)	0.06	0.36	1.40

Chromatographic purity values for Examples 27-53 at the six-month time point appear atypically low, particularly with respect to the three-month time points for these samples. Relative trends for stabilizing or destabilizing effects can be established by comparison with Example 27 and Example 31 as internal reference experiments, for which the chromatographic purity values are approximately 6-8% lower than consistently observed in other studies that have been conducted (see, e.g., Examples 2 and 9). The three month data provided in Table 16A for the same formulations shows more typical chromatographic purity values. Thus, the low chromatographic purity values at six months are likely due to an insufficient desiccant capacity at six months for these particular storage conditions. This hypothesis is supported by the impurity peaks that are observed and that are indicative of exposure to moisture.

Example 55: Linaclotide Formulation Stability Testing at 25°C/60 % RH for 24 months

For the formulations of Examples 8-15 and 17, gelatin capsules were filled with approximately 225 mg of active beads. Five filled capsules were placed in plastic bottles. The bottles contained 1 g of desiccant and were induction sealed. The bottles were stored at 25°C/60 % RH for 24 months.

Linaclotide content and purity as well as the amount of linaclotide-related substances were measured essentially as described in Example 21 or by an equivalent method. Results are provided in Table 17.

Table 17

Example	Assay [w/w] % of Initial	Area % by HPLC			
		Linaclotide (% of Initial)	Oxidation	Hydrolysis	Formaldehyde Imine
8	94.36	94.58 (101.7)	0.21	1.26	0.53
9	94.08	95.09 (97.86)	0.14	0.36	0.93
10	80.80	87.82 (90.84)	0.38	0.26	3.77
10a [1)]	89.29	91.55 (94.95	0.50	0.39	1.60
10b [2)]	88.41	91.19 (95.02)	0.44	0.34	1.61
10c [3)]	72.35	72.36 (75.76)	0.30	0.26	19.13
11	87.50	90.25 (92.82)	1.03	0.42	1.94
12	62.82	66.77 (68.62)	2.20	1.24	2.11
13	90.59	93.79 (95.93)	1.21	0.65	0.77
14	91.41	94.88 (97.09)	0.18	0.47	0.65
15	90.91	90.31 (96.18)	0.17	0.56	1.64
17	91.45	92.92 (96.81)	0.71	0.56	0.73

[1)] As for Example 10 with additional protective coating of Aquacoat (Aquacoat Ethylcellulose Aqueous Dispersion, 15% w/w, FMC Biopolymer, ECD-30)

[2)] As for Example 10 with additional protective coating of Opadry (Opadry AMB dispersion, 20% w/w, Colorcon).

[3] As for Example 10 with additional protective coating of Eudragit (Eudragit E PO, Degussa, Roehm Pharma Polymers; SLS, Stearic Acid)

Example 56: Linaclotide Tablet Formulation and Stability Testing

Active linaclotide granules were made by fluid bed granulation essentially as described in Example 26 using the reagents described in Table 18. The linaclotide granules were blended with the excipients described in Table 19 and compressed into tablets with a hardness of ~4kp.

35 tablets were packaged in a 60 cc bottle with 5 gram desiccant and stored at 40°C/75 % RH for up to 3 months or 30°C/65 % RH for up to 3 months.

Linaclotide content and purity as well as the amount of linaclotide-related substances were measured essentially as described in Example 21 or by an equivalent method. Results are provided in Table 20.

Table 18

Ingredients	**Function**	**Granule, 150 µg linaclotide/53.7 mg granules**
Linaclotide	API	0.15 mg
Mannitol, USP	Granule filler	50 mg
Leucine, USP	Stabilizer	0.64 mg
$CaCl_2 \cdot 2H_2O$, USP	Stabilizer	0.72 mg
PVP K30, USP	Binder	2.2 mg
HCl solution (pH 2.5)	--	--

Table 19

Ingredients	**Function**	**Tablet (200 mg total weight)**
Linaclotide granules	Active	53.4
Isomalt, USP	Tablet filler	134.1
Croscarmellose Sodium, USP	Disintegrant	10
Magnesium stearate, USP	Lubricant	1.5
Talc, USP	Glidant	1.0

Table 20

Condition	**Time**	**Change in Assay %**	**Total Degradation**

40°C/75 % RH	Initial	100	2.27
40°C/75 % RH	1 month	96.2	2.09
40°C/75 % RH	2 months	102	2.15
40°C/75 % RH	3 months	99.5	1.52
30°C/65 % RH	3 months	100.1	1.19

Example 57: Linaclotide Capsule Formulation

The linaclotide formulation of Example 57 was produced essentially as described in Example 16. Table 21 provides the coating solution ingredients and their theoretical weights (mg/g) and (kg/Batch) for the complete Linaclotide Beads Drug Layer Solution. Table 22 provides the ingredients and theoretical weights (mg/g) and (kg/Batch) for the preparation for the Linaclotide Active Beads. The linaclotide formulation was encapsulated in hard gelatin capsules, size 2 (weight 61 mg), essentially as described in Example 20. The 150 μg linaclotide capsules contained 56 mg linaclotide beads (600 μg linaclotide/225 mg beads) while the 300 μg linaclotide capsules contained 113 mg linaclotide beads (600 μg linaclotide/225 mg beads).

Table 21

Ingredients	Function	Theoretical Weight (mg/g)	Theoretical Weight (kg/batch)
Linaclotide	API	2.67	0.067
$CaCl_2 \cdot 2H_2O$, USP, EP, BP, JP	Stabilizer	15.41	0.385
L-Leucine, USP	Stabilizer	6.87	0.172
Hydroxypropyl Methylcellulose, USP (Methocel E5 Premium LV)	Binder	7.00	0.175
Purified Water, USP	--	--	16.666
HCl (36.5-38.0), NF	--	--	0.114

Table 22

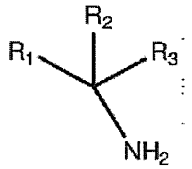
Ingredients	Function	Theoretical Weight (mg/g)	Theoretical Weight (kg/batch)
Linaclotide Beads Drug Layer Solution	Coating solution	31.95	0.799
Microcrystalline cellulose spheres NF (Celphere CP-305)	Beads	968.05	24.201
Final Total: Linaclotide Beads, 600 μg/225 mg)	Active beads	1000	25.000

CLAIMS

1. A solid pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide, a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+}, and a sterically hindered primary amine.

2. The solid pharmaceutical composition according to claim 1, wherein said Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} is provided as magnesium chloride, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, manganese chloride, potassium chloride, sodium chloride or aluminum chloride.

3. The solid pharmaceutical composition according to claim 1 or claim 2, wherein said cation is Ca^{2+} and is provided as calcium chloride.

4. The solid pharmaceutical composition according to any one of claims 1-3, wherein the sterically hindered primary amine is selected from an amino acid, a polymeric amine, or a compound of the formula: 

, wherein R_1, R_2 and R_3 are independently selected from: H;
-C(O)OH; C_1-C_6 alkyl, optionally substituted by $-CO_2H$, $-CONH_2$, or a 5-10 membered aryl or heteroaryl; C_1-C_6 alkoxyalkyl; or C_1-C_6 thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or $-NH_2$, and provided that no more than two of R_1, R_2 and R_3 are H.

5. The solid pharmaceutical composition according to claim 4, wherein the sterically hindered primary amine is a naturally-occurring amino acid selected from the group consisting of histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan and valine.

6. The solid pharmaceutical composition according to claim *5,* wherein the naturally-occurring amino acid is leucine.

7. The solid pharmaceutical composition according to any one of claims 1-6 further comprising a pharmaceutically acceptable glidant, lubricant or additive that acts as both a glidant and lubricant.

8. The solid pharmaceutical composition according to any one of claims 1-7 further comprising one or more of an antioxidant; a pharmaceutically acceptable binder; or a pharmaceutically acceptable filler.

9. The solid pharmaceutical composition according to claim 8, wherein the pharmaceutically acceptable binder is selected from polyvinyl alcohol, polyvinylpyrrolidone (povidone), a starch, maltodextrin or a cellulose ether.

10. The solid pharmaceutical composition of claim 9, wherein the pharmaceutically acceptable binder is a cellulose ether selected from methylcellulose, ethylcellulose, carboxymethylcellulose, hydroxyethyl cellulose, hydroxyethyl methylcellulose, hydroxypropyl cellulose and hydroxypropyl methylcellulose.

11. The solid pharmaceutical composition according to any one of claims 8-10, wherein the pharmaceutically acceptable filler is cellulose, isomalt, mannitol or dibasic calcium phosphate.

12. The solid pharmaceutical composition according to claim 11, wherein the cellulose is selected from microfine cellulose and microcrystalline cellulose.

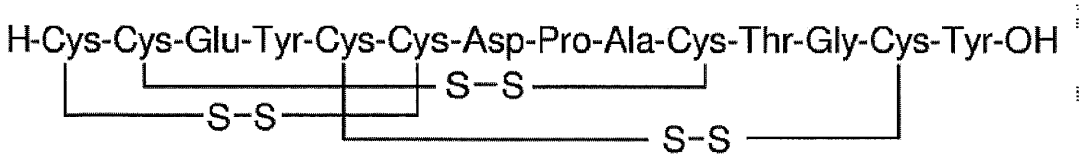
13. The solid pharmaceutical composition according to any one of claims 1-12, wherein the amine is leucine and the molar ratio of leucine to linaclotide is at least 10:1.

14. The solid pharmaceutical composition according to any one of claims 1-13, wherein the cation is Ca^{2+}, the amine is leucine and the molar ratio of Ca^{2+} to leucine is at least 1:1.

15. The solid pharmaceutical composition according to any one of claims 1-14, wherein the molar ratio of the cation: sterically hindered primary amine: linaclotide is 40-100:20-50:1.

16. The solid pharmaceutical composition according to any one of claims 1-15, wherein the cation is Ca^{2+}, the sterically hindered primary amine is leucine and the molar ratio of Ca^{2+}:leucine:linaclotide is 60:30:1.

17. The solid pharmaceutical composition according to any one of claims 1-16, further comprising: (i) a hydrolysis product having a structure of



comprising less than 2% by weight compared to the weight of the linaclotide; or

(ii) a linaclotide oxidation product having a molecular weight of 1542.8 comprising less than 2% by weight compared to the weight of the linaclotide.

18. A solid pharmaceutical composition comprising:
linaclotide;
Ca^{2+};
leucine; and
hydroxypropyl methylcellulose,
wherein the molar ratio of Ca^{2+}:leucine:linaclotide is between 5-100:5-50:1.

19. The solid pharmaceutical composition of claim 18, wherein the Ca^{2+} is provided as $CaCl_2$.

20. A solid pharmaceutical composition of any one of claims 1 -19 for use in treating a gastrointestinal disorder selected from impaired intestinal motility, irritable bowel syndrome, constipation, pain associated with constipation, dyspepsia, gastroparesis, chronic intestinal pseudo obstruction, Crohn's disease, ulcerative colitis, or inflammatory bowel disease.

21. Use of linaclotide or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a gastrointestinal disorder selected from impaired intestinal motility, irritable bowel syndrome, constipation, pain associated with constipation, dyspepsia, gastroparesis, chronic intestinal pseudo obstruction, Crohn's disease, ulcerative colitis, or inflammatory bowel disease, said medicament comprising a solid pharmaceutical composition comprising a pharmaceutically acceptable carrier, linaclotide, a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+}, and a sterically hindered primary amine.

22. The solid pharmaceutical composition of claim 20 or use of claim 21, wherein said irritable bowel syndrome is constipation-predominant irritable bowel syndrome or alternating irritable bowel syndrome.

23. The solid pharmaceutical composition of claim 20 or use of claim 21, wherein said irritable bowel syndrome is constipation-predominant irritable bowel syndrome.

24. The solid pharmaceutical composition of claim 20 or use of claim 21, wherein said constipation is chronic constipation, idiopathic constipation, post-operative ileus, or constipation caused by opiate use.

25. The solid pharmaceutical composition of claim 20 or use of claim 21, wherein said constipation is chronic constipation.

26. The solid pharmaceutical composition or use of any one of claims 20-25, wherein said pharmaceutical composition comprises 50 µg to 1 mg of linaclotide.

27. The solid pharmaceutical composition or use of claim 26, wherein said pharmaceutical composition comprises 67.5 µg, 133 µg, 150 µg, 266 µg or 300 µg of linaclotide.

28. The solid pharmaceutical composition or use of any one of claims 20-27, wherein said pharmaceutical composition is to be administered once daily or twice daily.

29. The solid pharmaceutical composition or use of any one of claims 20-28, wherein the pharmaceutical composition is to be administered once daily as one or two tablets or capsules.

30. The solid pharmaceutical composition or use of any one of claims 20-29, wherein said pharmaceutical composition comprises 266 μg of linaclotide.

31. The solid pharmaceutical composition or use of any one of claims 20-29, wherein said pharmaceutical composition comprises 133 μg of linaclotide.

32. The solid pharmaceutical composition or use of any one of claims 20-31, wherein said treatment is for a period of at least four weeks.

33. The solid pharmaceutical composition or use of any one of claims 20-32, wherein said treatment improves at least one symptom selected from reduced abdominal pain, an increase in the number of complete spontaneous bowel movements (CSBM) in a week, an increase in the number of spontaneous bowel movements (SBM) in a week, improved stool consistency, reduced straining, reduced abdominal discomfort, reduced bloating or reduced IBS-c symptom severity.

34. The solid pharmaceutical composition or use according to any one of claims 1-33, said composition further comprising coated beads, wherein the beads are coated with a coating solution comprising linaclotide.

35. The solid pharmaceutical composition, or use of claim 34, wherein the coating solution comprises:

linaclotide;
Ca^{2+};
leucine; and
hydroxypropyl methylcellulose,

wherein the molar ratio of Ca^{2+}:leucine:linaclotide is between 5-100:5-50:1.

36. The solid pharmaceutical composition, or use of claim 35, wherein the beads comprise microcrystalline cellulose.

37. A unit dosage form comprising a solid pharmaceutical composition according to any one of claims 1-19 or 34-36.

38. A sealed container comprising a plurality of unit dosage forms according to claim 37.

39. A method for preparing a solid pharmaceutical composition comprising linaclotide or a salt thereof, the method comprising:

(a) providing an aqueous solution comprising:

(i) linaclotide or a pharmaceutically acceptable salt thereof

(ii) one or more of a cation selected from Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, K^{+}, Na^{+} or Al^{3+} or a sterically hindered primary amine; and,

(iii) optionally, a pharmaceutically acceptable binder; and

(b) applying the aqueous solution to a pharmaceutically acceptable filler to generate linaclotide-coated filler.

40. The method of claim 39, wherein the aqueous solution is applied to the filler by spray drying.

ABSTRACT

LINACLOTIDE-CONTAINING FORMULATIONS FOR ORAL ADMINISTRATION

Solid, stable formulations of linaclotide suitable for oral administration are described herein as are methods for preparing such formulations. The formulations described herein contain a polypeptide consisting of the amino acid sequence Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr ("linaclotide") or a pharmaceutically acceptable salt thereof. The linaclotide formulations described herein are stable and have a sufficient shelf life for manufacturing, storing and distributing the drug.

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